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Research Article

Research of the Chronic Toxicity of Chlomepiroxane

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Abstract: The article presents the results of experimental studies, during a 3-month period of time, devoted to the chronic toxicity research of the first synthesized compound – 7-(3-chlorobutylene-2-yl-1)-8-N-methylpiperazino-3-methylxanthine (chlomepiroxane), possessing anthelmintic activity.
Keywords: 7-(3-chlorobutylene-2-yl-1)-8-N-methylpiperazino-3-methylxanthine, chronic toxicity, chlomepiroxane, synthesized compound, animals, rats.

INTRODUCTION

Each new, newly synthesized chemical compound requires an immediate toxicological assessment, as it is the basis for preventing the harmful effects of chemical compounds on the animal organism. In the course of the experiments connected with the examination of new synthesized compounds, the researcher in one form or another must register the reactions occurring in the animal body under the influence of the examined substance. At the same time, reliable material accumulates, allowing to make an opinion about the nature and a mechanism of action of the substances being studied [10,12].

The study of the chronic toxicity of newly synthesized substances has its own characteristics, depending on the tasks facing the researcher. The goal of chronic toxicological experiments is the characterization of the degree of damaging effect of a harmful substance during its long introduction, the detection of the most sensitive organs and systems of the organism, as well as the investigation of the degree of reversibility of the damage caused by it.

Objective

Chronic toxicity study of the first synthesized compound – 7-(3-chlorobutylene-2-yl-1)-8-N-methylpiperazino-3-methylxanthine (chlomepiroxane),

MATERIALS AND METHODS

The object of the study was 7-(3-chlorobutylene-2-yl-1)-8-N-methylpiperazino-3-methylxanthine first synthesized at the Department of Biological Chemistry of the Zaporizhzhya State Medical University, under the guidance of the Doctor of Pharmaceutical Sciences, Professor N.I. Romanenko [13].

Chronic toxicity of chlomepiroxane was studied on white mongrel rats of both sexes weighing 120-170 g. Four groups of animals with 10 rats in each were observed [3, 4, 8]. Daily for three months the animals of the first, second and third groups were injected chloperiopyroxane in doses of 45, 90 and 225 mg/kg intraperitoneally with a metal probe. The fourth group was a control group and received distilled water in the same volume as the experimental animals for three months daily.

After a macroscopic morphological study of the internal organs of white rats, pieces of tissue were taken and fixed in Carnoy's liquid, dehydrated in alcohols, and poured into paraffin according to the generally accepted method [7].

Histological sections of organs (5 μm thick) were stained with hematoxylin-eosin. The color of the chromatophilic substance in the cytoplasm of nerve cells was performed according to the method proposed by Nissl. Soft shells of nerve cells were stained by the Shpilmeyer method [9].

Animals of all groups were on a standard diet, followed closely by [5]. Every 15 days they were weighed, behavioral reactions were taken into account. Before the beginning of the experiments, after 90 days, laboratory tests of blood and urine, determination of kidney activity under water loading conditions according to E.B. Berkhin were carried out [1]. After the end of a three-month study of the chronic toxicity of

chlomepiroxane, the animals were sacrificed under ether anesthesia by instantaneous decapitation, morphological and histological studies of internal organs were performed [2, 6, 11].

During the experimental studies, the animals were in standard vivarium conditions in accordance with the norms and principles of the EU Council Directive on the protection of vertebrates, which were used for experimental and other scientific researches [2].

RESULTS

The three-month chronic toxicity research of chlomepiroxane showedd that the rats tolerate it well; no visible changes in the general state and behavior of the animals were detected during the experiment. The rats were normally developed and added to the mass (Table 1). During the entire period of the experiment they were active. At the end of three months all the rats remained alive. Laboratory and pathomorphological studies were carried out. The change in the weight of rats in the study of the three-month chronic toxicity of chlomepiroxane is shown in Table 1.

Table-1: The change in the mass of rats during the three-month chronic toxicity of chlomepiroxane research

Group	Mass of rats in grams in ... days						
	primary	15	30	45	60	75	90
1	91±1.6	96±1.8	101±2.1	108±2.3	114±2.4	120±2.1	127±2.2
2	94±1.4	100±2.1	105±1.8	111±1.9	116±2.2	122±2.3	129±2.3
3	92±1.3	98±1.9	105±1.9	112±2.0	119±2.1	125±1.8	132±2.1
4	93±1.2	97±1.8	104±1.7	110±1.7	117±1.6	124±1.9	130±2.4

Note: * - for p> 0.05 compared with the control

The analysis of the results of experimental data showed that chlomepiroxane at a dose of 45, 90 and 225 mg/kg with intraperitoneal administration to rats does not have a statistically significant (p> 0.05) change in body weight compared to the control group.

The effect of chlomepiroxane (25 mg/kg) on the function of the kidneys in rats in a three-month chronic experiment is given in Table 2.

Table-2: The effect of chlomeprioxane (25 mg/kg) on rat's kidney function in a three-month chronic experiment

Group	Diuresis for 4 hours in ….days, ml		
	30	60	90
1	2.6±0.12	3.2±0.11*	4.1±0.14*
2	2.8±0.14*	3.4±0.17*	4.5±0.17*
3	2.7±0.13*	3.1±10.13*	3.9±0.18*
4	2.1±0.12	2.6±0.10	2.9±0.12

Note: * - for p> 0.05 compared with the control

The analysis of the research results of changes in the main indicators of kidney activity compared with primary data showed, that after daily administration of chlomepiroxane to animals of three experimental groups, showed an increase in renal excretory function after 30 days by 23.8-33.3%, after 60 days - by 23.1 -30.7% and in 90 days - by 41.4-55.1% compared with the fourth control group. Consequently, in long-term use, chlomepiroxane does not have a toxic effect on the functional state of the kidneys in experimental animals.

After the autopsy and pathomorphological study of control and experimental animals, it was established that the brain tissue is moderately full-blooded on the incision. The mucous membrane of the larynx, trachea and bronchus is gray, shiny. Valve apparatus of the heart was without pathological changes. The liver, kidneys, organs of the endocrine system, the mucous membrane of the pelvis, ureters and the bladder were also without visible morphological changes in their structure.

Brain

At a histological examination of the brain structure it was established that the soft meninges of the usual blood filling and structure in the animals of the experimental and control groups. Neurons when stained with hematoxylin-eosin and Nissl have a bright nucleus with a well-contoured nucleolus, which in some neurons occupies a central position, while in others it is located closer to the nuclear envelope. The Nissl substance is well contoured. In some cells, moderate peripheral chromatolysis is noted, which is regarded as a functional change in neurons. The number of glial cells is normal. The ependyma is represented by a layer of ependent cells with rounded or oval cores. The vascular plexus has the usual histological structure.

Heart

Muscle fibers are of usual thickness. On the muscular section, under the thin strip of the endocardium, there are single smooth muscle fibers of a spindle shape with a rounded nucleolus in the center, and a large number of striated fibers are located below. The inner, middle and

outer walls of the vessels are well defined, unchanged. The structure of the walls of the blood vessels of the animals of the experimental groups corresponds to the control group.

Lungs

Histological examination of pulmonary tissue preparations (staining with hematoxylin-eosin) of experimental and control animals showed no deviations of the morphostructure. The interalveolar septa have the usual histological structure. The epithelium of the bronchi is multi-row, ciliate, the nuclei are located in the basal sections, rounded. The cytoplasm is homogeneous, pink in color. There are goblet cells.

Kidneys

Parenchyma of the kidneys is of normal blood filling. Capsules of glomeruli of usual size and structure, the lumen of it is free. The loops of the blood capillaries of the glomeruli have an identical structure in the animals of the control and experimental groups. The epithelium of the nephrons is of a cubic form, the nuclei are located basally.

Liver

The liver has a lobed structure of moderate blood filling. Hepatocytes of normal size, round-shaped nuclei are located in the center of the hepatic cells. The cytoplasm has separate vacuoles and gentle granularity. It is necessary to note the identical histological structure of the liver of animals in the control and experimental groups. Vacuolization of the cytoplasm of hepatocytes in the control and in the experimental groups can be explained, apparently, by the food ration of animals. Portal tracts have the usual histological structure.

Spleen

Both in the control and in the experimental groups, the spleen has the usual structure. The white pulp is well contoured, which is represented by a lymphoid tissue located near its arteries in the form of follicles. The central part of the lymphatic follicles of the spleen is colored lighter. The red pulp consists of a reticular tissue with free cellular elements of blood and connective tissue and blood vessels located in it in the form of venous sinuses.

Stomach

Histological examination of the stomach of experimental and control rats on the transverse section clearly shows the epithelium in the form of single-layered cylindrical cells. The nuclei of cells are round in shape. The cells of the epithelium form various gastric fossae. The plate of the mucosa is represented by a loose fibrous unformed connective tissue, in which the rounded forms of the artery and the flattened form of the veins, the nerve cells, are found in places. There were no deviations in the structure of histological preparations of the stomach of experimental animals when compared with the histological preparations of the stomach of the control group. In a single section of the preparation of the third group, a defect of a single-layered cylindrical epithelium and loose connective tissue was found.

Rectum

The nuclei of the epithelium are small, the oval are located in the middle and partly in the epithelial part. There are elongated forms of cells with flattened nuclei, occupying the middle or epic position. There are single goblet cells. In crypts there are many cells with slightly basophilic cytoplasm, cone-shaped. In the cover of the epithelium, a thin margin is visible. In all experimental groups, the structure of the rectum corresponds to control.

Thus, based on the chronic toxicity research of chlomepiroxane, it is established that, for a three-month application, it does not adversely affect the overall functional state of the animals, nor does it cause significant morphological and histological changes in the tissue structure of vital organs and systems.

CONCLUSIONS

- It has been established that LD_{50} of chlomepiroxane when administered intraperitoneally to rats is 910 mg/kg and refers to non-toxic substances.
- Chlomepiroxane for long-term use does not have a toxic effect on the functional state of the kidneys in experimental animals.
- With the pathomorphological study, it was established that the brain, heart, kidneys, liver, spleen, stomach, rectum, mucous membrane of the larynx, bronchi, lungs were without pathological changes.

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