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ANTIAPOPTOTIC MECHANISM FOR THE IMPLEMENTATION OF THE HEPATOPROTECTIVE EFFECT OF PYRIMIDINE DERIVATIVES

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Pyrimidine derivatives, Apoptosis, Carbon tetrachloride, Toxic liver damage

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ABSTRACT: The present study aimed to study the effect of the drug Xymedon and its conjugate with *L*-ascorbic acid exhibiting hepatoprotective activity on the apoptosis of rat liver cells against the background of the influence of hepatotoxic agent tetrachloromethane. Though the general effects of Xymedon and its conjugates with various biogenic acids have been studied, the molecular markers affected by the compounds have not yet been established. Experimental methods of investigation included modeling of toxic liver damage by oral administration of carbon tetrachloride to laboratory Wistar rats. To establish the molecular mechanisms of the antiapoptotic effect involved in the hepatoprotective activity of Xymedon and its conjugate with L-ascorbic acid, the level of early apoptosis markers Akt, BAD, BCL-2, p53, Active Caspase-8 Active Caspase-9 in rat liver homogenates was determined by multiplex analysis method using the MagPix system («MerkMillipore», USA). The study showed that Xymedon and its derivative with *L*-ascorbic acid exhibited an antiapoptotic effect, significantly reducing the number of early apoptosis markers BAD, Active Caspase-9. Also, the derivative with *L*-ascorbic acid reduces the expression of p53. Moreover, it was shown that some biochemical markers are normalized under the influence of the studied compounds. The results obtained have both scientific value, bringing us closer to the true mechanism of action of pyrimidine derivatives, and practical value - the possibility to create an effective hepatoprotective drug with a strong evidence base and degree of study.

INTRODUCTION: The liver is the body that plays a key role in the detoxification of exogenous and endogenous toxic substances. Late treatment of mild forms of liver pathologies caused by hepatotoxic substances can lead to more serious up to necrotic damage [1].

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The objects of the study were a drug based on the pyrimidine derivative Xymedon (compound 1), and it's conjugate with *L*-ascorbic acid (compound 2). The structural formula of compounds 1 and 2 is shown in **Fig 1**.

Xymedon (1, 2 – dihydro - 4, 6 – dimethy *L* – 1 - (2 - hydroxyethyl) - pyrimidin- 2 -one) is an original Russian drug compound a tissue regeneration stimulant. The chemical compound was synthesized at the Institute of Organic and Physical Chemistry in 1966. This compound has many activities, including membrane stabilizing, regenerating, immunostimulating, *etc.* [2].

Earlier data have shown that conjugation of Xymedon with some bioactive acids leads to an increase in the protective effect of the Xymedon molecule itself [3-5]. As a result of exploratory studies, Xymedon conjugate with *L*-ascorbic acid, among other studied conjugates, has a more pronounced and stable hepatoprotective effect. It has been shown *in vivo* that the conjugate of xymedon with L-ascorbic acid helps to reduce the severity of morphological signs of liver damage induced by carbon tetrachloride (CCl_4) [6]. It was also found that Xymedon conjugate with *L*-ascorbic acid contributes to the normalization of biochemical markers of liver tissue damage in the blood of animals against the background of liver damage induced by CCl_4 [6]. Moreover, a more pronounced hepatoprotective effect was shown for Xymedon conjugate with *L*-ascorbic acid than for the comparison drug – Tiotriazolin [10]. Thus, understanding the fact that we know the hepatoprotective potential of Xymedon conjugate with *L*-ascorbic acid, we attempted to establish the mechanism by which the protective effect of the studied conjugate is carried out. This study aimed to study the effect of Xymedon and its conjugate with *L*-ascorbic acid, which has hepatoprotective activity, on apoptosis of rat liver cells against the background of injuries caused by CCl_4.

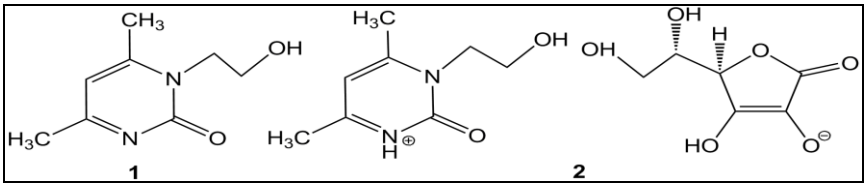

FIG. 1: STRUCTURAL FORMULA OF THE STUDIED COMPOUNDS. 1: xymedon; 2: conjugate of xymedon with l-ascorbic acid

MATERIALS AND METHODS:

Synthesis of the Studied Compounds: Xymedon (1, 2-dihydro-4, 6-dimethyl-1-(2-hydroxyethyl)-pyrimidin-2-one) and the conjugate of Xymedon with *L*-ascorbic acid were synthesized by previously described methods from 2-dihydro-4, 6-dimethylpyrimidin-2-one and 2- chloroethanol [7, 8].

Design of the Experiment Involving the Experimental Animals: The experiments were performed on adult male Wistar rats weighing 300-350 g obtained from the Laboratory Animal Breeding Station of FIBH RAS (Pushchino, Russia). Animals were kept in accordance with the provisions of preclinical test guidelines [9] and European Community guidelines for the housing and care of animals used for experiments and other research purposes (EC Committee guidance on management and care of animals used for experiments and other research purposes, 2007/526/EC, of June 18th 2007.) in standard vivarium conditions with a 12-hour light day and unrestricted access to food and water. Animals were fed a complex feed made according to the Specification (protein 22%, fiber no more than 4%, fat no more than 5%, ash no more than 9%, moisture no more than 13.5%, Caloric value 295 kcal per 100 g. All animal studies and protocols were approved by the local ethical committee of Kazan Federal University (Protocol № 4 of May 18, 2017). In total, 24 animals participated in the experiment (6 animals in each of the four experimental groups). The experimental scheme was as follows: administration of CCl_4 for 3 days, then administration of drugs for 6 days (a total of 9 days). Material (blood for biochemical studies, liver samples) was taken the day after the last CCl_4 injection (day 4 of the experiment), 3 days after stopping CCl_4 injection and 3 days of drug administration (day 7 of the experiment) and 6 days after stopping CCl_4 administration and 6 days of drug administration (day 10 of the experiment), when animals were euthanized.

Carbon tetrachloride was administered orally as a 35% oil solution at a 1.5 ml/kg dose. Investigated compounds 1 and 2 were injected intraperitoneally at 0.24 and 0.5 mg/kg doses. The drug solutions were prepared every day immediately before administration to the animals. Serum was prepared by double centrifugation of blood at 3000 rpm and +4°C. Serum was stored in a freezer at -25°C before analysis. Biochemical parameters were studied using an automatic biochemical analyzer ARD 200 (ARD, Russia) and reagent kits.

Histological Studies: Histological processing - preparation of paraffin sections, staining with

Garris hematoxylin (BioVitrum, Russia), alcohol eosin (BioVitrum, Russia) were performed according to standard protocols described in more detail in our previous works [3-7]. Frozen sections were also prepared and stained with Sudan 4 (BioVitrum, Russia) according to standard protocols. For microscopy and microphotography the Nikon (Nikon, Japan) microscope, digital camera (Nikon, Japan) and NIS Elements software (Nikon, Japan) were used.

Study of Molecular Markers of Apoptosis: Markers were studied in rat liver lysates according to the protocol of Early Apoptosis Magnetic Bead Kit (Merck / Millipore Sigma, USA) with measurement on Mag Pix device (Merk / Millipore, USA). To study apoptosis markers, 5 mg of liver tissue taken from animals during euthanasia was lysed beforehand according to the instructions of Micro Rotofor Lysis Kit. (BioRad, USA) with Protease Inhibitor Cocktail (Merck/Sigma-Aldrich, USA) added. Next, all samples were aligned for total protein concentration to 100 µg/ml by measuring the protein concentration in the samples by the Bradford method using the Quick Start Bradford Protein Assay Kit (BioRad, USA) and an Epoch microplate spectrophotometer (BioTech, USA). Bovine serum albumin (Amresco, USA) was used to construct a calibration chart.

Statistical Analysis: All experimental data obtained underwent statistical analysis. We calculated the mean values and standard errors of the mean. Figures contain mean values for each standard parameter and standard error. For each sample the normality of the distribution was evaluated using the Kolmogorov-Smirnov criterion. In cases of normal distribution, samples were compared using Student's t-criterion. In cases of non-normal distribution, the comparison was done using Mann-Whitney U-test. Statistical processing was performed using SPSS 13.0. Data visualization was performed in Origin Lab 2018, the layout of figures in Adobe Photoshop 18.00.

RESULTS:

Evaluation of the Clinical Condition of the Animals: After a three-day course of CCl_4 injection, the animals showed a picture of acute toxic damage to the liver, confirmed on histological sections, where foci of steatosis, hepatocyte dystrophy and necrotic changes were visible **Fig. 2**.

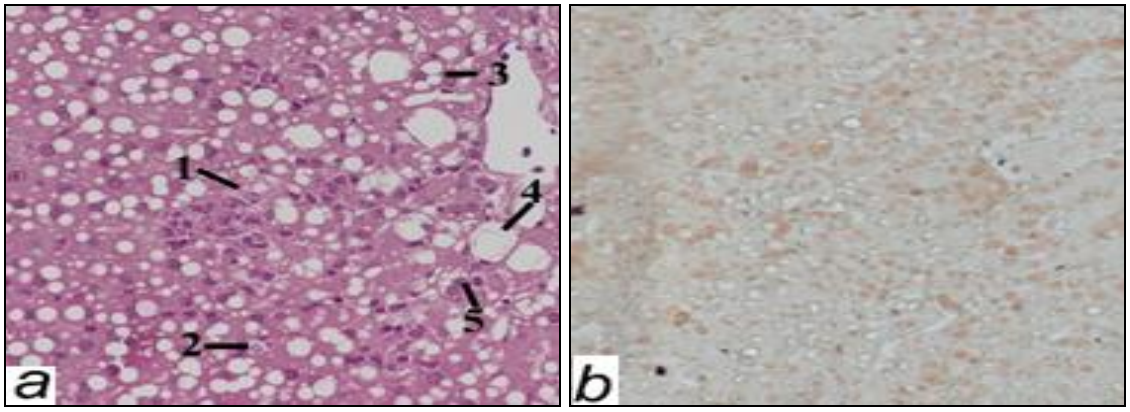

FIG. 2: MICROPHOTOGRAPHS OF RAT LIVER SECTIONS AFTER THREE-DAY EXPOSURE TO CCL_4. a: hematoxylin and eosin staining (1-hydropic dystrophy, 2-caunselmain cell, 3-large dropletadipose degeneration, 4-balloon dystrophy, 5-small droplet adipose degeneration); b: sudan iv staining (orange drops are lipid droplets); lens 20x. zoom 300x

Among the changes in the biochemical blood parameters, we observed a twofold increase in the activity of ASAT (aspartate aminotransferase) **Fig. 3A**, while ALAT activity decreased in comparison with the reference values of the intact group of animals **Fig. 3B**. By day 10 of the experiment, the level of ALAT (alanine aminotransferase) activity is practically unchanged, but ASAT activity by day 10 sharply decreases more than twofold compared with the reference values of the intact group of animals. Against the background of introduction of compounds 1 and 2, the changes in the activity of aminotransferases were not as pronounced as in the control animals. Thus, the activity of ALAT when compounds 1 and 2 were injected on day 7 decreased less significantly relative to intact animals, but its level was significantly higher than in the control group on the same day of the experiment. On day 10 of the experiment, ALAT activity in the experimental groups remained at the

level of day 7, but did not statistically differ from the control group on the same day **Fig. 3B**. ASAT activity against the background of administration of compounds 1 and 2 practically did not differ from the reference values of the intact group of animals. However, administration of compound 2 on day 10 of the experiment resulted in a decrease in ASAT activity compared to the intact group of animals, but its activity was significantly higher than that in the control group on the same day **Fig. 3A.**

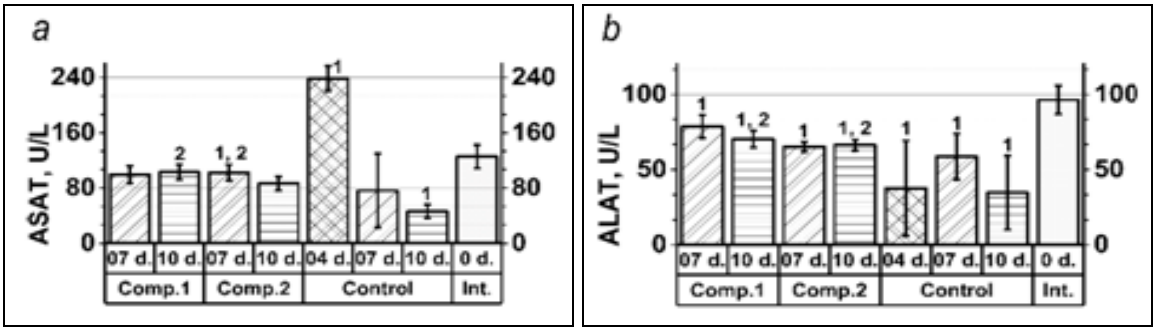

FIG. 3: EFFECT OF COMPOUNDS 1 AND 2 ON CELL CYTOLYSIS MARKERS. a: aspartate aminotransferase (asat) activity, b: alanine aminotransferase (alat) activity; 1 differences from intact group are significant by t-test, $p < 0.05$; 2 differences from control group of the same day are significant by t-test, $p < 0.05$.

After a three-day course of CCl_4 administration, we observed signs of biliary excretion and bilirubin excretion in animals. Namely, accumulation of alkaline phosphatase and bilirubin in blood plasma. The increase in total bilirubin is mainly associated with an increase in the fraction of conjugated bilirubin **Fig. 4A-D.** On day 7 of the experiment, compounds 1 and 2 showed no significant improvement in any of the indices. However, while the control group on day 10 of the experiment showed a fairly strong decrease in the ALP activity (alkaline phosphatase) **Fig. 4A** and concentration of all bilirubin fractions **Fig. 4B-D**, the use of compounds 1 and 2 on day 10 of the experiment showed their stabilization in comparison with the indices of the intact group of animals. ALP activity and concentration of bilirubin fractions are significantly higher than those of the control group on the same day but still remain lower than in the intact group of animals **Fig. 4A-D.**

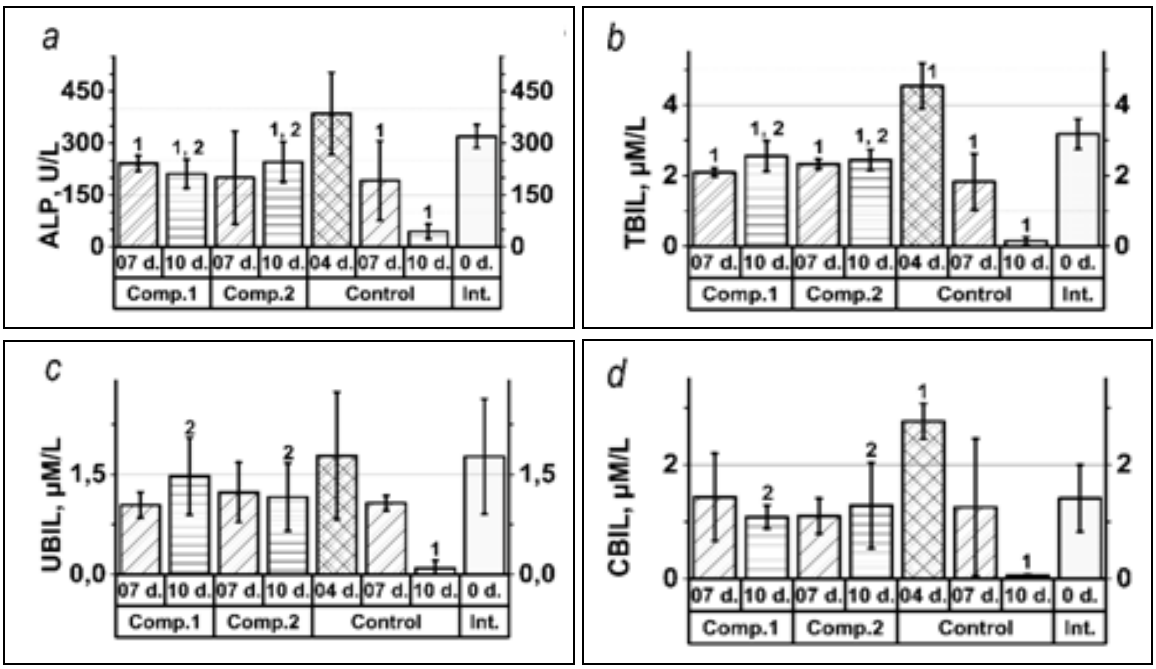

FIG. 4: EFFECT OF COMPOUNDS 1 AND 2 ON MARKERS OF BILIARY EXCRETION AND BILIRUBIN EXCRETION. a: alkaline phosphatase (alp) activity; b: total bilirubin concentration; c: unconjugated bilirubin concentration; d: conjugated bilirubin concentration; 1 difference from the intact group are reliable by t-test, $p < 0.05$; 2 differences from the control group of the same day are reliable by t-test, $p < 0.05$.

Disruption of albumin synthesis and a decrease in its concentration in blood serum in animals injected with CCl_4 is observed immediately after the cancellation of the toxicant on day 4 of the experiment. With each subsequent day after the cancellation of CCl_4, the albumin concentration in the blood serum decreased and reached its minimum on the last, tenth day of observation. Administration of compounds 1 and 2 for 6 days after cancellation of CCl_4 contributed to the fact that albumin concentration decreased to a lesser extent than in the control group, and on day 10 of the experiment, this index in the experimental groups was statistically significantly higher than in the control group **Fig. 5A**. The concentration of total serum protein **Fig. 5B** in the control group of animals also decreased; however, on days 4 and 7 of the experiment, not as critical as the albumin concentration. The minimum protein concentration was also recorded on the last, 10th day of the experiment. The use of compounds 1 and 2 against the background of CCl_4-induced toxic damage resulted in a smaller decrease in protein concentration than the control (the differences with the control group on day 10 are statistically reliable, with the intact group - not reliable). At the same time, the character of changes in protein concentration under the influence of substances was similar to that of albumin concentration **Fig. 5B**. The serum glucose concentration was not significantly affected by CCl_4 on days 4 and 7 of the experiment **Fig. 5B**. However, on day 10 of the experiment, the blood glucose concentration reached minimum values. Quite interesting results were obtained using compounds 1 and 2 against the background of toxic liver damage. If against the background of CCl_4 exposure, by day 10 of the experiment, a natural decrease in this index was observed in control; no such decrease was observed with compounds 1 and 2. Moreover, we noted that the compounds used led to a significant increase in glucose concentration in the blood serum compared to the intact group of animals in all control points of the experiment **Fig. 5B**.

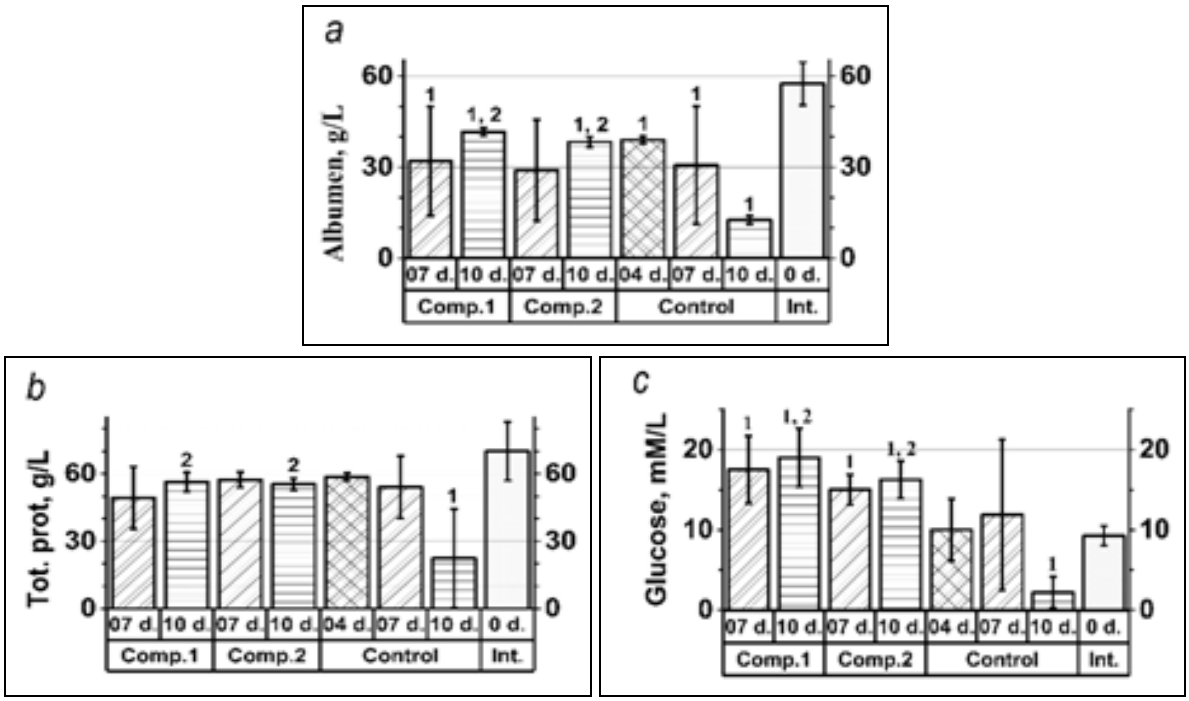

FIG. 5: EFFECT OF COMPOUNDS 1 AND 2 ON MARKERS OF PROTEIN METABOLISM AND GLUCOSE. a: albumin concentration; b: total protein concentration; c: glucose concentration; 1 differences from the intact group are significant by t-test, $p < 0.05$; 2 differences from the control group of the same day are significant by t-test, $p < 0.05$.

Molecular Markers of Early Apoptosis: The study of markers of early apoptosis in liver lysates allowed us to see how a three-day CCl_4 injection promotes the emergence of such a trend as an increase in MFI (Mean intensity of fluorescence) of the proapoptotic BAD protein of BCL family on days 7 and 10 of the experiment **Fig. 6A**. At the same time, the more days pass since the cessation of the introduction of the toxicant, the greater the MFI of BAD is detected in the liver homogenates of the control group rats. In turn, administration of compounds 1 and 2 contributed to a decrease in the

MFI of this marker of early apoptosis. Compound 1 was found to have no significant effect on the 7th day of the experiment **Fig. 6A**. But on day 10, compound 1 produced a sharp, significant decrease in BAD-protein MFI both in comparison to the same parameters in the control group and in comparison to the intact group of animals **Fig. 6A**. When compound 2 was used, a significant decrease in the BAD-protein MFI was already observed on day 7 of the experiment, both compared to the control group of animals on the same day and compared to the intact animals **Fig. 5A**. On day 10 of the experiment, when compound 2 was injected, a significant decrease in this MFI BAD protein was also observed **Fig. 6A**. Considering the antiapoptotic markers BCL-2 and Akt in this study, we cannot speak about any reliable effect of CCl_4 and compounds 1 and 2 on them. However, interesting, quite logical correlations between proapoptotic BAD and antiapoptotic BCL-2 and Akt are observed. A decrease in the MFI of antiapoptotic BCL-2 and Akt markers increases the MFI of proapoptotic BAD. It is worth noting that this statement is not supported by statistical analysis **Fig. 6 B, C**.

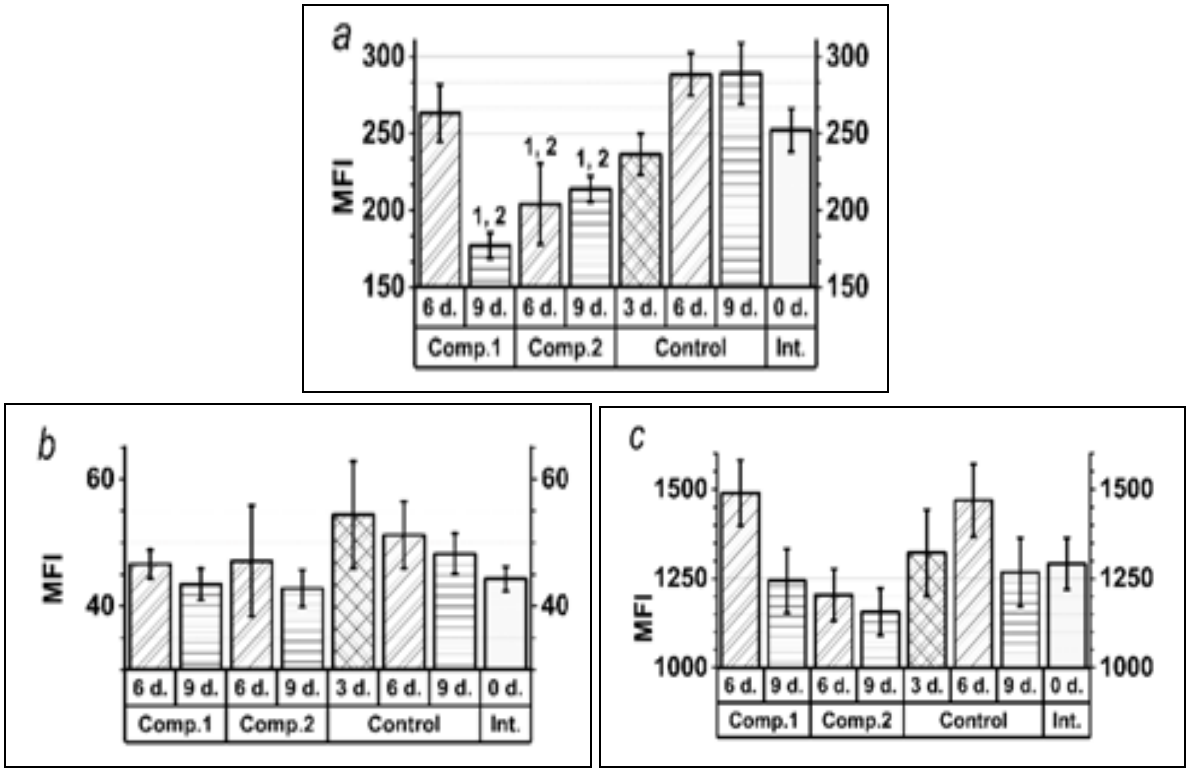

FIG. 6: EFFECT OF COMPOUNDS 1 AND 2 ON MEAN FLUORESCENCE INTENSITY (MFI) OF EARLY APOPTOSIS MARKERS. a: MFI BAD; b: MFI BCL-2; c: MFI Akt; 1 differences from intact group are significant by U-test, $p < 0.05$; 2 differences from control group of the same day are significant by U-test, $p < 0.05$.

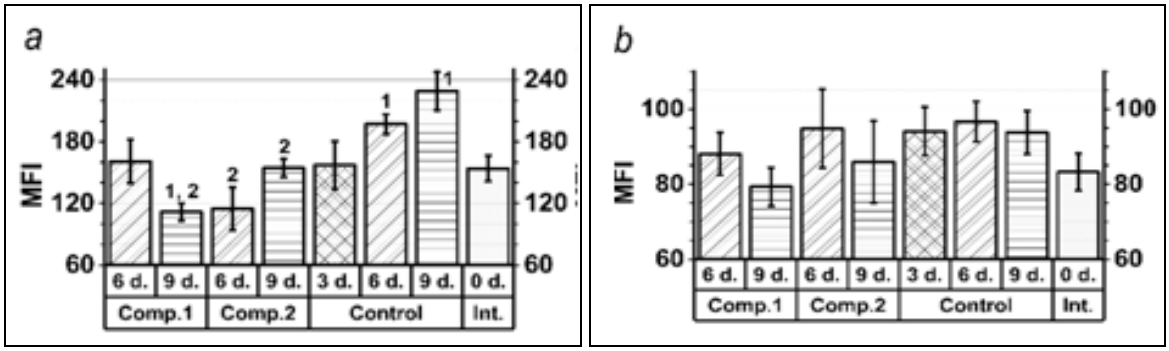

FIG. 7: EFFECT OF COMPOUNDS 1 AND 2 ON THE MEAN FLUORESCENCE INTENSITY (MFI) OF ACTIVATED CASPASES 9 AND 8. a: MFI of activated caspase 9 (CASP9); b: MFI of activated caspase 8 (CASP8); 1 differences from intact group are significant by U-test, $p < 0.05$; 2 differences from control group of the same day are significant by U-test, $p < 0.05$.

Three-day injection of CCl_4 contributed to a significant increase in the MFI of activated CASP9 on days 7 and 10 of the experiment relative to the reference values of the intact group of animals **Fig. 7A**. Administration of compound 1 contributed to a significant decrease in CASP9 activation on day 10 of the experiment relative to the control group on the same day of the study **Fig. 7A**. As early as day 7 of the experiment, administration of compound 2 contributed to a significant decrease in CASP9 activation **Fig. 7A**. On day 10 of the experiment, compound 2 also contributed to a decrease in CASP9 activation relative to the animal control group (**Fig. 7A**. In turn, administration of CCl_4 and drugs did not result in a significant change in the MFI of activated CASP8 **Fig. 7B**. We also observed a significant decrease in the MFI of p53 protein when using compound 2 relative to controls on similar days of the study **Fig. 8**.

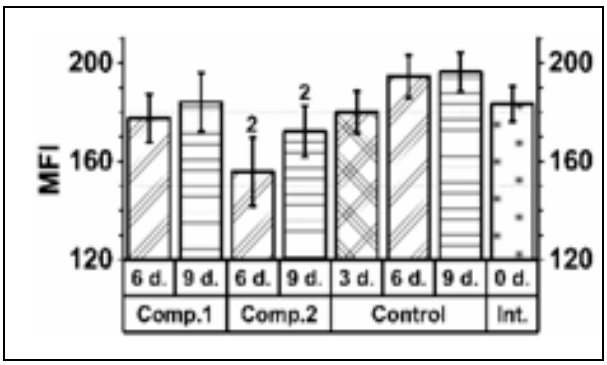

FIG. 8: EFFECT OF COMPOUNDS 1 AND 2 ON THE AVERAGE FLUORESCENCE INTENSITY OF MFI P53; 2 DIFFERENCES FROM THE CONTROL GROUP OF THE SAME DAY ARE SIGNIFICANT BY U-TEST, P < 0.05.

DISCUSSION: The mechanism of action of the Xymedon molecule is presumably related to the regulation of adenylate cyclase (AC) activity since there is evidence that Xymedon reduces the activity of this signaling molecule in immunocompetent cells [10]. Speaking about further consequences of AC activation or inhibition, it is worth mentioning that there are different AC isoforms whose regulation is mediated by G-proteins [11]. The regulation of AC activity through G-protein subunits is top-specific, *i.e.*, it depends on the AC isoform and cell type [11]. AC activity is associated with cAMP production (cyclic adenosine monophosphate) in cell [12]. cAMP, in its turn, activates regulatory processes in a cell, depending on its type [13]. For example, there is evidence that high cAMP concentrations stimulate the proliferation of epithelial cells, hepatocytes, adipocytes and pancreatic β-cells [14]. But at the same time, high cAMP production by fibroblast, smooth muscle, and neoplastic B-cells, on the contrary, suppresses proliferation [14]. After a three-day course of CCl_4 injection in animals, there is a picture of acute toxic damage to the liver, expressed in a change in the activity of ASAT and ALAT enzymes. The sharp increase in ASAT level after a course of toxicant injection can be explained because ASAT, like ALAT, is contained in large quantities in liver cells [15]. Its highest activity is observed in the mitochondria of the peripheral zone of the acini [16]. Consequently, acutely toxic damage to the cells of this zone will cause a sharper increase in serum ASAT activity than ALAT.

A further decrease in ASAT activity is because the half-life from the bloodstream is about 17 h for total ASAT and an average of 87 hours for mitochondrial ASAT [17]. While pathological changes in ALAT and ASAT activities were observed in the control group, Xymedon compounds and their conjugate with *L*-ascorbic acid resulted in more stable preservation of the activities of these aminotransferases. This indicates less necrotic damage to the liver parenchyma. Increased necrotic damage on day 4 of the experiment led to the occurrence of stagnation. It increased levels of ALP, as well as impaired excretion of bilirubin from the blood, which is a sign of jaundice [18].

A sharp decrease in ALP activity and the level of total bilirubin and its fractions on day 10 in the control group, in all probability, is caused by the decrease of functional activity of hepatocytes as a result of toxic damage. More stable ALP activity and concentrations of bilirubin fractions when using Xymedon and its conjugate with *L*-ascorbic acid, especially on day 10 of the experiment, testify to the fact that under the influence of these compounds, either the activity of undamaged hepatocytes increased or the necrotic damage to the organ in general decreased, so the metabolism of bilirubin passes in the liver [18]. The decrease in the concentrations of total protein and albumin noted in our experiment against the background of exposure to a toxicant looks quite logical since a decrease in synthetic liver function is observed in liver

parenchyma damage [19]. As follows from the data obtained, when using Xymedon and its conjugate with *L*-ascorbic acid, the preservation of synthetic liver function or intensification of such processes in undamaged hepatocytes is observed. In favor of the theory about the intensification of hepatocyte metabolism processes, there are data on blood glucose concentration. It is known that the liver is the main depository of the main energy source for eukaryotic cells - glucose in the form of glycogen [20]. From liver glycogen, under the influence of the enzyme system of hepatocytes and external signaling factors, glucose is formed again, which covers the energy deficit of cells of the whole organism [21].

In our case, however, after a course of drug administration, the level of glucose rises considerably above the reference values obtained in intact animals. This may be evidence of glycogenolysis activation, which allows supporting the theory about the intensification of metabolic processes in liver cells. Activation of glycogenolysis can also be explained by an increase in cAMP under the influence of the molecule Xymedon. cAMP in a hepatocyte can increase glucose production by increasing the transcription of gluconeogenic enzymes [22, 23]. The increase in glucose production after AC activation and the increase in cAMP concentration in hepatocytes after exposure to Xymedon conjugate with *L*-ascorbic acid can explain the earlier data that this compound leads to a 440% increase in the performance of rats without a decrease in blood glucose levels [24].

Our studies of early apoptosis markers revealed that Xymedon (on day 10 of the experiment) and its conjugate with *L*-ascorbic acid (on days 7 and 10 of the experiment) reduced the MFI of the proapoptotic protein BAD. Protein containing only BH3, BAD inhibits antiapoptotic BCL-2 and BCL-xL and promotes BAX/BAK activation in response to apoptotic stimuli [25]. This is evidence that these compounds reduce the initiation of apoptotic processes in cells. Thus, compensating not only for the effect of CCl_4, but also leading to a decrease in the expression of the proapoptotic protein BAD below the values of the intact group of animals. However, in this case, the decrease in MFI of the proapoptotic protein BAD is not related to MFI Akt, which is considered an inhibitor of apoptosis because of its ability to inactivate proapoptotic molecules, including caspase-9 (CASP9) and BAD protein [26]. Thus, the decrease in BAD MFI under the influence of Xymedon and its conjugate with *L*-ascorbic acid is most likely related not to its Akt phosphorylation but a decrease in the expression of this protein in the cell. We also found no significant increase in MFI of the antiapoptotic protein BCL-2. This protein prevents apoptosis either by sequestering the proformas of caspases that cause cell death or by preventing the release of mitochondrial apoptogenic factors such as cytochrome C and AIF (apoptosis-inducing factor) into the cytoplasm [28].

In the experiment conducted, CCl_4 promoted activation of predominantly internal liver cell apoptosis pathway related to CASP9 activation [28]. The observed significant decrease in fluorescence intensity of both BAD protein and activated caspase-9 when injecting Xymedon on day 10 of the experiment and the conjugate of Xymedon with *L*-ascorbic acid on days 7 and 10 of the experiment may be evidence of less damage of mitochondrial membranes and less release of cytochrome C that trigger activation of apoptotic processes [29]. Enhanced antiapoptotic properties of the conjugate of Xymedon with *L*-ascorbic acid, expressed in the fact that these properties of the conjugate occur earlier in comparison with Xymedon, firstly, probably due to the high bioavailability of ascorbic acid for cells, which probably contributes to the bioavailability of the Xymedon molecule as well.

Secondly, there is evidence that *L*-ascorbic acid can act synergistically with other substances. For example, ascorbic acid, together with phenylephrine and metaproterenol, can enhance the synthesis and proliferation of primary hepatocyte culture DNA [30]. Phenylephrine and metaproterenol themselves do not accelerate the synthesis and proliferation of primary hepatocyte culture DNA [30]. Besides, it was shown that *L*-ascorbic acid itself can accelerate the regeneration of rat liver tissue [21].

CONCLUSIONS: It was shown that Xymedon and its conjugate with *L*-ascorbic acid, against the background of damage caused by three-day administration of CCl4, contribute to the preservation of liver cell function, which is

manifested in the reduction of the activity of cytolysis markers ASAT and ALAT, normalization of synthetic liver function, preservation of function of bilirubin metabolism in the liver. Also, these compounds cause a significant increase in blood glucose. As a result of this study, Xymedon and its conjugate with *L*-ascorbic acid against the background of exposure to CCl_4 contribute to a decrease in the lysates of rat liver tissue proapoptotic markers BAD and CASP9.

The antiapoptotic activity of Xymedon conjugate with *L*-ascorbic acid always occurred earlier (on day 7 of the experiment) than that of Xymedon (manifestation of activity on day 10 of the experiment). The derivative of Xymedon with *L*-ascorbic acid was found to significantly decrease the expression of the proapoptotic protein p53, while Xymedon, in turn, does not affect this marker.

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Ethical Compliance: All animal studies and protocols were approved by the local ethical committee of Kazan Federal University (Protocol № 4 of May 18, 2017).

CONFLICTS OF INTEREST: The author declares no conflict of interest.

REFERENCES:

1. Arias IM: The liver: biology and pathobiology. Wiley-Blackwell, Edition 2020; 6.
2. Izmajlov SG: Ksimedon: nastoyashche eibudushchee. Nizhegorodskij medicin skijzhurnal. In Russ 2002; 3: 81-7
3. Porfiriev A: The Influence of the Xymedon Conjugate with l-Methionine on the Regeneration of Schmidtea mediterranea Planarians. Bio Nano Science 2020; 10(2): 397-402. doi: 10.1007/s12668-020-00735-z
4. Vyshtakalyuk AB: Conjugate of pyrimidine derivative, the drug xymedon with succinic acid protects liver cells. J of Biochem and Mol Tox 2021; 35(3): 22660. doi: 10.1002/jbt.22660
5. Parfenov AA: Xymedone conjugate with para-aminobenzoic acid. Estimation of hepatoprotective properties. Rus Chem Bul 2019; 68(12): 2307-15. doi: 10.1007/s11172-019-2704-z
6. Vyshtakalyuk AB: Recovery of liver damaged by ccl4 under treatment by conjugate of drug xymedon with l-ascorbic acid. Int J of Pharmaceutical Sci and Res 2018; 9(10): 4117-26. doi: 10.13040/IJPSR.0975-8232.9(10).4117-26
7. Vyshtakalyuk AB: Synthesis and primary evaluation of the hepatoprotective properties of novel pyrimidine derivatives. Russ J of Bio Chem 2017; 43(5): 604-11. doi: 10.1134/S106816201704015X
8. Reznik VS: Reactions of pyrimidinols and pyrimidinethiols with 2-chloroethanol and with 2-chloro-1-propanol. Bul of the Academy of Sci of the USSR, Division of Chem Sci 1966; 15(9): 1554-7. doi: 10.1007/BF00848915
9. Mironov AN: Rukovodstvo po proved eniyudoklinicheskihissledovanijlekarstvennyhsredstv. Grifi K, First edition 2012 (Russian)
10. Slabnov YD: Effect of pyrimidine derivatives on adenylate cyclase system of immunocompetent cell regulation in vitro. Bul of Exp Bio and Med 1998; 125(6): 588-90. doi: 10.1007/BF02445248
11. Khannpnavar B: Structure and function of adenylyl cyclases, key enzymes in cellular signaling. Current Opinion in Structural Biology 2020; 63: 34-41. doi: 10.1016/j.sbi.2020.03.003
12. Muthal AP: Cyclic adenosine monophosphate: Recent and future perspectives on various diseases. Journal of Applied Pharmaceutical Science 2022; 12(03): 001-15. doi: 10.7324/JAPS.2022.120301
13. Khannpnavar B: Structure and function of adenylyl cyclases, key enzymes in cellular signaling. Current Opinion in Structural Biology 2020; 63: 34-41. doi: 10.1016/j.sbi.2020.03.003
14. Zou T: A perspective profile of ADCY1 in cAMP signaling with drug-resistance in lung cancer. Journal of Cancer 2019; 10(27): 6848. doi: 10.7150/jca.36614
15. Nho K: Association of altered liver enzymes with Alzheimer disease diagnosis, cognition, neuroimaging measures and cerebrospinal fluid biomarkers. JAMA Network Open 2019; 2(7): 197978-.
16. Tokushige and Katsutoshi: Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. Journal of Gastroenterology 2021; 56(11): 951-963. doi: 10.1007/s00535-021-01796-x
17. Garcia-Cortes M: Drug induced liver injury: an update. Archives of Toxicology 2020; 94(10): 3381-407. doi: 10.1007/s00204-020-02885-1
18. Vítek L: Bilirubin as a signaling molecule. Medicinal Research Reviews 2020; 40(4): 1335-51. doi: 10.1002/med.21660
19. Ruiz AR: Measurement and clinical usefulness of bilirubin in liver disease. Advances in Laboratory Medicine/ Avancesen Medicina de Laboratorio 2021; 2(3): 352-61. doi: 10.1515/almed-2021-0047
20. Zaccherini G and Bernardi M: The role and indications of albumin in advanced liver disease. Acta gastro-enterologica Belgica 2019; 82(2): 301-8.
21. Bhagavan NV: Carbohydrate Metabolism II: Gluconeogenesis, Glycogen Synthesis and Breakdown, and ALA Ternative Pathways. In Med Biochem Academic Press London UK San Diego CA, 2001; 275-305. USA. doi: 10.1016/B978-012095440-7/50017-2
22. Blanco A: Carbohydrate metabolism. In Medical Biochemistry Academic Press London UK San Diego CA USA2017 283-323. doi: 10.1016/B978-0-12-803550-4.00014-8

23. Jitrapakdee S: Transcription factors and coactivators controlling nutrient and hormonal regulation of hepatic gluconeogenesis. The Int J of Biochem & cell Bio 2012; 44(1): 33-45. doi: 10.1016/j.biocel.2011.10.001
24. Zobov VV: Effektivnost' vliyaniyanovy-proizvodnyhpirimidinana-fizicheskuyurabotos posobnost' krys vu sloviya hvypolneniyatesta «plavanie do otkaza». Ekologiyacheloveka 2015; 1: 28-35.
25. D'Arcy MS: Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biology International 2019; 43(6): 582-92. doi: 10.1002/cbin.11137
26. Junaid M: Biological Role of AKT and Regulation of AKT Signaling Pathway by Thymoquinone: Perspectives in Cancer Therapeutics. Mini Reviews in Medicinal Chemistry 2021; 21(3): 288-301.
27. Singh R: Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nature reviews Molecular Cell Biology 2019; 20(3): 175-93. doi: 10.1038/s41580-018-0089-8
28. McGlorthan L: Progesterone induces apoptosis by activation of caspase-8 and calcitriol *via* activation of caspase-9 pathways in ovarian and endometrial cancer cells *in-vitro*. Apoptosis 2021; 26(3): 184-94.
29. Kalpage HA: Cytochrome c phosphorylation: Control of mitochondrial electron transport chain flux and apoptosis. The International Journal of Biochemistry & Cell Biology. 2020; 121: 105704. doi: 10.1016/j.biocel.2020.105704
30. Moteki H: Signal transduction mechanism for potentiation by α1-and β2-adrenoceptor agonists of l-ascorbic acid-induced DNA synthesis and proliferation in primary cultures of adult rat hepatocytes. European Journal of Pharmacology 2013; 700(1-3): 2-12. doi: 10.1016/j.ejphar.2012.12.010
31. Kimura M: L-ascorbic acid-and L-ascorbic acid 2-glucoside accelerate *in-vivo* liver regeneration and lower serum alanine aminotransaminase activity in 70% partially hepatectomized rats. Biological and Pharmaceutical Bulletin 2014; b13-00839.doi: 10.1248/bpb.b13-00839.

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