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# RECOVERY OF LIVER DAMAGED BY $CCl_4$ UNDER TREATMENT BY CONJUGATE OF DRUG XYMEDON WITH L-ASCORBIC ACID

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**Keywords:**

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**ABSTRACT:** The aim of the study is to investigate the capacity of pyrimidine derivatives like the active ingredient of Xymedon (I) and its derivative, salt-like conjugate with L-ascorbic acid (II) to stimulate liver recovery when damaged by toxic hepatitis. The study was done on out breed white rats. The liver damage was induced by carbon tetrachloride (injected 50% oil solution subcutaneously, once per day, 2-4 times, dose of solution 2 ml/kg). Tested compounds ((I), (II), Thiotriasolinum) were administered orally in the dose 20 mg/kg within 5 days. The new derivative (II) possesses pronounced hepatoprotective properties. It was established the obvious advantages of (II) in comparison with both Xymedon and Thiotriasolinum. Application of (II) improves of general medical condition of the animals (increase in the survival rate up to 100 %), the structure and morphology of their liver (reducing the symptoms of destructive, degenerative and irreversible changes to hepatocytes) and biochemical markers of cytolysis, synthetic and metabolic liver ability in blood (recovery in the levels of alanine-aminotransferase, aspartate-aminotransferase, γ-glutamyl-transpeptidase, total protein, cholesterol, alkaline phosphatase).

**INTRODUCTION:** The incidence of liver disease, of different aetiology, has been steadily on the increase worldwide. The probability of toxic liver damage is often linked to taking medications, including those designed for curing such socially significant disease as tuberculosis [1] and cancer [2, 3, 4]. It was proved that chemotherapeutic agents can produce liver toxicity through different pathways, resulting in different categories of liver injuries [5].

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In [6] the economic effect of prevention was demonstrated, the one based on hepatoprotectors as part of chemotherapy for lymph adenoma. On the other hand, there are reports [1] that administration of such hepatoprotectors as silymarin, glucurone and inosine during anti-tuberculosis medication therapy show very low effectiveness.

In addition to this, clinical findings from 2003 on 789 patients [7] proved that effectiveness was practically zero when the well-known EPL hepatoprotectors were used, in comparison with the placebo, for curing alcohol-induced or combined aetiology hepatitis. In connection with this, searching for more effective hepatoprotective agents was and still remains a very important line of medical and pharmaceutical studies.

In a review in [8] we can find reports that extracts taken from a wide range of plants have been evaluated as hepatoprotective agents against damage induced by $CCl_4$, EtOH, d-galactosamine, and/or acetaminophen. Authors of the same paper examined natural plant substances like silymarin, curcumin, aringenin, resveratrol and N-trans-caffeoyldopamine as hepatoprotective agents to be taken together with anti-tuberculosis medications [8]. It is widely held that antioxidant activity comprises the principal mechanism of action of natural substances possessing a hepatoprotective effect [9].

As of lately, customary has become the use of medications based on ursodeoxycholic acid [10], the mechanisms of action of which are diverse [11]. For instance, the cytoprotective effect of ursodeoxy-cholic acid's preparations is based on the phenomenon of displacement of injected hydrophobic and toxic bile acids (capable of destroying cell membranes) by ursodeoxycholic acid from bio exchange. The choleretic effect of ursodeoxycholic acid-based therapy is ensured by activation of synthesis and a quicker release of bile acids from hepatocytes during therapy. We also have reports on hepatoprotective properties of lactulosa and inulin's prebiotics, *etc.* [12, 13, 14]

Pyrimidine derivatives are interesting as potential hepatoprotective agents due to their wide range of bio activity, namely, to their capability to stimulate tissue regeneration. There are studies describing various forms of bio activity of synthetic derivatives of pyrimidine [15, 16, 17, 18]. On the hepatoprotective action of pyrimidine derivatives, however, the literatures provide very modest information. We know the hepatoprotective effects of the metabolic agents of potassium orotate that stimulates synthesis of nucleic acids and albumin in liver. Besides, some papers describe weak hepatoprotective effects of uracil's derivatives (methyluracil, 4-methyl-5-oximethyluracil) [19, 20] along with those of synthetic derivatives 2,4-dioxo-5-arylidenimino-1,3-pyrimidines [21].

In this paper we present our findings on how liver is recover from toxic hepatitis by pyrimidine derivatives one of which (1-(2-oxyethyl)-4,6-dimethyl-1,2-dihydro-2-oxopirimidon) has been registered and adopted in the Russian Federation as a regenerative-reparative medication Xymedon [22], whereas the second one (L- ascorbate 1-(2-hydroxyethhyl)-4,6-dimethyl-1,2-dihydropyrimidin -2-ona) is the former's further derivative namely Xymedon's co-crystal with *L*-ascorbic acid. Recent clinical results have given some grounds for Xymedon's prescription for the cure of scleroderma [23], psoriasis [24], obstructive lung disease [25], gastro-duodenal ulcer [26], osteomyelitis [27, 28], suppurative inflammations [29].

By its action, Xymedon affects the key bio-chemical 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. The medication also affects the control of active calcium transport within a cell, has an impact on tissue respiration, lipid peroxidation and the work of the antioxidant system [22]. Formerly, we had findings related to the stimulative action of Xymedon upon liver recovery after toxic damage by carbon tetrachloride [30]. New data are on Xymedon's derivaties with biogenic acids (ascorbic, p-amino-benzoic acid) that have a better specific capability if compared with Xymedon to stimulate spinal marrow recovery after trauma [31], improve body's adaptation under physical stress [32, 33], as well as demonstrate hepato-protective properties [34, 35, 36]. In this study we focus on the mechanisms of action of pyrimidine's derivatives, we investigate neurotransmission mediated through P2-neuroreceptors based on such ligands as purine and pyrimidine compounds [37].

## MATERIALS AND METHODS:

**Compounds Under Test:** In this study we considered the capability of two pyrimidine derivatives - the active substance of Xymedon medication (1-(2-oxyethyl)-4,6-dimethyl-1,2-dihy-dro-2-oxopirimidon; hereinafter) - (I) and its derivative, salt-like conjugate with Vitamin C (*L*-ascorbate 1-(2-hydroxyethhyl)-4,6-dimethyl-1,2-dihydropyrimidin-2-ona (Formula 1); here in after (II) - to promote liver recovery. Both compounds (the active ingredient of Xymedon and its derivative with L-ascorbic acid) were synthesized in A. E. Arbuzov Institute of Organic and Physical Chemistry. For the reference compound we used the Thiotriasolinum medication (active substance - morpholine-methyl-triazolyl-thioacetate) purchased from a pharmacy chain.

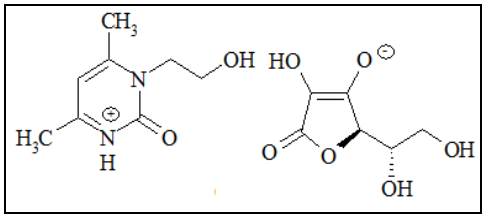

**FIG. 1: XYMEDON CONJUGATE WITH VITAMIN C**

**Experimental Design:** The trials were done on outbreed white rats, mature males and females, with the weight of 250 - 400 g, obtained from the vivarium of A.E. Arbuzov Institute of Organic and Physical Chemistry, Kazan Research Centre of the Russian Academy of Sciences. Animal care conditions and experimental conditions were in compliance with [38, 39, 40]. Animals had constant access to water and food. The diet comprised all-in-one granulated feed (raw protein 22%, raw fat max. 5%, raw fibre max. 4%, crude ash max. 9%, moisture max. 13.5%, energy value 295 kcal/100 g), with added oats, dried bred and vegetables. The study and protocols were approved by the Local Ethical Committee of Kazan Federal University (Record No. 4, of May 18th2017).

In order to simulate toxic hepatitis in the animals, a hepatotoxin was injected subcutaneously - carbon tetrachloride $CCl_4$ at the dose of 1 ml/kg mixed with vegetable oil 1:1. The hepatotoxin was administered to 57 animals in compliance with [41] during 2 - 4 days, subject to age and sex factors upon which $CCl_4$ tolerance depends. When priming $CCl_4$ we observed weight loss in the animals; in addition to that, 10 rats (17.5%) died. Autopsy revealed an enlarged liver (up to 6% relative to body weight), whereas in the normal control rats the weight of liver to the weight of body was 3.03 ± 0.06%. The revealed pathoanatomical changes testified to a pronounced toxic damage to the liver.

After the simulation of liver toxic damage, with reliance on the randomization method, the rats were divided into five equivalent groups comprising 8 - 10 animals:

**Group I:** Normal control (distilled water, no $CCl_4$)

**Group II:** Control group 1 ($CCl_4$ + water)

**Group III:** Control group 2 ($CCl_4$ + Thiotriazolinum 20 мг/кг)

**Group IV:** Control group 3 ($CCl_4$ +drug Xymedon (derivative I) 20 мг/кг)

**Group V:** Experimental group ($CCl_4$ +derivative II 20 мг/кг)

The compounds under test were administered orally, through a feeding tube during 5 days. All the substances were given in the same doze of 20 mg/kg as 2% solutions at the rate of 0.1 ml per 100 g of the body weight. The control group was also given an equivalent amount of water.

**Test Indicators:** To evaluate how the substances under test impact the general clinical status of the animals with toxic liver damage, we carried out daily observation and tracked body weight changes and survival rate in each of the groups. The effect of the tested substances on liver recovery, *i.e.* their hepatoprotective capabilities, was evaluated by biochemical markers of blood serum and changes to liver morphology (mass coefficient *i.e.* the ratio of liver weight to body weight, structural changes on histological sections).

**Sample Preparation:** To determine liver pathology due to damage by $CCl_4$, some of the animals were sacrificed on the following day of the last $CCl_4$ injection. To determine reference indices, animals from normal control group were sacrificed not having been subject to any injections. The other rats were sacrificed on the following day of the last injection of the compounds under test, *i.e.* on the 6th day after injection of $CCl_4$.

Blood samples were taken in plastic test tubes by sacrificing the animals by exsanguinations through carotid artery under anaesthesia. 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. Liver samples of the rats for histological examination were fixed with 4% buffered formalin for 24 h.

**Biochemical Tests:** In the blood serum, the following indicators were determined: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (g-GT), glucose, total protein and cholesterol. By calculation, the de Ritis ratio was defined as AST/ALT. Biochemical indices were determined using an automated clinical chemistry analyzer Daytona Randox and a set of special kits Randox.

**Histological Studies:** The fixed samples of rats liver processed in a series of solutions: ethanol in an increasing concentration from 50 up to 100 %, xylene and paraffin molten at 58 °C. Then, tissue slices were prepared with thickness of 5 - 7 µm, coloured by haematoxylin and eosin. For microscopy and microphotography the Carl Zeiss microscope and digital camera Axiocam were used.

**Statistical Analysis:** The determined indicators were compared with the reference values from normal control animals along with values from the $CCl_4$ control group rats who were affected in the same way as the experimental group but did not receive any treatment. For statistical data processing the Origin 6.0 software package was used; the samples were compared by student's t-test.

## RESULTS:

**Clinical State of Animals:** Our observations showed that injection of $CCl_4$ leads to poorer general health of rats: loss of appetite, fatigue, heavy breathing, shivering, ruffled fur, occasionally death (17.5% of the total amount of the rats in the experiment). During the 5 days of cure by the substances under test, in the experimental group where pyrimidine derivative (II) was used, an obvious improvement of the clinical condition was observed already on day 2, unlike other groups where such improvement took place only on days 4-5. During the experiment, we also observed occasional deaths of rats (25-30%) in the control group, in the group where (I) was used and in the one where Thiotriasolinum was applied **Table 1**. In the group of rats treated by pyrimidine derivative (II), 100% survival was observed.

**TABLE 1: COMPOUND IMPACT UPON ANIMAL'S GENERAL CLINICAL CONDITION**

| Group | Animals in group, heads | Survived, rats / (%) | Final body weight, % of the initial |
|---|---|---|---|
| Group I | 10 | 10 (100) | 101.2 ± 5.1 |
| Group II | 8 | 6 (75) | 90.3 ± 3.1 |
| Group III | 10 | 7 (70) | 86.4 ± 4.9 |
| Group IV | 8 | 6 (75) | 91.8 ± 2.1 |
| Group V | 9 | 9 (100) | 88.6 ± 2.6 |

The rats average body weight by the end of the experiment in the control group was 90.28% of the initial values, which was naturally due to toxic damage. In the experimental groups, body weight loss relative to the initial values was not significantly different from the control group.

A study of the structural and morphological changes in the liver demonstrated that the mass coefficient of the normal control group of rats was 3.05 ± 0.05%. Liver tissue specimen analysis (normal group of rats) showed a pronounced structure in the form of lobules. Cell boundaries were well defined. The cells were polygonal with distinct edges. Cytoblasts were located near the centre of cells, homogeneous cytoplasm of moderate density, visible was a thin reticular basophilic pattern, which is characteristic of healthy liver cells **Fig. 2a**.

Among the pathomorphological liver changes after a toxic impact by carbon tetrachloride, we observed the following: mass coefficient increase by up to 6% in the rats that died and by up to 4.9% in those who survived. Histological liver specimen revealed pronounced changes to liver cell morphology, such as steatosis (large vesicular liposis), emergence of cells with homogeneous cytoplasm due to the destruction of cell organelles, the changed form and increased size of cells, hydropic and ballooning degeneration manifested by a shift of cell nuclei to periphery, emergence of gigantic (goblet) cells, vast necrosis **Fig. 2b**.

Cell morphology in the control group (the $6^{th}$ day of observation) showed persistent pathologic changes even 5 days after the $CCl_4$ injection. The mass coefficient (3.62%) was more than in the normal control group but less than that on the $1^{st}$ day after $CCl_4$ injection. *I.e*. the mass coefficient partially have been recovered spontaneously within 5 days. Among other structural and morphological changes, we observed steatosis with large lipoid vesicles at the rate of 50% of total liver tissue in 2 out of 8 rats (25%) and fatty + hydropic liver cell degeneration in 6 out of 8 rats (75%) **Table 2**, **Fig. 2c**, **2d**.

In all the rats groups where the medications were used, we observed a pronounced improvement of the structure and morphology of liver in comparison with the control group, first and foremost in fewer symptoms of destructive, degenerative and irreversible changes to hepatocytes **Table 2**. Instead of large vesicular fatty infiltration, we revealed small-vesicle infiltration, fewer symptoms of liver structure disorganization; instead of vast necrosis there were

focal liver cell necrosis. Instead of hydropic and ballooning cell degeneration, hepatocytes' enlargement took place with the cell structure remaining intact. By liver mass coefficient, no significant differences between the rats groups were revealed.

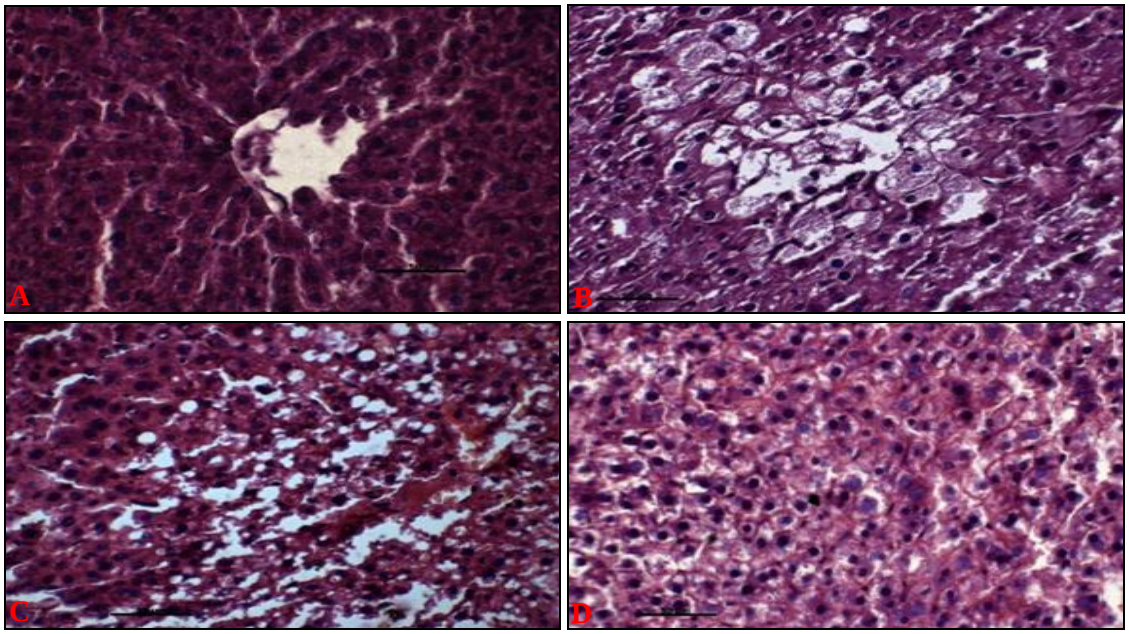


**FIG. 2: MICRO MORPHOLOGY OF RATS LIVER FROM THE INTACT CONTROL GROUP**

(a) and pathomorphological changes of liver in control group (b-d): (b) steatosis, hydropic degeneration and ballooning degeneration on the following day of the last injection of carbon tetrachloride; (c) steatosis and necrosis of liver cells on day 6 after last carbon tetrachloride injection; (d) fatty + hydropic liver cell degeneration on day 6 after last carbon tetrachloride injection. Stained with haematoxylin and eosin. Lens 40x. Zoom 600x.

In the groups where Pyrimidine derivatives (I) and (II) were used, the structural and morphological changes were uniform and differed from those in the group where Thiotriasolinum was administered. Some rats in all the groups demonstrated enlargement of liver cells, in some cases with symptoms of focal necrosis in individual cells. This pathomorphological change was most frequent (33.3%) in the group under Thiotriasolinum. Small-drop liposis infiltration of hepatocytes most frequently occurred in the groups treated by pyrimidine derivatives, particularly substance (II).

After administration of Thiotriasolinum, to the contrary, fatty liver infiltration symptoms were minimal: we found it in a single rat out of 6 (16.7%) and in isolated cells only (**Table 2**, **Fig. 3b**). Focal liver cell necrosis in the group under Pyrimidine derivative (II) occurred less frequently (22.2%) if compared with the group where Xymedon or Pyrimidine (I) were administered (50%) **Fig. 4** and **Fig. 5**. In the group where Thiotriasolinum therapy was applied, the most frequent symptoms were liver cell shrinkage and deformation with cytoplasmic condensation and infiltration of mononuclear leukocytes into the periportal area **Table 2**, **Fig. 3c**.

The toxic action of $CCl_4$ resulted in a sizeable shift of biochemical markers: activation of transaminases that are markers of cytolysis (ALT and AST), ALP; at the same time the de Ritis coefficient went down **Table 3**. The revealed changes are statistically valid at $p < 0.001$ and do testify to development of toxic hepatitis in the rats. In 5 days in the control group, we observed spontaneous partial recovery of the biochemical marker values; however, some deviations still persisted. For instance, we found an increased ALT ($p < 0.001$), increased de Ritis ratio ($p < 0.01$) and a higher level of the g-GT ferment.

These findings indicate a progress in pathologic destructive changes in the liver. In addition to this, in the control group we saw a change in the indicators that characterize metabolism, the synthetic work of liver and its role in the homeostasis: increased level of glucose (the differences were not statistically valid), decreased level of protein ($p<0.01$) and cholesterol.

**TABLE 2: IMPACT OF THE SUBSTANCES UNDER TEST UPON LIVER STRUCTURE AND MORPHOLOGY AFTER TOXIC EXPOSURE TO $CCl_4$**

| Group | Liver mass coefficient % | Pathomorphological changes and their incidence in the sample, pcs. / total number of animals in group (%) |
|---|---|---|
| Group I | 3.05 ± 0.05 | No abnormalities (Fig. 2a) |
| all group, 1 day after $CCl_4$, before treatment | 4.86 ± 0.31*** | Steatosis, the changed form and increased size of cells, hydropic and ballooning degeneration, emergence of gigantic (goblet) cells, vast necrosis (Fig. 2b) |
| Group II ($6^{th}$ day) | 3.62 ± 0.25** | 1) Steatosis + necrosis (50% of tissue - large vesicle fat degeneration, 50% small vesicle fat infiltration of liver cells) - 2/8 (25%) (Fig. 2c)<br>2) Fatty and hydropic liver cell degeneration - 6/8 (75%) (Fig. 2d) |
| Group III ($6^{th}$ day) | 4.03 ± 0.20*** | 1) Enlarged liver cells, focal necrosis - 2/6 (33, 3%) (Fig. 3a)<br>2) Small vesicle fat infiltration of individual cells - 1/6 (16.7%) (Fig. 3b)<br>3) Cell shrinkage and deformation + small vesicle fat degeneration of individual liver cells + mononuclear periportal infiltrate - 3/6 (50%) (Fig. 3c) |
| Group IV ($6^{th}$ day) | 3.81 ± 0.10*** | 1) Enlarged liver cells - 1/6 (16.7%) (Fig. 4a)<br>2) Focal liver cell necrosis - 3/6 (50%) (Fig. 4b)<br>3) Vast small-vesicle fat infiltration - 2/6 (33, 3%) (Fig. 4c). |
| Group V ($6^{th}$ day) | 3.59 ± 0.08*** | 1) Enlarged liver cells, focal necrosis - 2/9 (22.2%) (Fig. 5a)<br>2) Focal liver cell necrosis with symptoms of hydropic degeneration - 2/9 (22.2%) (Fig. 5b)<br>3) Extensive small-vesicle fat infiltration- 5/9 (55.6%) (Fig. 5c) |

** - differences with Group I (normal control) of rats are statistically significant at $p < 0.01$; *** - idem, at $p < 0.001$.

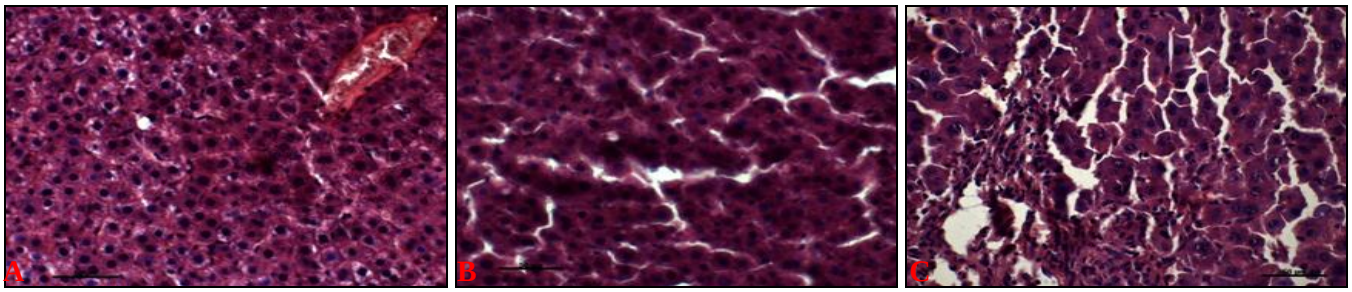


**FIG. 3: MICRO MORPHOLOGY OF RATS LIVER IN THE GROUP TREATED BY REFERENCE PREPARATION THIOTRIASOLINUM AT A DOSE OF 20 mg/kg (a-c)**

(a) enlarged liver cells with focal necrosis; (b) small-drop liposis of individual cells; (c) cell changes accompanied by infiltration of mononuclear leukocytes into the periportal area.Stained with haematoxylin and eosin. Lens 40x. Zoom 600x.

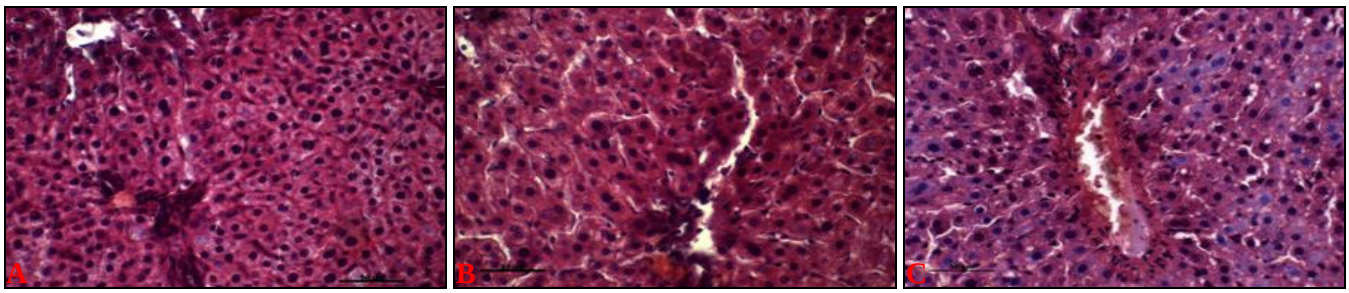


**FIG. 4: MICRO MORPHOLOGY OF RATS LIVER IN THE GROUP TREATED BY PYRIMIDINE DRUG XYMEDON PYRIMIDINE DERIVATIVE I (a-c)**

(a) enlarged liver cells; (b)with focal necrosis of individual cells; (c) small-drop liposis of individual cells. Stained with haematoxylin and eosin. Lens 40x. Zoom 600x.

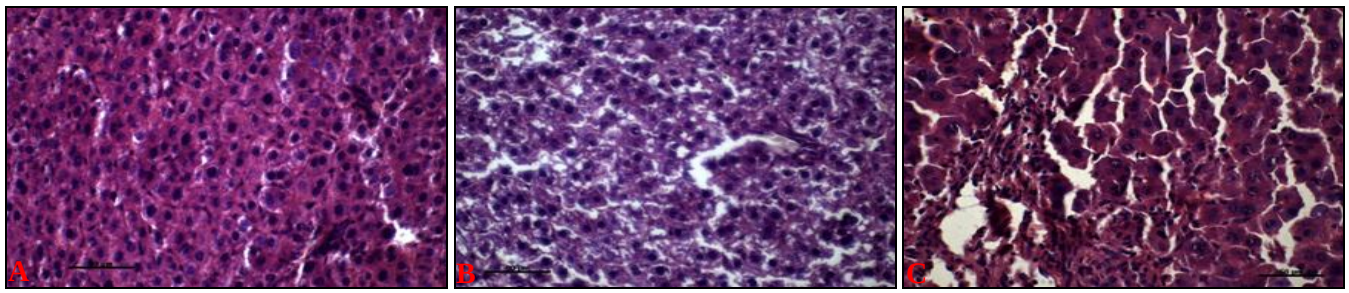


**FIG. 5: MICRO MORPHOLOGY OF RATS LIVER IN THE GROUP TREATED BY DRUG XYMEDON DERIVATIVE WITH L-ASCORBIC ACID (PYRIMIDINE II) (a-c) AT DOSES OF 20 mg/kg**

(a) enlarged liver cells; (b)with focal necrosis of individual cells; (c) small-drop liposis of individual cells. Stained with haematoxylin and eosin. Lens 40x. Zoom 600x.

In the groups of rats who were given the substances under test during 5 days, we observed recovery of biochemical indicators. Under the impact of pyrimidine (I), the level of transaminases dropped in comparison with the control group, but this was not statistically significant, while the de Ritis ratio and the g-GT activity had not changed in practical terms. At the same time, the level of ALT remained higher ($p<0.01$, statistically valid) and the de Ritis coefficient lower ($p<0.05$, statistically valid) than normal values in the normal control group of rats **Table 3**.

Under the hepatoprotective medication Thio-triasolinum, the level of g-GT went down and the de Ritis value reached its normal rate; however, the level of transaminases remained high ($p<0.001$). The de Ritis ratio went up to the normal value due to the increase in AST.

Under derivative (II), the transaminase level was in correspondence to normal values, at that ALT differed from the control values at $p<0.01$ (statistically valid) **Table 3**. AST level was below the control value and that of normal control rats, but statistically insignificantly. The levels of g-GT and alkaline phosphatase were normal. The findings of this experiment speak of the end to the progressive pathologic destructive process (cytolysis) of liver cells, and of a more pronounced action on biochemical markers of liver damage, in comparison with Xymedon (pyrimidine I) and the Thiotriasolinum medication.

**TABLE 3: IMPACT OF THE COMPOUNDS UNDER TEST UPON LIVER STATUS BIOCHEMICAL MARKERS**

| Group of animals | ALT, U/L | AST, U/L | de Ritis ratio (AST/ALT) | g-GT, UE/л | ALP, UE/L | n |
|---|---|---|---|---|---|---|
| Group I | 37.38 ± 2.69 | 148.32 ± 8.95 | 4.38 ± 0.28 | 4.40 ± 0.85 | 311.5± 25.0 | 10 |
| all group, 1 day after | 134.85 ± | 231.62 ± | 2.16 ± | 39.12 ± | 433.2 ± | 13 |
| $CCl_4$, before treatment | 23.95*** | 20.02*** | 0.49*** | 12.15* | 65.2* | |
| Group II | 75.76 ± | 158.51 ± | 2.28 ± | 22.50 ± | 339.7 ± | 7 |
| ($6^{th}$ day) | 7.72*** | 21.65 | 0.41** | 10.47 | 18.9 | |
| Group III | 69.23 ± | 226.83 ± | 3.35 ± | 12.39 ± | 400.6 ± | 6 |
| ($6^{th}$ day) | 10.38*** | 45.30** | 0.60 | 5.17 | 59.1 | |
| Group IV | 60.54 ± | 118.07 ± | 2.19 ± | 33.82 ± | 337.6 ± | 6 |
| ($6^{th}$ day) | 9.90** | 13.08 | 0.40* | 10.02 | 66.1 | |
| Group V | 42.41 ± | 122.75 ± | 2.95 ± | 9.26 ± | 345.5 ± | 9 |
| ($6^{th}$ day) | 5.14(**) | 13.54 | 0.16* | 3.17(*) | 34.9 | |

* - differences with Group I (normal control) are statistically significant at $p<0.05$; ** - idem., at $p<0.01$; *** - idem., at $p<0.001$. In parentheses - comparison of the indicators with the values of Group II (control group 1, $CCl_4$ + water).

The substances under test, when applied, also led to better metabolism indicators. Under these agents, the levels of protein and cholesterol went up - the ones that were below normal values in the control group. At that, the smallest differences with the reference figures of the normal control group were found in the groups where the rats were given pyrimidine derivatives (I) and (II) **Table 4**.

**TABLE 4: IMPACT OF THE SUBSTANCES UNDER TEST UPON LIVER METABOLIC STATUS**

| Group of animals | Glucose, мМ/л | Total protein, г/л | Cholestrol, мМоль/л | n |
|---|---|---|---|---|
| Group I | 8.65 ± 0.65 | 65.30 ± 0.85 | 1.14 ± 0.05 | 10 |
| Group II ($6^{th}$ day) | 10.27 ± 0.37 | 57.99 ± 1.00** | 0.97 ± 0.06* | 7 |
| Group III ($6^{th}$ day) | 10.07 ± 1.16 | 61.60 ± 2.16 | 2.47± 0.59*** (*) | 6 |
| Group IV ($6^{th}$ day) | 11.59 ± 0.27** (*) | 63.04 ± 0.77(**) | 1.21 ± 0.09(*) | 6 |
| Group V ($6^{th}$ day) | 11.15 ± 0.40** | 64.10 ± 1.44(**) | 1.47 ± 0.27 | 9 |

* - differences with Group I (normal control) are statistically significant at $p<0.05$; ** - idem., at $p<0.01$; *** - idem., at $p<0.001$. In parentheses - comparison of the indicators with the values of Group II (control group 1, $CCl_4$ + water).

**DISCUSSION:** A review of recent literature has shown that liver diseases linked to toxicity have a rather high incidence rate. Described are liver injuries due to anti- tuberculosis [1] and anticancer [2, 3, 4]; medications alcoholic liver disease still remains high on the agenda. This is what accounts for an active ongoing study of and search for effective hepatoprotective agents to prevent and cure toxic liver injuries of different aetiologies. In this paper, we study pyrimidine derivatives as hepato-protective agents that can help liver recovery after toxic exposure to carbon tetrachloride. Data on

hepato-protective effects of pyrimidine derivatives have been scarce in the literature; due to this reason our study is what shall help expand our knowledge of biological activity of this type of compounds. The findings on hepatoprotective effects of pyrimidine's synthetic derivatives from [21], as well as our previous data on Xymedon effectiveness [30], indicate that this line of research is important and promising.

Presumably, one of the expected results of a synthesis of the Xymedon derivative with the well-known antioxidant ascorbic acid should be stronger hepatoprotective effects, which is the object of this study. The reference substance - hepatoprotective medication Thiotriasolinum, of metabolic effect, is the closest one to the substances under test by its pharmacology and action.

Our findings confirmed that the hepatoprotective medication Xymedon - a derivative of pyrimidine - is effective, which is in line with our previous results [30], and that the Xymedon's derivative with vitamin C is more effective if compared with Thiotriasolinum and with the original substance, which is in accord with findings presented in [34, 35] where hepatoprotective effects are described of pyrimidine (I) and (II) using the simulation of $CCl_4$-induced liver injury, with a prevention-therapy pattern.

Quite recently hepatoprotective agents have been quite actively studied and searched for using the models of medicine-induced liver injury by anti-tuberculosis [8, 9], anti-cancer [5, 6] medications and paracetamol [42, 43]. The model of toxic $CCl_4$-induced hepatitis in this paper is well-known, widely adopted and recommended for studies on hepatoprotective effects of various substances [41]. The use of a toxic $CCl_4$-induced hepatitis for evaluation of hepatoprotective effects is mentioned also in more recent works [44, 45, 46].

According to our findings, in oral administration to rats with a toxic liver injury, all the three substances under test contributed to a significant improvement in liver structure and morphology and had a beneficial effect on the biochemical markers of liver condition. Our results helped reveal pharmacological effects of Xymedon's pyrimidine and its derivative with vitamin C, if compared with thiotriasolinum used for curing $CCl_4$-induced toxic liver injury. Unlike the control group where we observed destruction of hepatic tubules and a large amount of cells with symptoms of degeneration, structural liver recovery under the three substances occurred along with an increase in the number of unaffected hepatocytes. The revealed injury to liver cells in experimental groups was reversible, it did not lead to cell death. The damage to liver cells revealed under the substances had certain specific features. In the group under Thiotriasolinum therapy, we observed fewer symptoms of small- and large-drop fatty infiltration of hepatocytes. Among pathomorphological abnormalities, most prevalent was larger infiltration of liver tissue by reticuloendothelial cells, which may be accounted for by activation of immune responses and might with a high probability lead to the growth of inflammation. Besides, we observed shrinkage of liver cells and their deforation **Table 2**, **Fig. 3**.

In the groups where pyrimidine derivatives (**I**) and (II) were administered - Xymedon and its derivative with vitamin C - the size of liver cells was normal or enlarged, the structure and morphology of liver tubules intact, while the symptoms of destruction and degeneration in liver cells much fewer that in the control group. Among the pathomorphological changes we revealed reversible symptoms of fatty infiltration of liver tissue; at this, differences in such changes between the groups that were treated with both pyrimidine derivatives are insignificant **Table 2**, **Fig. 4**, **5**.

More pronounced hepatoprotective properties in the group treated by Pyrimidine (II), if compared with both other groups under the original substance (I) or Xymedon and Thiotriasolinum, are confirmed by the clinical condition of rats affected by $CCl_4$ and by the changes in biochemical parameters. With regard to survivability factor - 100% for (II), only around 75% for control and (I) and Thiotriasolinum **Table 1**- the new derivative pyrimidine (II) demonstrated a better hepato-protective effect.

In the group under pyrimidine (II), the biochemical indicators were closer to the reference values of the non-involved sample, which also confirms a more pronounced hepatoprotective effect of the substance. Under pyrimidine (II), hepatocyte

cytolysis (ALT and AST) got normalized, which speaks of a cytoprotective effect, along with alkaline phosphatase and g-GT, which is a testimony to the fact that an antiholestatic effect is also present. Besides, under both pyrimidines as well as thanks to the administration of Thiotriasolinum, the levels of protein and glucose went up and that of cholesterol in blood serum became normal. Our findings demonstrate that the pyrimidine (I) and (II) derivatives have a metabolic effect leading to metabolic liver function recovery after a toxic injury by $CCl_4$ including protein, carbohydrate and lipid metabolism. A more prominent effect upon recovery of indicators characterizing liver cell cytolysis (ALT, AST), or pyrimidine (II)'s cytoprotective action, is most likely due to the increase in its antioxidant properties if compared with (I), thanks to a fragment of Vitamin C in the compound.

**CONCLUSION:** In our study, it has been established that administration of the new pyrimidine derivative (II) of the active agent of Xymedon preparation (I) with ascorbic acid - to rats with toxic carbon tetrachloride-induced hepatitis during 5 days leads to the improvement of general medical condition of the animals, which is confirmed by an increase in the survival rate up to 100 percent. Pyrimidine (I) and (II) and Thiotriasolinum, have had a positive effect at a dose of 20 mg/kg upon the structure and morphology of liver at the same time reducing the symptoms of destructive and degenerative and irreversible changes to hepatocytes.

Blood biochemistry testifies to obvious advantages of the new pyrimidine derivative (II) if compared with both medications: Xymedon and Thiotriasolinum. With application of (II) at the dose of 20 mg/kg during 5 days we observed recovery in the levels of ALT, AST, g-GT, total protein, cholesterol, ALP, *i.e*. all of the basic biochemical values that are the markers of hepatocyte cytolysis, of synthetic and metabolic liver ability.

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