

Toxicity of Substance BKP-115 on Rats and Mice of Both Sexes at Long Term Intra-gastric Introduction

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Abstract

Today there is a large number of scientific publications in which the heterocyclic systems of 1,2,4-triazole and their derivatives are studied. Many of the medications, which contain compounds with the 1,2,4-triazole ring, were discovered and applied in practice. For many years, 1,2,4-triazole derivatives remain the object of close attention of scientists of various scientific fields. The unique properties of 1,2,4-triazole derivatives include high reactivity, which allows different modification of this system, practical absence of toxicity of these derivatives, and the presence of a wide range of biological and pharmacological properties.

For our research, we find the most promising compound for the study of acute toxicity. There is BKP-115, which shows interesting results in the classification of substances for toxicity class IV-V. The purpose of the work is to study the toxicity of substance BKP-115 on rats and mice of both sexes at long-term intra-gastric introduction, macroscopic examination of internal organs, and histostructure of internal organs.

Keywords: Derivatives of 1,2,4-triazole, BKP-115, Acute toxicity, Histostructure of internal organs

INTRODUCTION

In the scientific world in recent years there has been a tendency to increase the number of publications related to the study of the properties of the heterocyclic system of 1,2,4-triazole and its derivatives [1, 2]. Interest in these compounds is shown not only by scientists involved in organic synthesis but also by pharmacologists, biologists, specialists in the field of veterinary medicine, pharmacy, and agronomy [3-7]. Such vigilant attention from the wider circle of the scientific community to the derivatives of 1,2,4-triazole is a reasoned explanation, first, 1,2,4-triazole and its derivatives are highly reactive [8, 9], and second, their insignificant toxicity and high pharmacological activity is purposeful, and creates conditions for a long-term search of potential biologically active compounds [8, 10]. It should be noted that today there is a lot of information that confirms the fact that the most promising search for substances of potential drugs is among the water-soluble derivatives of 1,2,4-triazole [11-13]. At the first stage of practical implementation, the necessary condition is the study of acute toxicity of the compound [14, 15]. Previously, by using computer forecasting, about 100 new compounds were analyzed for various types of pharmacological activity [16]. With a high probability of manifestation of biological activity, the results indicate the water-soluble substances among the derivatives 1,2,4-triazole. Therefore, we selected one of the most promising results of the prediction of the compound for which acute toxicity was studied. The purpose of the work is to study the toxicity of substance BKP-115 on rats and mice of both sexes at long-term intra-gastric introduction, and macroscopic

examination of internal organs and histostructure of internal organs.

MATERIALS AND METHODS

The research was conducted following the national "General Ethical Principles of Animal Experiments" (Ukraine, 2001), which is consistent with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes" (Strasbourg, France, 1985), as well as following the EU Council Directive [17].

The study of acute toxicity of a water-soluble compound was conducted following the recommendations of Stefanova O.V. [15], and the statistical treatment of the results of the study by Prozorovsky VB [14]. As the dynamics of animal death did not have a strict linear dependence, the mean airborne dose

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(LD50), latter was calculated using the Kerber method. For male rats LD50 was 4667 mg/kg, for females 3333 mg/kg. For male mice, LD50 was 7000 mg/kg, for females 4000 mg/kg.

RESULTS AND DISCUSSION

It was established that when intra-gastric introduction, the substance BKP-115 causes the death of animals, which increases with increasing the dose.

Observations on the state of animals showed that after 5-10 minutes after intra-gastric introduction of the minimum doses of BKP-115 in both rats and mice there was an inhibition of motor activity, slowed breathing. At the lower doses, the indicated symptoms of intoxication continued in animals for 1-2 h; at higher doses, this period was shorter - 30-60 minutes.

The death of animals was fixed on the first day after the introduction of the substance BKP-115. The further condition of surviving animals was normalized and did not differ from such intact animals. Macroscopic examination of lost rats revealed no apparent pathological changes, mucous membrane (CO) of the stomach and intestines without much change, parenchymatous organs in the abdominal and chest cavities were blood-filled.

Based on the obtained mid-lethal doses it is possible to conclude about the sexual sensitivity of animals of both studied species to the action of BKP-115. The most sensitive to the action of the drug were females, both rats, and mice. If in male rats, death began at doses of 4000-7000 mg/kg, then the female rats died when BKP-115 was the introduction at doses of 2000-5000 mg/kg. The dynamics of the mass of rats and surviving mice is shown in **Tables 1-4**. The weight of animals varied within the limits of the values of the IR group (intact control), the weight gain of the body had no reliable values, except for the male mice in the group of IK (**Table 3**).

Table 1. Dynamics of Mass (g) of Body of Male Rats at Intra-gastric Introduction of BKP-115 Substance, $M \pm m$

Animal Groups	The Term of Observation			
	Source Data	3 Days	7 Days	14 Days
IntactControl	159±6,6	159±6,9	166±6,6	175±6,8
BKP-115, 3000 mg / kg	163±9,1	162±9,8	165±10,0	168±12,4
BKP-115, 4000 mg / kg	164±12,6	158±1,7	167±3,3	172±3,3
BKP-115, 5000 mg / kg	158±3,6	140±0,0	140±0,0	140±0,0
BKP-115, 6000 mg / kg	157±8,4	168±14,5	172±15,9	178±22,0
BKP-115, 7000 mg / kg	160±8,8	-	-	-

Table 2. Dynamics of Mass (g) of Body of Female Rats at Intra-gastric Introduction of BKP-115 Substance, $M \pm m$

Animal Groups	The Term of Observation			
	Source Data	3 Days	7 Days	14 Days
Intact Control	159±3,5	162±4,0	163±3,6	165±5,9
BKP-115, 1000 mg / kg	163±8,8	164±9,6	166±10,8	172±13,5
BKP-115, 2000 mg / kg	161±5,2	148±4,3	148±4,8	154±5,5
BKP-115, 3000 mg / kg	160±6,6	154±7,5	151±8,5	151±8,5
BKP-115, 4000 mg / kg	163±14,4	148±18,8	152±18,8	155±16,1
BKP-115, 5000 mg / kg	163±8,7	-	-	-

Table 3. Dynamics of Mass (g) of Body of Male Mice at Intra-gastric Introduction of BKP-115 Substance, $M \pm m$

Animal Groups	The Term of Observation			
	Source Data	3 Days	7 Days	14 Days
IntactControl	21,5±0,43	21,6±0,20	22,2±0,31	22,7±0,20 <i>p₁=0,0391</i>
BKP-115, 5000 mg/kg	21,5±0,69	22,0±0,60	22,3±0,63	22,6±0,63
BKP-115, 6000 mg/kg	21,8±0,20	26,1	26,2	26,3
BKP-115, 7000 mg/kg	21,8±0,70	22,8±0,97	22,9±0,97	23,5±0,80
BKP-115, 8000 mg/kg	21,4±0,51	21,2±1,80	21,3±1,95	21,9±1,60
BKP-115, 10000 mg/kg	21,7±0,44	-	-	-

Notes:

1. Intergroup comparisons were performed using the dispersion analysis and the Newman-Keells criterion, $p < 0.05$.
2. p_1 - level of statistical significance concerning the initial values;

Table 4. Dynamics of Mass (g) of Body of Female Mice at Intra-gastric Introduction of BKP-115 Substance, $M \pm m$

Animal Groups	The Term of Observation			
	Source Data	3 Days	7 Days	14 Days
IntactControl	21,1±0,27	22,4±0,93	22,8±0,31	23,1±0,30
BKP-115, 2000 mg/kg	22,7±1,02	23,0±0,89	23,2±1,15	23,7±1,09
BKP-115, 3000 mg/kg	22,5±1,12	22,12±1,03	21,7±1,06	22,4±0,92
BKP-115, 4000 mg/kg	21,9±0,63	21,8±0,90	22,4±0,87	22,9±0,90
BKP-115, 5000 mg/kg	21,5±0,67	23,1	21,0	21,3
BKP-115, 6000 mg/kg	21,0±0,89	-	-	-

The obtained data provides the basis for the conclusion that in the case of an overdose of the tested substance BKP-115 in males, the target organs are the liver and heart and to a lesser

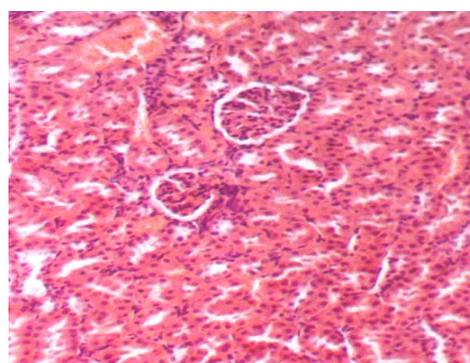
extent - lungs and thymus; in females, they are kidneys, heart, adrenal glands and, to a lesser extent, spleen.

In rats that survived the intra-gastric introduction of BKP-115 substance, on the 14th day of observation, the visual condition of the wool is normal, there is no separation from the natural apertures. No marked violations of the macroscopic state and topography of the internal organs were detected. Hypotonic hypertrophy of organs, signs of inflammation, circulatory disorders, tumor growth have not been noted. Leaves of the pleura, pericardium, and peritoneum are thin, shiny, and smooth. Thymus is a whitish-grayish tint, of moderately dense consistency. The heart muscle is moderately dense and uniformly brownish. The cavity of the heart contained a small amount of liquid blood. The surface of the lungs had a homogeneous pale pink color. The mucous membrane of the stomach is shiny, smooth, and pink, without signs of irritation. Enlargement of the stomach is filled with chyme. Enlargement of the small and large intestines is unchanged, the mucous membrane is common in color, the contents correspond to divisions. The surface of the liver is smooth, homogeneous, and dark red. Liver Capsule is thin and clear. The consistency of the organ is of normal density. The pancreas is visually unchanged. The surface of the spleen is a homogeneous dark cherry shade. The consistency of the spleen is moderately dense. The capsule of the kidneys is easily removed. The surface of the organ is smooth, homogeneous in a brownish-grayish color. On the cut of the organ, the cortical and cerebral matter differed significantly. The consistency of the kidneys is moderately dense. The supplements are without peculiarities. The bladder is filled with clear and light urine. Macroscopic examination of the internal organs of surviving rats did not reveal any pathological changes. Following Order No. 944 of the DEC on the Ministry of Health of 14.12.2009, samples of tissues of rats receiving the maximum dose were selected for histological examination: for males, this dose was 6000 mg/kg, for females - 4000 mg/kg. Examples of samples of the heart, lungs, liver, thymus, spleen, kidneys, adrenals, pancreas, stomach, empty rectum, testicles, ovaries of rats, which survived after a single intra-gastric introduction of BKP-115 substance at a dose of 4000 mg/kg at the light-optical level - females and 6000 mg/kg – males. As a control, samples of similar organs and tissues of intact rats of both sexes were studied. Samples for further study were withdrawn on day 14 after a single injection of the test substance.

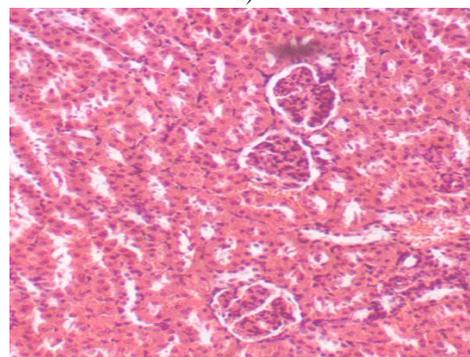
Liver: The histological structure of the organ in all rats that survived the experiment after the introduction of the test substance at a dose of 6000 mg/kg (males) and 4000 mg/kg (females), unchanged compared with intact control. The boundary between the segments is not clear, which is typical for rats, the area of the portal paths is narrow. The radial direction of hepatocytes can be traced. Signs that are characteristic of tension, activation, or suppression of hepatocyte function have not been identified. The processes of local microcirculation in most surviving rats are not

affected. However, in one male rat, the focal hypertrophy of sinusoidal capillaries was noted. All the rats in the cytoplasm of the cells observed different-sized optic cavities. The core of localization did not change. Such hollows are associated with the dissolution of the water-soluble fraction of glycogen in the preparation of samples for light-optical research.

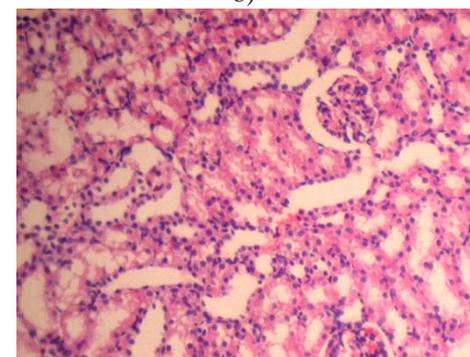
Kidneys for the condition of glomeruli, tubular system of nephrons are common in males and females of rats that survived after the introduction of BKP-115 substance at doses of 6000 mg/kg and 4000 mg/kg, respectively. The flowers moderately varied in size, the density of the arrangement. Nuclear saturation, clarity of the pattern of glomerular loops is normal. The level of loosening of the apical sections of the nephrocytes of the convoluted tubules did not exceed the intact. Channels of the brain layer were without features (**Figure 1**).



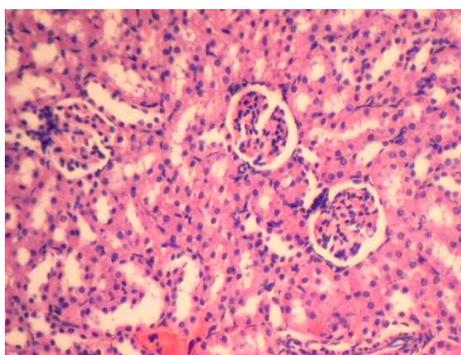
a)



b)



c)



d)

Figure 1. Rat kidney after intra-gastric introduction of BKP-115 substance (a - male, dose 6000 mg / kg; b - female, dose 4000 mg / kg) and intact rats (c - male, d - female). The normal state of glomeruli and tubules of nephrons. Hematoxylin-eosin. x200

The myocardium of rats, both males, and females, after the introduction of the test substance in these doses, retained the usual histological structure. The cardio-muscle fibers are evenly colored, tightly arranged. Transverse membrane myofibrils can be traced. Expression of endosomal and perimemic is common. Venous-type vessels are full-blooded, stromal cellular response is not detected

The alveolar pattern is fairly clear in the respiratory lung of all experimental and intact rats. Signs of alveolar edema or an increase in cellular saturation between alveolar partitions were not observed. Lymphocytic response in the stroma of the bronchial tree is moderate. Epithelium of the bronchi and bronchioles were without signs of damage.

In the spleen, lymphoid nodules are common in size. They are visible periarterial T-dependent, B-dependent marginal zone, and germinal center. In the red pulp, there are many erythrocytes and visible nuclear forms of cells. Signs of the pathology of the immune response after the introduction of the BKP-115 substance were not detected.

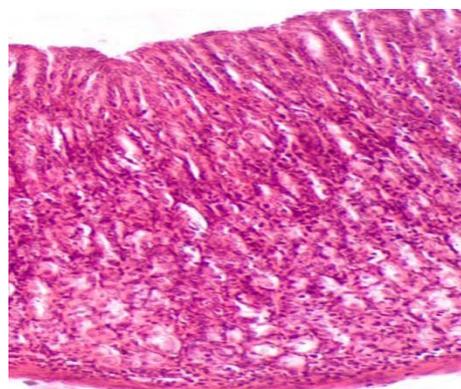
In the adrenal glands of the rats, there are no histological signs indicating changes in the production of mineral and glucocorticoids. The functional state of chromophil cells varies moderately physiologically.

In the pancreas of all experimental and intact rats, the exocrine and endocrine parts are distinctly different. In acinus pancreas with a typical dyzonal cytoplasmic paint, the acinus pattern is not changed. Pancreatic islets, in shape and size variation, meet the norm. Insulinocytes are not visually altered.

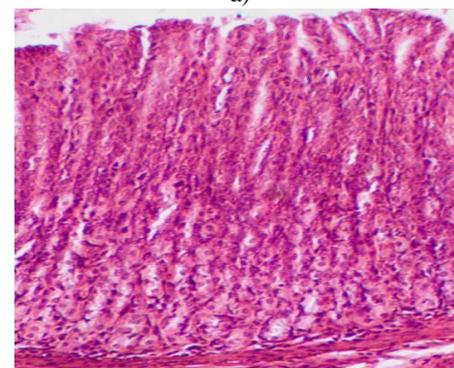
Testes of males were survived after introduction of BKP-115 substance, without pathology. The tape of the spermatogenic epithelium is wide enough, containing the entire pool of germ cells. Sex cells are located in concentric layers according to

the stages of spermatogenesis. Leydig and Sertoli cells are without pathological changes. In ovaries of the female rats, different degrees of development of egg follicles, yellow bodies of different cycles are visible. In the eyes, the level of atresia of follicles corresponds to normal.

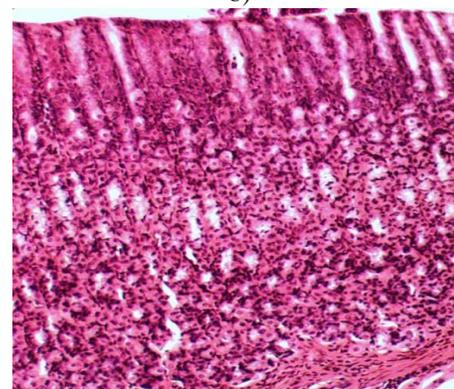
Microscopic examination of the mucous membrane of all rats' stomach did not reveal desquamation processes in the cover epithelium, changes in morphological characteristics of glandular cells of the own and pyloric glands. Microcirculatory processes in the stroma are common (**Figure 2**).



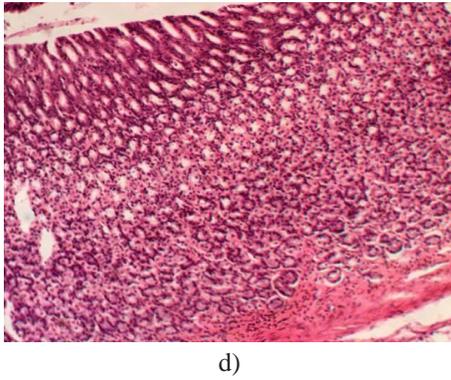
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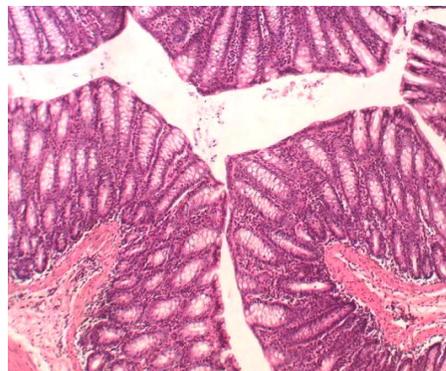


d)

Figure 2. Stomach of rats after intra-gastric introduction of substance BKP-115 (a - male, dose 6000 mg / kg; b - female, dose 4000 mg / kg). The normal state of the mucous membrane. Hematoxylin-eosin. x200 Stomach of intact rats (c - male, d - female). The normal state of the mucous membrane. Hematoxylin-eosin. x200

In the empty intestine visually, the condition of the villi of the mucous membrane is normal. Epitheliocytes of villi and intestinal crypts are unchanged. Cupeloid cells are sufficient in number. Stroma of mucous membrane of the usual type.

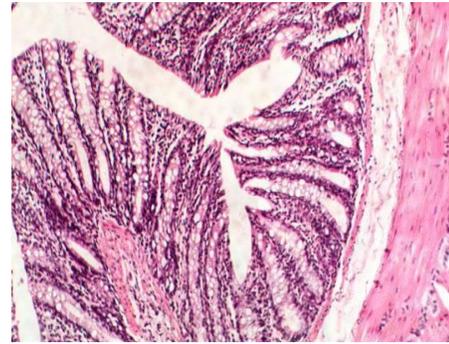
The mucous membrane of the rectum of all the examined rats is lined with a cubic single layer epithelium with a clear cuticular border, a significant content of the cellophageal cells. Intestinal cryptum is moderately deep, the lymphoid saturation of the stroma is also moderate (**Figure 3**).



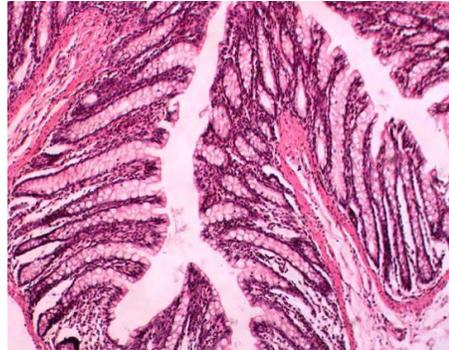
a)



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Figure 3. The rectum of rats after intra-gastric introduction of the substance BKP-115 (a - male, dose 6000 mg / kg; b - female, dose 4000 mg / kg) and intact rats (c - male, d - female). The structure of the mucous membrane has not changed. Hematoxylin-eosin. 200

CONCLUSION

- Following the classification of substances for toxicity, the substance BKP-115 with intra-gastric introduction is:
 - for rats (males and females) - up to grade IV (1000 < LD₅₀ < 5000 mg / kg);
 - for male mice - up to grade V (5000 < LD₅₀ < 15000 mg/kg);
 - for females of mice - IV grade (1000 < LD₅₀ < 5000 mg/kg).
- Based on the data obtained, it can be concluded that male rats who survived after a single intra-gastric introduction of BKP-115 in a dose of 6000 mg/kg and in females of rats that survived after an introduction of a substance at a dose of 4000 mg/kg, on 14 days of the experiment notable on the light-optical level of changes in the histological structure of the investigated internal organs compared with intact control was not found.

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CONFLICT OF INTEREST: None

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ETHICS STATEMENT: The research was conducted following the national "General Ethical Principles of Animal Experiments" (Ukraine, 2001), which is consistent with the

provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes" (Strasbourg, France, 1985), as well as following the EU Council Directive.

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