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DEPENDENCE OF GENERAL ACTION AND ACUTE TOXICITY ON CHEMICAL STRUCTURE IN A ROW OF NEW DERIVATIVES OF 3-METHYL-7-SUBSTITUTED-8-N-ETHYL PIPERAZIN XANTHINE

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Summary. It has been established that the acute toxicity of the synthesized for the first time derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine is in the range from 560 to 1325 mg/kg. In accordance with the classification of K. K. Sidorov, three compounds belong to practically non-toxic substances and four compounds are low-toxic. After a single administration of toxic doses of the studied compounds, the performed pathomorphological studies did not reveal statistically significant changes in the mass of the internal organs. The derivatives of 3-methyl-7-substituted-8-ethyl piperazin xanthine are a promising group of heterocyclic compounds to create safe anthelminthic drugs on their basis.

Keywords: 3-methyl-7-substituted-8-N-ethyl piperazin xanthine derivatives, general action, acute toxicity

Introduction. In modern medical practice, in the treatment of helminthiases in humans and animals, anthelmintic preparations of different classes of chemical compounds are used to treat helminthic invasions of humans and animals (piperazine, male fern extract, chloroxyl, etc.). There are drugs that are used primarily for the treatment of nematodes, cestodoses and trematodes, as well as anthelmintic agents of a wide spectrum of action that are effective against different types of helminths. From the salts of piperazine, piperazine adipate is mainly used, which is widely used for mass dehelminthization at ascaridosis and enterobiasis, because it does not require the preliminary designation of an appropriate diet, laxatives, and also has low toxicity to humans and rarely causes side effects (Arkhipov, 2009; Mashkovskiy, 2009; Drogovoz et al., 2010). In connection with this, the development of new and improvement of the already used anthelmintic agents is an actual problem and is practically necessary. In the second half of the twentieth century, the main direction of pharmacy was the creation of new medicines based on existing substances of anthelmintic drugs (Kornienko, Tarasevičius and Samura, 2013; Kornienko, Samura and Romanenko, 2013; Romanenko et al., 2013).

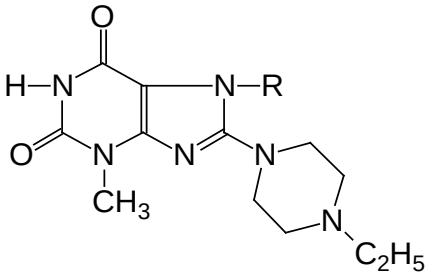
The number of new, newly synthesized organic heterocyclic derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine, developed by us, is of theoretical and practical interest for further targeted synthesis of new substances with predicted anthelmintic activity (Aleksandrova et al., 2016; Ponomarenko et al., 2016; Ivanchenko et al., 2012).

At the stage of pharmacological screening in the study of general action and acute toxicity, the information can be obtained for further modeling of the chemical structures of the xanthine series in order to select more active and less toxic substances for testing specific activity and safety.

The aim of this study: To study in experiments on mice, the general effect and acute toxicity of the newly synthesized heterocyclic derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine.

Materials and methods. The new synthesized heterocyclic derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine (compounds No. 1–7) were used as the object of the study. Synthesis of substances was carried out at the Department of Biological Chemistry of the Zaporizhia State Medical University under the direction of Doctor of Pharmaceutical Sciences, Professor N. I. Romanenko.

Chemical structure of 3-methyl-8-N-ethyl piperazin xanthine derivatives is:



The structure of the synthesized substances was confirmed with the help of modern physicochemical methods of elemental analysis, UV, IR, PMR, and mass spectrometry, counter synthesis, and the purity of the synthesized substances was monitored by thin-layer chromatography.

A study of the acute toxicity of new derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine was carried out on white mongrel mice weighing 20–24 g (Sernov and Gatsura, 2000; Arzamastsev, 1985; Kovalenko et al., 2001).

The substances were dissolved in physiological saline in the form of 3–5% aqueous suspension stabilized by

Tween-80 and were administered intraperitoneally to the animals. The control group of mice was injected with the same saline solution as the experimental groups. The animals were monitored for 14 days after a single administration of the test compounds (Kozhemiakin et al., 2002; Arzamastsev, 1985; Kovalenko et al., 2001).

During the entire experimental period, the animals were monitored. At the same time, the condition of animal fur and visible changes in the mucous membranes were taken into account. The dynamics of changes in the body weight of mice, the character of secretions and the duration of life were monitored. An evaluation of the overall effect of 7 compounds studied was carried out according to behavioral reactions, neuromuscular excitability and some vegetative effects. The number of surviving and dead animals was recorded every 24 hours. Acute toxicity (LD_{50}) was calculated by the Kerber method (Kozhemiakin et al., 2002).

Experimental studies were conducted in accordance with the 'Regulations on the use of animals in biomedical research' (Strasbourg, 1986) and the 'General ethical principles of experiments in animals' (Kiev, 2001), agreed with the requirements of the 'European convention for the protection of vertebrate animals, used for experimental and scientific goals' (Reznikov et al., 2006).

The statistical verification of the data was carried out using the standard analysis package for the statistical processing of the results (Microsoft Office Excel 2003). The results are presented as a sample mean and a standard error of the mean value. The reliability of differences between the experimental groups was assessed using Student's t-test and the Mann-Whitney U-test, the computer program Statistica for Windows 7.0. (Statsoft Inc., No. AXXR712D833214 Fan.5), differences were considered statistically significant for all types of analysis with a significance level of at least 0.05 (Lapach, Chubenko and Babich, 2002; Samura, Korniienko and Romanenko, 2012).

Results. The studies of the general effect of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine derivatives showed that signs of toxic effect were manifested after 5–15 min after single administration of toxic doses of test substances. Compound No. 1 (7-α-methylbenzyl-8-N-ethyl piperazin theophylline) and Compound No. 7 (1-p-fluorobenzyl-8-(4-ethylpiperazinyl-1-) theobromine) caused to signs of an exciting action, which was expressed in increased motor activity, the appearance of tremor, and immediately before the death, clonic-tonic convulsions were observed. In addition, the alertness of animals, increased sensitivity to sound and pain stimuli, the reflex of the withdrawal of the head while touching the mustache were noted. Breathing somewhat increased, the color of the ears became pale, the corneal reflex was preserved, the pupils remained unchanged, the cornea of the eyes remained transparent, moist. In some animals, diarrhoea appeared, the fur became disheveled, lost its luster. After the administration of toxic doses of Compound No. 7, a sharp excitation was first observed with a movement disorder: fibrillar contractions of individual muscle groups of skeletal muscles, indicating abrupt functional shifts in the sphere of vegetative innervation, changes in the tone of skeletal muscles and development of ataxia of the central genesis. In 30–45 min after the administration of the test compounds, excitation was followed by inhibition. Mice became inactive, died from stopping breathing and heart activity. Under the action of Compound No. 3 (7-α-naphthylmethyl-8-N-ethyl piperazin theophylline), the mice fell into a drowsy state with muscular hypotension and died from stopping respiratory and cardiac activity.

After the administration of Compound No. 3, a decrease in the motor activity of mice was observed, respiratory distention appeared, cyanosis of visible mucous membranes was noted, body temperature decreased by 1–2 °C, corneal and pupillary reflexes were absent, which indicates a sedative effect in the spectrum of pharmacological action of these compounds.

Compound No. 4 (7-β-phenylethyl-8-N-ethylpiperazin theophylline), Compound No. 5 (3-methyl-7-n-heptyl-8-N-ethyl piperazin theophylline), and Compound No. 6 (3-methyl-7-n-dodecyl-8-N-ethyl piperazin theophylline) showed a less pronounced soothing effect. Under the influence of these substances, a decrease in spontaneous motor activity was observed in mice. Most animals responded to sound and pain stimuli, and when the corneas were irritated, the animals pulled back the heads. After the injection of toxic doses of these substances, tonic convulsions periodically occurred. The mice took a lateral position, convulsions were replaced by individual twitching of the limbs and the animals died from stopping breathing.

The dead animals were dissected and pathomorphological studies were carried out. The attention was paid to the blood supply and the mass of internal organs, the state of the gastrointestinal tract.

As a result of the conducted experimental studies, there were no statistically significant changes in the mass of the brain, heart, liver, kidneys and spleen after a single administration of toxic doses of the studied compounds.

The calculation of LD_{50} was carried out based on study acute toxicity data (Table 1).

It was found that LD_{50} of the derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine is in the range from 580.0 to 1325.0 mg/kg. There was found that following compounds are almost non-toxic: Compound No. 5 — LD_{50} is 1325.0 mg/kg. Replacement at the 7^{th} position of the molecule of this compound of the heptyl (Compound No. 5) radical by phenylethyl (Compound No. 4), methylbenzyl (Compound No. 1), leads to an increase in acute toxicity (LD_{50} increased from 1325.0 to 1125.0 mg/kg).

Table 1 — Acute toxicity of 3-methyl-8-N-ethyl piperazin xanthine derivatives at intraperitoneal administration to mice

Compounds		LD_{50}, mg/kg
No. 1	7-α-methylbenzyl-8-N-ethyl piperazin theophylline	1125.0 ± 28.9
No. 2	7-(3-chlorobuten-2-yl-1)-8-N-ethyl piperazin theophylline	685.0 ± 32.4*
No. 3	7-α-naphthylmethyl-8-N-ethyl piperazin theophylline	560.0 ± 24.7*
No. 4	7-β-phenylethyl-8-N-ethyl piperazin theophylline	1210.0 ± 37.5
No. 5	3-methyl-7-n-heptyl-8-N-ethyl piperazin theophylline	1325.0 ± 41.5
No. 6	3-methyl-7-n-dodecyl-8-N-ethyl piperazin theophylline	740.0 ± 21.8*
No. 7	1-p-fluorobenzyl-8-(4-ethyl piperazinyl-1-) theobromine	620.0 ± 19.7*

Note: * — $p < 0.05$ statistically significant differences in comparison with the control.

The most toxic was Compound No. 3 — LD_{50} is 560.0 mg/kg. In accordance with the classification of Sidorov (1973), three compounds are related to practically non-toxic substances and four compounds are low-toxic.

Compounds No. 2 (LD_{50} is 685.0 mg/kg), No. 6 (LD_{50} is 740.0 mg/kg), No. 7 (LD_{50} is 620.0 mg/kg) were moderately toxic.

Thus, as a result of the study of the general action and acute toxicity of the newly synthesized derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine, compounds were selected to study anti-helminth activity. The dependence of acute toxicity on the chemical structure in the series of studied new heterocyclic derivatives of 3-methylxanthine was established.

Conclusions. 1. Acute toxicity of the derivatives of 3-methyl-7-substituted-8-ethyl piperazin xanthine is in the range from 560 to 1325.0 mg/kg. Three compounds belong to practically non-toxic and four compounds are low-toxic substances.

2. After a single administration of toxic doses of the studied compounds, the performed pathomorphological studies did not reveal statistically significant changes in the mass of the internal organs.

Prospects for further research. Derivatives of 3-methyl-7-substituted-8-ethyl piperazin xanthine are a promising group of heterocyclic compounds for the creation on their basis of safe and more effective anthelmintic drugs.

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