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## Research of the Act-Protective Properties of Xymedon and Its New Analogs.

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### ABSTRACT

This paper deals with the effects of the medicinal agent xymedon and its six new two-fragment analogs containing fragments of xymedon and biogenic acids, in the stimulation of rats' physical performance in the test of forced swimming with weights. It was noted that single and multiple injection (for 21 day) of the tested substances did not have any statistically significant act-protective effect. The most pronounced effect was observed for act-protective L-ascorbate compound 1- (2-hydroxyethyl) -4,6-dimethyl-1,2-dihydropyrimidine-2-one (20 mg / kg) in the 11-day intraperitoneal injection. The effect of the swimming time stimulation for rats, on day 14 of the experiment, was 440% higher relative to the control group. Compound is a conjugate, comprising the fragments of xymedon and ascorbic acid. With the injection of the compound for 11 days, in a debilitating physical exertion conditions, no influence on the processes of leucopoiesis was noticed. Increase in the number of erythrocytes and hemoglobin level in blood indicate the stimulation of erythropoiesis. Revealed biochemical signs indicating improvement in the exercise tolerance upon the injection of the substance to animals and its anti-catabolic action. The absence of cardio- and hepatotoxic action of the substance was disclosed.

**Keywords:** act-protection, environmental pharmacology, pyrimidine derivatives, xymedon, generalized linear models.

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## INTRODUCTION

In modern conditions of technological change, pollution and accelerating urbanization, work activity of a person is characterized by the daily psycho-physical stress of the whole organism. The highest risk of extreme and critical conditions have the liquidators of consequences of accidents, the workers of shelf areas and polar zones, military and law enforcement officers, submariners, divers, astronauts, whose work is connected with constant ecological, toxicological, physical and psycho-emotional stress [1,2,3]. Currently, the combined impact of environmental hazards of man and his work requires the development of adequate and safe means of pharmacological support to the adaptive capacity of the organism, which is studied in the framework of a new trend of human ecology - ecological Pharmacology [3]. To meet these challenges there is a promising direction of research which is to study the properties of act-protective pyrimidine derivatives [4,5,6,7,8].

The mechanism of action of anabolic pyrimidine derivatives underlies in stimulation of the synthesis of nucleic acids, performing a key function in the creation of protein molecules. The application of pyrimidine as protein anabolic is more rational, since the use of steroids causes redistribution of liver and skeletal muscle proteins, and pyrimidine contributes to the preservation of proteins in the liver and muscle [9,10,11]. Pyrimidine derivatives contribute to the restoration of functional activity of the liver and its morphological structure under its acute and chronic poisoning with carbon tetrachloride and sodium selenite [9,12].

Low toxicity is considered to be the one of the pyrimidine derivatives features [13]. In addition, the substances in this group have the ability to boost the immune-biological body defense, stimulate the hematopoiesis, as well as have anti-stress and adaptogenic influence, adjust the progress for inflammatory and regenerative processes. Uracil moiety of ligand has all the unique properties, inherent to N-heteroaromatic systems, required to improve the selectivity of action [14,15].

## MAIN PART

Prior to the experiments investigating the efficiency of working ability the initial evaluation of compounds' toxicity was performed by calculating the half-lethal dose ( $LD_{50}$ ) under intraperitoneal and intragastric methods of injection in the form of aqueous solutions in the acute experiments on white mice of both sexes weighing  $19,0 \pm 2,0$  g. Then for 72 subsequent hours the monitoring of the mice condition was carried out and the signs of intoxication were registered.

To evaluate the act-protective properties in this study the technique of "forced swimming to complete failure" was used [16] in terms of intraperitoneal injection of the compounds to the outbred white laboratory rats in the acute (single injection) and chronic (21 and 11 days) experiments. In total, there were investigated 6 new pyrimidine derivatives synthesized on the basis of xymedon.

Assessment of the performance was carried out on the basis of changes in the length of rats' swimming time to their complete failure to swim. The animals with a load attached, equal to 7% of body weight, were placed into a swimming cylindrical vessel with water ( $t = 29-30$  °C). The load was attached to the tail of rats.

The scheme of the experiment after a single dose: 1) 1st swimming to failure; 2) splitting into groups according to the 1st time swimming record through the pairwise selection; 3) exhausting swimming in 5 minutes after the first; 4) the intraperitoneal injection of the compounds at doses of 1% of the  $LD_{50}$ ; 5) 3rd swimming 40 minutes after the compound injection.

The procedure for conducting the chronic experiment: 1) swimming to failure on the 1st day of the experiment; 2) splitting into groups of 12 individuals on the basis of the duration of the first swimming by pairwise selection; 3) intermediate swimming to failure on the 7th day of the experiment; 4) depending on the scheme: swimming on the 14th and 26th day. Injection of the compounds was performed at doses of 1% of the  $LD_{50}$  for 11 and 21 day. In the control group of rats they were injected 0.9% NaCl saline.

A statistically significant increase in the duration of the 3rd swimming of the acute experiment, compared with a control group, is considered as a criterion of efficacy of the compound as a potential remedy

to enhance physical performance. Increasing the length of the first swimming on the 26th and the 14th day in the chronic experiments with the test group of rats, compared to the control, is regarded as a criterion for the effectiveness of the studied medication as a potential remedy having act-protective properties.

To study the effects of the leading-compounds to the main hematological and biochemical indices of rat blood, during the 11-day injection and extreme physical exertion, the repeated experiment was conducted on a 14-day scheme with analyzing the blood samples from the tail. The first rats' blood analysis was carried out a week before the forcing to swim experiments to determine the background values of the basic indexes. The second sampling was performed immediately after the experiment of forced swimming on the 14th day of the experiment.

The number of red blood cells and white cells was counted using Mythic 18 Vet hematology analyzer. With the help of the Dayton Randoh biochemical analyzer was determined the concentration of glucose, lactic acid, albumin, urea, AST, ALT, and total protein levels in serum blood samples of rats.

Statistical processing and analysis of the data was performed using the statistical program R. To create a mathematical model of evaluating the effectiveness of the influence of the test substances on the physical performance, compared to the control group, was used the GLM function - generalized linear model, allowing to work with arbitrary distributions of data, as well as dependent and heterogeneous data. In this paper we used a generalized linear model of the form:

$$E[\ln(T_3)] = a_0 + a_1 Wts1 + ID_i + \text{offset}(\ln(T_{1\_FON}))$$

i.e., model T3 = T1\_FON \*  $\alpha$ ,

where,  $\alpha = \exp(a_0 + a_1 Wts1 + ID_i)$ ,  $Wts1$  - the mass of the animal,

$ID_i$  - identification number of the animal,

$T_{1\_FON}$  - background time of swimming,

$T_3$  - time of swimming on the 26th and 14th day,

$a_0$  - the average value of the time swimming in the groups studied,

$a_1$  - difference in the average values of the swimming time in the control and test groups.

Statistical analysis of the changes in hematological and biochemical parameters, under exhausting physical activities and injection of the compounds that have a stimulating effect, was performed using linear regression model developed in the program R and has the following form:

$$y_{ij} = a_0 + \alpha_i + \alpha_t + \beta_{it} + \varepsilon_{ij}$$

Where,  $y_{ij}$  - is the value of the  $j$  index in the  $i$  group at a definite time moment  $t$  (background - 14th day);

$a_0$  - the average index value in the control group;

$a_1$  - the difference in the average index values in the study group relative to the control group;

$\beta_{it}$  - the difference in the average index change in the studied group on the 14<sup>th</sup> day, relative to the average change of the index in the control group on the 14<sup>th</sup> day;

$\varepsilon_{ij}$  - random error.

The differences between the index values of blood parameters were considered statistically significant at  $\beta_{it} \leq 0.05$ .

#### SUMMARY

From the obtained values of LD<sub>50</sub> (per os) (Table 1) follows that by the degree of impact on the body in accordance with GOST 12.1.007-76 [17] all the studied compounds belong to the 4th class of hazard - low hazard substances.

In a series of acute experiments (under single injection) was obtained experimental data on the changes in rats' swimming time in accordance with the method used. In the control group there was a shorter duration of the third swimming, in relation to the background one. This is due to the fact that the experimental animals do not have time to recover within 40 minutes after two exhausting swims. The same pattern was observed in the groups treated with the new test substances, except for the groups treated with compounds 26D and 29D. However, in these groups was admitted a statistically non-significant ( $p = 0.32$ ) trend towards stimulation of physical performance at 33 and 34% respectively, relative to the control group values. Xymedon and all its derivatives at selected doses do not cause unambiguous act-protective effects in laboratory rats under single injection.

During the 26-day chronic experiment all the studied compounds show a statistically significant ( $p < 0.05$ ) tendency to stimulate physical performance.

During the 14-day chronic experiment the background values of the swimming time of rats in the control group and 29-D group were at the same level, which was a result of paired animals' selection when forming experimental groups. On day 14 in the group of rats, which were injected compound 29-D for 11 days, the average swimming time varied from 3000 to 5000 seconds, and in the control group – from 200 to 500 sec. (Fig. 1).

As a result of the statistical analysis, conducted in the conditions of a multiple (11 days, intraperitoneal) injection, the best results in act-protective activity were obtained for the compound of L-ascorbate 1- (2-hydroxyethyl) -4.6-dimethyl-1.2-dihydropyrimidine-2- it (code 29-D, Fig. 2) representing two-fragment conjugate containing xymedon drug moiety and the ascorbic acid moiety. This is a single connection of all studied that in a dose of 20 mg/kg showed a statistically significant ( $p < 0.001$ ) stimulatory effect on exercise performance compared with the control group.

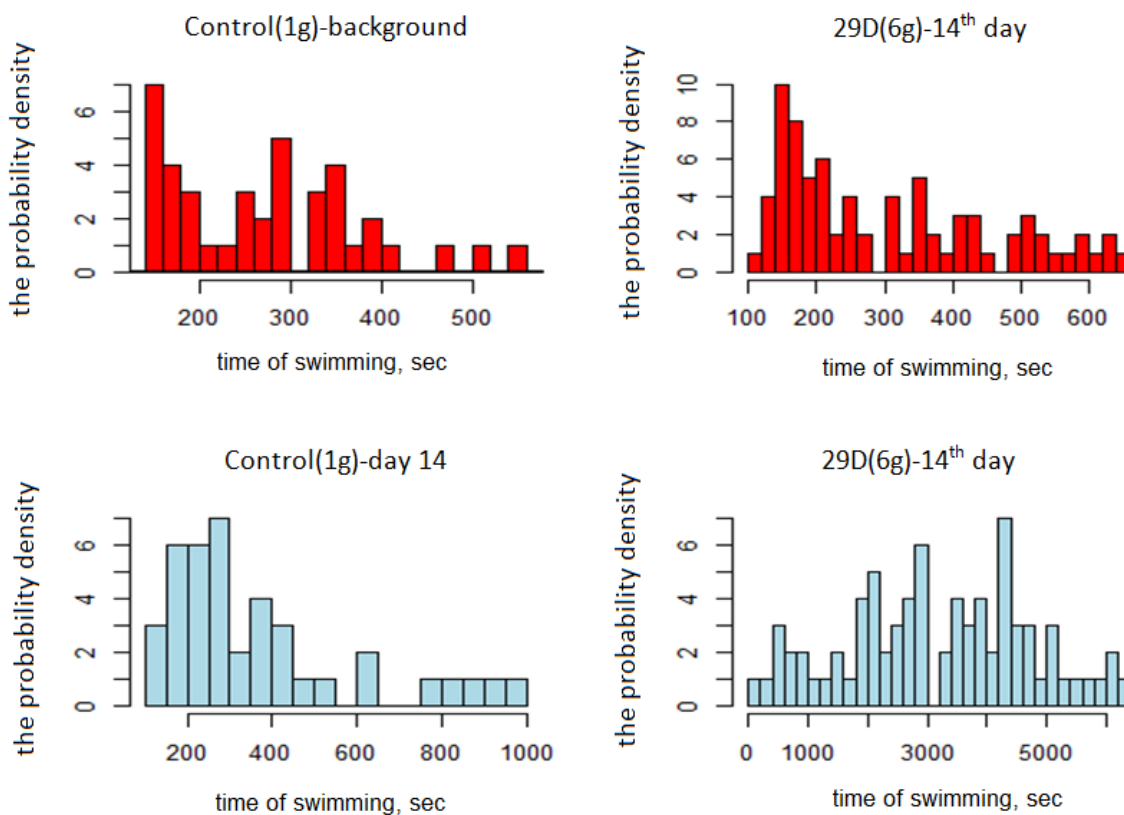


Figure 1: The histogram of the swimming time distribution in the control group, and under the injection of 29-D.

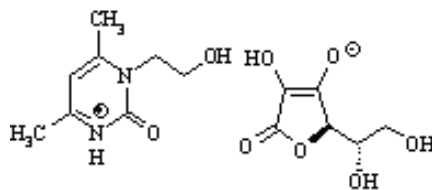
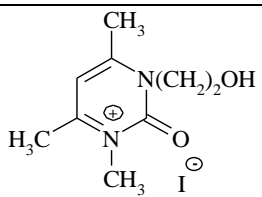
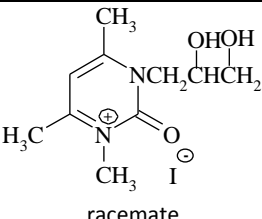
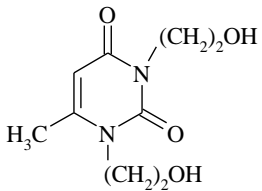
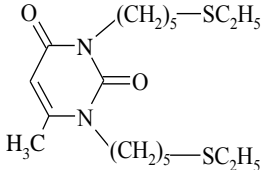
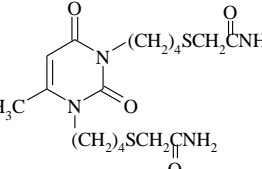
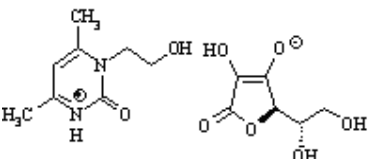
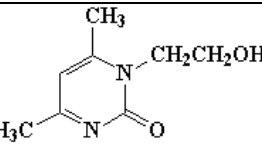


Figure 2: The structural formula of Compound 29-D

Table 1: Acute toxicity and the time change of rats swimming in the conditions of the compounds course injection

Laboratory connection code	Chemical structure	LD <sub>50</sub> , mg / kg (mice)		Changing in the time of swimming (in % to the control)	
		i/p	per os	Day 26	Day 14
1D		519 (388÷6 37)	5572 (5104÷ 5753)	211% increase	310% increase
7D	 racemate	512 (380÷6 33)	5431 (4876÷ 5571)	190% increase	270% increase
11D		535 (410÷6 38)	5668 (4855÷ 5985)	320% increase	380% increase
25D		492 (328÷6 17)	5350 (4701÷ 5790)	205% increase	270% increase
26D		538 (386÷6 83)	5860 (4986÷ 6043)	395% increase	365% increase
29D		2102 (1775÷ 2551)	7522 (7193÷ 7964)	425% increase	440% increase (p<0.001)
XYMEDON		> 7000	> 10000	615% increase	575% increase

**Table 2: Changes in hematological and biochemical index of rat blood in the conditions of exhausting physical activities and compound 29-D course injection**

Blood index	Rat group					
	Intact		Control		29-D	
	Background	14 <sup>th</sup> day	Background	14 <sup>th</sup> day	Background	14 <sup>th</sup> day
Red blood cells, 10 <sup>12</sup> /l	7.05±0.36	7.13±0.22	6.9±0.45	7.02±0.66	7.02±0.46	8.02±0.37
White cells, 10 <sup>9</sup> /l	1.31±0.25	1.33±0.22	1.28±0.28	1.68±0.21	1.22±0.23	1.71±0.21
Hemoglobin, g/l	137.8±6.0	138.9±7.6	139.3±7.7	117.4±7.7	138.0±3.8	148.9±8.2
Glucose, mmol /l	7.61±0.92	7.28±0.85	7.19±1.24	5.21±1.32	7.21±0.57	7.23±0.45
Lactic acid, mmol /l	4.93±1.10	4.83±0.61	4.93±0.92	7.94±0.68	4.98±0.56	3.89±0.55
Urea, mmol /l	5.14±0.74	5.95±1.76	5.15±0.64	7.46±1.14	5.61±0.58	3.71±0.38
Total protein, g/l	57.33±3.94	58.95±5.97	58.63±4.52	59.28±5.09	60.47±4.42	60.97±5.27
Albumin, g/l	29.47±4.16	31.64±3.73	27.90±1.80	28.76±2.99	28.15±2.98	28.79±3.11
ALT, u/l	26.72±6.7	25.79±3.38	27.73±9.15	27.76±5.69	28.93±7.27	28.72±5.95
AST, u/l	139.64±17.15	136.32±16.36	139.13±23.45	144.82±14.94	138.74±14.31	139.41±13.75

For the main hematological and biochemical indices of the blood of the contact group of rats there were no statistically significant changes. The number of erythrocytes in the blood, in conditions of intense exercise in chronic injection of the compound 29-D, increased by 14% ( $\beta$ it <0.001). In the control group of rats, at the end of exhausting physical activities, decreased hemoglobin level at 16% ( $\beta$ it <0.001) and glucose by 28% ( $\beta$ it <0.001). In the group of rats, treated with compound 29-D, was revealed an increase in hemoglobin significance at 6.5% ( $\beta$ it <0.001). In the group of rats treated with 29-D, the blood glucose concentration maintained at the initial level (Table 2). The lactic acid concentration in blood of rats of the control group relative to background values increased by 38% ( $\beta$ it <0.001). In the group of experimental animals was revealed the decrease of lactic acid concentration by 20% ( $\beta$ it <0.001). The contents of urea in blood serum under intense physical stress of rats in the control group increased by 36% ( $\beta$ it <0.001). Under experimental conditions was revealed the decrease ( $\beta$ it <0.001) of urea concentration in blood serum of rats by 36% (Table 2).

In the context of intense exercise implementation during 11-day schedule of 29-D compound injection to rats, on the 14th day of the experiment was indicated the preservation of the original level of enzyme activity of aminotransferases (ALT,  $\beta$ it = 0.94; AST,  $\beta$ it = 0.91), total protein content ( $\beta$ it = 0.80) and albumin ( $\beta$ it = 0.61) in serum, as compared to the control group (Table 2).

Repeated injection of the compound 29-D does not affect the process of white blood cells formation. At the same time, the compound has the ability to stimulate erythropoiesis processes. These findings are consistent with the results of scientific work on the properties of other pyrimidine compounds to stimulate erythropoiesis [5,9,18].

The absence of cardio and hepatotoxicity of 29-D shows no difference between the activities of aminotransferases in the control and experimental, as well as in intact groups of rats.

### CONCLUSION

Thus, it was determined that the degree of impact on the body in accordance with GOST 12.1.007-76 [17] all 6 new pyrimidine derivatives, that are based on xymedon, belong to the group of low-danger chemicals (class 4). The studied pyrimidine derivatives under single injection (intraperitoneally) do not exhibit pronounced actoprotective properties in the test "rats swimming to failure". Under the conditions of a single intraperitoneal injection (for 21 day) was revealed no effect of the the investigated compounds performance stimulation. However, in conditions of repeated intraperitoneal injection for 11 days, among the studied compounds, L-ascorbate 1- (2-hydroxyethyl) -4.6-dimethyl-1.2-dihydropyrimidine-2-one (29-D) at a dose of 20 mg / kg showed a statistically significant act-protective effect. Blood test results are indicative of a milder

exercise tolerance for the body on the background of the 29-D introduction. Repeated injection of the compound, under exhausting stress, has no effect on leukopoiesis but stimulates the process of hematopoiesis. Injection of the substance in these conditions contributes to the prevention of glucose level decrease and increase of urea and lactic acid level. It is revealed the absence of hepato- and cardiotoxic properties of the substance in rats.

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