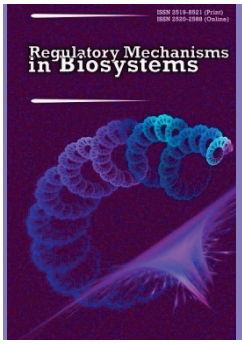

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2024, 15(1), 166–170
doi: 10.15421/022424

Toxicity parameters of a new 1,2,4-triazole derivative when subcutaneously injected to guinea pigs

V. V. Zazharskyi*, O. A. Bigdan**, V. V. Parchenko**, Y. V. Karpenko**, N. M. Zazharska*, Y. O. Mykhailiuk**, O. M. Kulishenko*, P. O. Davydenko*, S. M. Kulish**, B. V. Gutyj***

**Dnipro State Agrarian and Economic University, Dnipro, Ukraine*
***Zaporizhzhia State Medical and Pharmaceutical University, Zaporizhzhia, Ukraine*
****Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine*

Article info

Received 10.01.2024
Received in revised form 08.02.2024
Accepted 27.02.2024

Dnipro State Agrarian and Economic University, Sergiy Efremov st., 25, Dnipro, 49000, Ukraine. Tel.: +38-056-713-51-74. E-mail: zazharskiyv@gmail.com

Zaporizhzhia State Medical University, Mayakovsky av., 26, Zaporozhye, 69035, Ukraine. Tel.: +38-066-405-52-64. E-mail: parchenko@ukr.net

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Pekarska st., 50, Lviv, 79010, Ukraine. Tel.: +38-068-136-20-54. E-mail: bvh@ukr.net

Zazharskyi, V. V., Bigdan, O. A., Parchenko, V. V., Karpenko, Y. V., Zazharska, N. M., Mykhailiuk, Y. O., Kulishenko, O. M., Davydenko, P. O., Kulish, S. M., & Gutyj, B. V. (2024). Toxicity parameters of a new 1,2,4-triazole derivative when subcutaneously injected to guinea pigs. Regulatory Mechanisms in Biosystems, 15(1), 166–170. doi:10.15421/022424

The results of the literature analysis indicate a wide spectrum of biological activity of 1,2,4-triazole derivatives. Low toxicity, reactivity, and high biological activity of 1,2,4-triazole derivatives make this class of compounds very attractive. The obtained data determine the relevance of further studies of 1,2,4-triazole derivatives to find new highly effective biologically active substances that can become the basis for new medicines. Our further work aimed to study some toxicity parameters of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4] thiadiazine when administered subcutaneously to guinea pigs, which is a necessary condition for further implementation in practice of this compound. In this work, we observed an absence of toxic effects after a single subcutaneous injection to guinea pigs at a dose of 40 mg/kg experimental drug. The results of macro- and microscopic examinations of internal organs 14 days after a single subcutaneous injection of the studied compound at a dose of 20, 40 mg/kg showed the absence of any anatomical and morphological disorders in the tissue structures of guinea pigs. The calculated value indicates a high degree of safety of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4] thiadiazine and its prospects for use in veterinary practice as an effective and safe tuberculocidal agent.

Keywords: safety; acute toxicity; chronic toxicity; enzymes; biochemical parameters.

Introduction

The chemistry of heterocyclic compounds is very diverse. Among them, many substances with a number of unique properties have been found, which are valuable as objects of scientific research (Khanage et al., 2012). In the modern 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 triazole and its derivatives (Neofytos et al., 2010). The advent of triazoles has led to a revolution in the treatment of people with invasive and fungal infections (Zazharskyi et al., 2019). Interest in these compounds is shown not only by scientists engaged in organic synthesis, but also pharmacologists, biologists, specialists in veterinary medicine, pharmacy and agronomy (Bihdan et al., 2019; Palchykov et al., 2020). Such close attention from a wide range of the scientific community to 1,2,4-triazole derivatives needs a reasoned explanation; firstly, 1,2,4-triazole and its substitutes are very reactive (Tan et al., 2021), and secondly, their low toxicity and high pharmacological activity purposefully creates conditions for the prospective search for potential biologically active compounds (Xu et al., 2017).

Analysis of scientific sources shows that scientists pay a lot of attention to the study of antimicrobial, antifungal, antihypoxia, hypoglycemic, analgesic, and other types of activity in a number of 1,2,4-triazole derivatives. The results of the literature analysis indicate a wide spectrum of biological activity of 1,2,4-triazole derivatives. The obtained data determine the relevance of further studies of 1,2,4-triazole derivatives for antimicrobial, antifungal, antihypoxic, hypoglycemic, analgesic, anti-inflammatory and other activities with the aim of finding new highly effective biologically active substances that can become the basis of new medicines (Gotsulya et al., 2020).

Earlier, scientists reported that among the 1,2,4-triazole derivatives a series of new compounds were found that are active against different strains of mycobacteria (*Mycobacterium bovis* and *M. fortuitum*) (Zazharsky et al., 2018; Gotsulya et al., 2020). In addition, it should be noted that the last decade has seen progress in the world with the introduction of new and very promising anti-TB drugs, including bedaquiline (inhibition of mycobacterial ATP synthase), delamanide (inhibition of mycolic acid) and pretomanide (inhibition of cell wall synthesis).

However, despite this fact, resistant cases appeared a year after the introduction of these drugs in clinical practice, which reflects that the current efforts in the development of drugs are insufficient to completely eliminate the tuberculosis epidemic (Tan et al., 2021).

Scientists who have been able to synthesize new vinyl sulfones based on triazole and its allyl isomer (Doherty et al., 2017), as well as a series of new derivatives of 4-(azidomethyl)-2H-chromen-2-ones active against *Mycobacterium tuberculosis*, are searching for new promising anti-TB molecules among triazole derivatives (Khanapurmath et al., 2019).

Preliminary studies indicate that 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine is promising a compound with anti-TB activity has also been investigated for the minimal inhibitory concentration (MIC) of this compound against some strains of mycobacteria (*Mycobacterium bovis* and *M. fortuitum*). Thus, the aim of our further work was to study some toxicity parameters of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1, 3,4]thiadiazi-

ne when administered subcutaneously to guinea pigs, which is a necessary condition for further implementation in practice of this compound.

Materials and methods

The research was conducted in accordance with the Council of Europe's Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18 March 1986, Directive 2010/63 of the European Parliament and the Council of Europe of 22 September 2010 on the Protection of Animals used for Scientific Purposes and the Law of Ukraine of February 21, 2006 No. 3447-IV (as amended on June 22, 2017 No. 2120-VIII) "On protection of animals from cruel treatment". The guinea pigs were housed in cages (one guinea pig per cage) for adaptation under controlled light, temperature and humidity conditions with free access to water and food.

Acute toxicity of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine was determined on 12 nonlinear guinea pigs of both sexes with an average weight of 250 g. 4 groups of animals were formed, 3 animals in each group:

Group 1: subcutaneous administration of the studied compound at a dose of 20 mg/kg of animal weight – double the dose for treatment of tuberculosis;

Group 2: at a dose of 40 mg/kg of animal weight;

Group 3: use of the studied compound in a dose of 80 mg/kg of animal weight; Control group: clinically healthy animals (KZD) – subcutaneous injection of isotonic sodium chloride solution (6 ml/kg).

Evaluation of the action of the studied compound was performed on the following indicators:

a) mortality (terms of death of animals in each group, daily);

b) assessment of toxicity (daily), including assessment of the appearance of the injection site (presence of irritation, redness, edema);

c) the dynamics of changes in body weight (in the initial state at 4, 7 and 14 days after administration);

d) macroscopy of internal organs, mass coefficients of internal organs in guinea pigs (14 days).

Chronic toxicity of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine was determined in 12 guinea pigs on average weighing 250 g. Four groups of animals were formed, 3 animals in each group, which were reinjected with the studied compound in doses, as in the experiment, studying acute toxicity for 3 months.

Toxicity of the studied compound was studied for irritation, emotional and behavioral reactions of laboratory animals and we determined the coefficients of mass of internal organs of animals with prolonged administration of this compound.

The animals were kept under standard conditions. Experiments performed on live vertebrates were in line with the principles of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986) (Stephanov, 2001).

The content of total protein in the blood serum was examined by the biuret method, the concentration of albumin by the method of B. Doumas et al., The content of residual nitrogen was measured by the color reaction with Nesler's reagent, the nitrogen concentration of free amino acids by the method of GA Uzbekova in the modification of ZS Chulkova, urea content – according to Marsh, creatinine concentration – according to Popper's method, activity of ALT and AST – according to Reitman and Frenkel.

Results

After administration of the studied compound, the animals were observed for 14 days and assessed their general condition, mortality, weight dynamics. Acute toxicity of the studied compound when administered subcutaneously to guinea pigs in different doses is presented in Table 1.

As a result, the study found that after a single subcutaneous injection of the studied compound into animals of the experimental groups, changes in behavior and vital functions did not occur. Also, neither signs of intoxication nor death of animals were observed, except for group III – 1 died. Thus the LD_{50} for the studied compound in the acute phase toxicity was determined at 40.0 mg/kg animal weight. According to the method of studying acute toxicity, to assess the toxic effects of potential drugs on the body of mammals we studied the dynamics of body weight of animals of all groups (Fig. 1).

Table 1

Acute toxicity of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine (n = 10)

Group of animals	Dose, mg/kg	Number of animals killed	Number of surviving animals	Mortality, %
Control	0.0	0	3	0
I	20.0	0	3	0
II	40.0	0	3	0
III	80.0	1	2	33.3

We determined that the animals in groups I and II on the 7th day of observation did not differ from the control, and on the 14th day slightly lagged behind in body weight by 6.6 and 7.5 g, respectively. In animals of group III, on the contrary, body weight on the 7th day of observation was higher than control by 3.3; and on the 14th day – by 31.4 g ($P < 0.05$), respectively. On the 14th day of observation, all animals had a neat coat, unchanged mucous membranes of natural openings. Subcutaneous lymph nodes were normal in size and touch. The results of morpho-biochemical parameters of the blood of guinea pigs in the long-term introduction of the studied compound are shown in tables 2 and 3.

Most plasma proteins are synthesized by the liver. Note the increase in total protein in the third experimental group after the use of the studied compound in comparison with I, II and intact group of animals by 10.8%, 18.9% ($P < 0.05$) and 7.9% due to globulins by 22.3% ($P < 0.05$), 28.2% ($P < 0.05$) and 15.0% ($P < 0.05$), respectively. There is also a sharp decrease in the protein coefficient in the third experimental group by 30.8% ($P < 0.05$) compared to animals of group II 23.1% ($P < 0.05$) and 15.4% in control.

AST in animals of experimental group III with long-term administration of the studied compound exceeded intact guinea pigs in 1.8 times ($P < 0.05$). Aspartate aminotransferase catalyzes the formation of glutamic acid from 2-oxoglutarate by amino group transfer. AST in the maximum concentration is in the liver, heart and skeletal muscles. In our opinion, the increase in the content of serum AST concentration in group III animals by 3.3 times ($P < 0.05$) is associated with dysfunctional changes in the liver.

After the use of the studied compound in group III guinea pigs there was an increase in alkaline phosphatase by 1.7 times ($P < 0.01$), which is 4.6 ($P < 0.01$); 11.1 ($P < 0.001$) and 2.9 times higher than in I, II and controls, respectively. Alkaline phosphatase catalyzes the hydrolysis of organic phosphate monoethers in an alkaline medium. The enzyme is present in almost all tissues of the body, especially in cell membranes, and is found in particularly high concentrations in the placenta, intestinal epithelium, renal tubules, osteoblasts and liver. Increased alkaline phosphatase in group III may be associated with disorders of the hepatobiliary system – hepatotoxicity caused by the studied compound.

Urea is synthesized in the liver as a by-product in the amino acid deamination reaction. Its elimination in the urine is the main route of nitrogen excretion. The reduced concentration of urea in the plasma of group III guinea pigs is a consequence of reduced protein catabolism and weak dehydration. This figure is higher than in groups I, II and intact animals by 11.8%, 2.9% and 17.6% ($P < 0.05$), respectively.

Plasma creatinine concentration determines the rate of glomerular filtration – an integral indicator of renal excretory function. We note an increase in the amount of creatinine in animals in the III experimental group in comparison with group I, II and intact animals in 2.6 ($P < 0.05$), 3.2 ($P < 0.01$) and 2.6 times ($P < 0.05$), respectively. An increase in this indicator indicates the presence of an inflammatory reaction in the body of group III.

Biochemical parameters of blood in animals of I and II experimental groups are within the physiological norm (Table 3). Analyzing the morphological parameters of guinea pigs' blood with long-term administration of the studied compound, we note an increase in the number of leukocytes in animals of group III compared with I, II and intact guinea pigs in 1.9; 1.5 and 1.4 times due to rod-shaped neutrophils by2.0 ($P < 0.05$), 1.9 ($P < 0.05$) and 2.0 times ($P < 0.05$) on the background of a decrease in lym-

phocytes by 2.3 ($P < 0.05$); 2.1 ($P < 0.05$) and 2.0 ($P < 0.05$) times, respectively. There was also a decrease in the number of monocytes in 3.3 ($P < 0.05$), 2.2 ($P < 0.05$) and 2.0 ($P < 0.05$) times, respectively.

Mass coefficients of the internal organs of guinea pigs with prolonged administration of the studied compound are shown in Table 4. Mass coefficients (MC) – the percentage of organ mass to body weight, an integral indicator that was used to assess the condition of internal organs. The calculation of mass coefficients was determined by the formula:

MC = Body weight (g) / body weight (g) × 100%

We determined an increase in all indicators of internal organs in animals of the III experimental group in comparison with I, II groups and control, especially the mass coefficient of the liver is higher by 12.6%, 8.1% and 9.1%, as well as the spleen – by 31.0% ($P < 0.05$), 27.6% ($P < 0.05$) and 31.0% ($P < 0.05$), respectively. Analysis of this indicator in toxicological studies makes it possible to identify the target organ of the toxicant, to identify signs of endocrine-related effects.

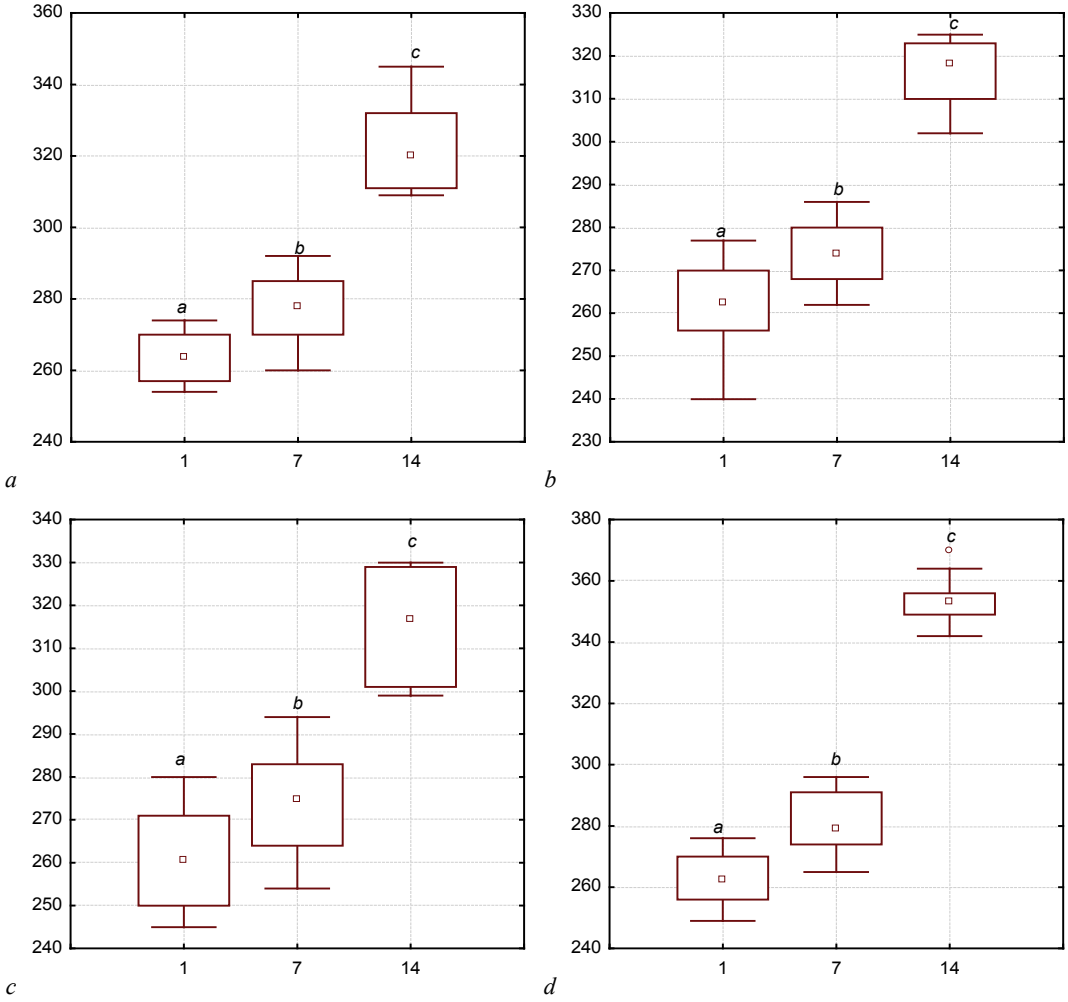

Fig. 1. Dynamics of animal body weight, g): *a* – control; *b* – I group; *c* – II group; *d* – III I group (n = 10)

Table 2

Biochemical parameters of guinea pigs blood with long-term administration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine (n = 10, x ± SD)

Indexes	Group of animals							
	control		I		II		III	
	before	after	before	after	before	after	before	after
Total protein, g/L	54.44 ± 3.13^{a}	58.11 ± 2.54^{a}	54.04 ± 2.93^{a}	56.33 ± 3.42^{a}	53.81 ± 3.93^{a}	51.24 ± 3.52^{a}	54.91 ± 3.34^{a}	63.13 ± 4.11^{b}
Albumins, g/L	33.02 ± 3.31^{b}	35.14 ± 4.13^{c}	33.21 ± 3.14^{b}	$35.22 \pm 2.7I^{c}$	33.14 ± 3.42^{b}	31.48 ± 3.67^{a}	32.96 ± 2.85^{b}	36.24 ± 3.73^{d}
Globulins, g/L	21.42 ± 3.44^{a}	22.97 ± 2.96^{b}	20.83 ± 1.46^{a}	21.11 ± 1.87^{a}	20.67 ± 3.26^{a}	19.76 ± 2.89^{a}	21.95 ± 3.73^{a}	26.89 ± 4.16^{c}
Protein coefficient, units	1.54 ± 0.18^{b}	1.52 ± 0.37^{b}	1.59 ± 0.34^{b}	1.67 ± 0.29^{c}	1.60 ± 0.21^{b}	1.59 ± 0.42^{b}	1.50 ± 0.33^{b}	1.35 ± 0.25^{a}
Urea, mmol/L	3.81 ± 0.48^{a}	4.02 ± 0.82	4.81 ± 0.37	3.93 ± 0.92^{a}	3.81 ± 0.46^{a}	3.52 ± 0.54^{a}	3.82 ± 0.29^{a}	3.43 ± 0.55^{a}
Urea nitrogen, mg%	6.51 ± 0.82^{a}	6.73 ± 0.88^{a}	6.52 ± 0.94^{a}	6.71 ± 1.04^{a}	6.22 ± 0.77^{a}	6.34 ± 1.25^{a}	6.41 ± 0.67^{a}	6.52 ± 1.30^{a}
Creatinine, μmol/L	61.24 ± 3.92^{a}	79.23 ± 6.51^{b}	67.13 ± 3.22^{a}	79.01 ± 4.3^{b}	65.63 ± 6.32^{a}	93.22 ± 9.61^{c}	63.94 ± 5.43^{a}	204.01 ± 11.42^{d}
AST, U/L	79.13 ± 6.11^{b}	57.52 ± 7.34^{a}	78.73 ± 7.41^{b}	$101.43 \pm 6.82^{*}$	82.61 ± 8.14^{b}	58.23 ± 7.81^{a}	79.54 ± 5.82	$104.91 \pm 8.44^{*}$
ALT, U/L	72.73 ± 6.94^{b}	39.62 ± 8.13^{a}	80.12 ± 5.71^{b}	47.62 ± 6.34^{a}	74.66 ± 7.27^{b}	41.83 ± 6.92^{a}	75.31 ± 6.84^{b}	131.28 ± 5.49^{c}
De Ritis Index (AST/ALT), units	1.09 ± 0.18^{a}	1.45 ± 0.18^{b}	0.98 ± 0.23^{a}	2.13 ± 0.29^{c}	1.11 ± 0.17^{a}	1.39 ± 0.41^{b}	1.06 ± 0.17^{a}	0.80 ± 0.08^{a}
Alkaline phosphatase, U/L	200.6 ± 11.7^{c}	140.5 ± 14.6^{b}	186.6 ± 14.2^{c}	88.3 ± 15.9^{a}	214.8 ± 12.4^{d}	136.5 ± 12.9^{b}	240.1 ± 13.4^{d}	406.9 ± 21.7^{e}
Total bilirubin, μmol/L	3.42 ± 0.48^{b}	4.01 ± 0.44^{c}	3.44 ± 0.62^{b}	2.21 ± 0.39^{a}	3.53 ± 0.57^{b}	4.41 ± 0.22^{c}	3.54 ± 0.55^{b}	9.82 ± 1.60^{d}
Glucose, mmol/L	5.83 ± 0.76^{c}	2.02 ± 0.30^{a}	4.81 ± 0.56^{b}	1.60 ± 0.72^{a}	5.53 ± 0.68^{c}	2.21 ± 0.11^{a}	6.02 ± 0.08^{c}	4.01 ± 0.82^{b}
Calcium, mmol/L	3.30 ± 0.54^{b}	3.04 ± 0.29^{b}	3.24 ± 0.62^{b}	2.90 ± 0.43^{a}	3.12 ± 0.39^{b}	3.31 ± 0.44^{b}	3.22 ± 0.62^{b}	2.13 ± 0.39^{a}
Inorganic phosphorus, mmol/L	3.70 ± 0.48^{b}	4.41 ± 0.32^{c}	3.93 ± 0.38^{b}	4.51 ± 0.64^{c}	3.62 ± 0.71^{b}	4.34 ± 0.42^{c}	3.82 ± 0.28^{b}	2.74 ± 0.57^{a}
Ca/P, un	0.89 ± 0.27^{c}	0.69 ± 0.17^{a}	0.82 ± 0.19^{c}	0.64 ± 0.17^{a}	0.86 ± 0.15^{c}	0.76 ± 0.17^{b}	0.84 ± 0.24^{c}	0.78 ± 0.18^{b}
Cholesterol, mmol/L	1.56 ± 0.28^{c}	1.93 ± 0.19^{e}	1.64 ± 0.33^{d}	1.63 ± 0.42^{d}	1.54 ± 0.37^{c}	1.40 ± 0.45^{b}	1.63 ± 0.38^{d}	0.92 ± 0.28^{a}

Note: different letters within a row indicate that data samples significantly ($P < 0.05$) differ one from another according to the results of the Tukey test with the Bonferroni correction.

Table 3
Morphological parameters of guinea pigs blood with long-term administration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4] -triazolo- [3,4-b] [1,3,4] thiadiazine (n = 10, x ± SD)

Indexes	Group of animals							
	control		I		II		III	
	before	after	before	after	before	after	before	after
Hemoglobin, g/L	118.9 ± 11.4^{b}	122.3 ± 9.4^{b}	118.5 ± 8.6^{b}	112.4 ± 12.3^{b}	109.4 ± 9.5^{b}	82.3 ± 12.5^{a}	115.3 ± 9.8^{b}	151.8 ± 8.7^{d}
Hematocrit, %	36.52 ± 3.71^{b}	39.91 ± 4.24^{c}	37.87 ± 4.46^{b}	37.51 ± 5.24^{b}	36.04 ± 3.92^{b}	28.11 ± 3.32^{a}	36.94 ± 3.52^{b}	46.93 ± 4.84^{d}
Erythrocytes, 10^{12}/L	3.94 ± 0.13^{b}	4.16 ± 0.65^{c}	4.52 ± 0.33^{d}	3.51 ± 0.44^{b}	4.63 ± 0.62^{d}	2.71 ± 0.43^{a}	4.22 ± 0.50^{c}	4.53 ± 0.42^{d}
ESR, mm/g	1.04 ± 0.13^{b}	1.02 ± 0.11^{b}	1.01 ± 0.12^{b}	0.96 ± 0.10^{a}	1.03 ± 0.11^{b}	0.91 ± 0.14^{a}	1.02 ± 0.13^{b}	0.93 ± 0.12^{a}
Platelets, 10^{9}/L	269.0 ± 13.6^{b}	279.0 ± 13.4^{b}	280.1 ± 13.2^{b}	380.1 ± 21.3^{c}	283.6 ± 14.7^{b}	240.6 ± 14.4^{a}	281.3 ± 12.7^{b}	490.7 ± 12.3^{d}
Leukocytes, 10^{9}/L	4.93 ± 0.37^{b}	5.22 ± 0.18^{c}	5.51 ± 0.24^{c}	3.94 ± 0.18^{a}	4.92 ± 0.44^{b}	5.13 ± 0.28^{c}	4.81 ± 0.34^{b}	7.52 ± 0.19^{d}
Leukocyte formula, %								
Basophils	0	0	0	0	0	0	0	0
Eosinophils	0 ± 0	0 ± 0	0 ± 0	1.01 ± 0.12	1.02 ± 0.10	2.03 ± 0.11	1.01 ± 0.12	1.03 ± 0.11
Neutrophils								
Myelocytes	0	0	0	0	0	0	0	0
Young	0	0	0	0	0	0	0	1
Stick-core	1.62 ± 0.14^{b}	1.51 ± 0.15^{a}	1.61 ± 0.14^{b}	1.54 ± 0.10^{a}	1.72 ± 0.19^{c}	1.60 ± 0.26^{b}	1.52 ± 0.11^{a}	3.04 ± 0.223^{d}
Segmental	35.36 ± 3.47^{b}	24.02 ± 4.32^{a}	35.6 ± 4.7^{b}	27.02 ± 2.71^{a}	36.01 ± 3.64^{b}	30.02 ± 4.63^{b}	36.11 ± 4.73^{b}	34.02 ± 5.11^{b}
Lymphocytes	74.04 ± 7.52^{b}	71.31 ± 6.73^{b}	75.31 ± 6.42^{b}	81.02 ± 7.24^{c}	76.53 ± 5.81^{b}	75.02 ± 10.56^{b}	70.80 ± 8.42^{b}	35.05 ± 8.67^{a}
Monocytes	5.32 ± 0.36^{c}	3.51 ± 1.40^{b}	5.12 ± 0.34^{c}	2.14 ± 0.38^{a}	5.01 ± 0.42^{c}	3.23 ± 0.36^{b}	5.51 ± 0.41^{c}	7.04 ± 0.18^{d}

Note: see Table 2.

Table 4
Mass coefficients of the internal organs of guinea pigs with prolonged administration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4] -triazolo- [3,4-b] [1,3,4] thiadiazine (x ± SD)

Indexes	Group of animals			
	control	I	II	III
Heart	0.442 ± 0.051^{a}	0.455 ± 0.014^{a}	0.453 ± 0.041^{a}	$0,450 \pm 0.013^{a}$
Lungs	1.286 ± 0.064^{b}	1.173 ± 0.021^{a}	1.306 ± 0.022^{c}	$1,160 \pm 0.032^{a}$
Liver	5.401 ± 0.543^{a}	5.193 ± 0.322^{a}	5.464 ± 0.718^{a}	5.941 ± 0.542^{b}
Spleen	0.203 ± 0.032^{a}	0.216 ± 0.041^{a}	0.212 ± 0.031^{a}	0.292 ± 0.013^{b}
Kidneys	0.924 ± 0.111^{b}	0.807 ± 0.014^{a}	0.928 ± 0.145^{b}	0.941 ± 0.064^{b}

Discussion

Current developments in the pharmaceutical industry in Ukraine necessitate the introduction of novel, highly efficient, and minimally toxic medications. Presently, derivatives of 1,2,4-triazole maintain a prominent position amid a vast array of biologically active substances. For several decades, experts from not only the pharmaceutical industry but various fields have been captivated by this class of heterocyclic compounds. It is widely acknowledged that compounds resulting from the combination of the 1,2,4-triazole nucleus with diverse functional substituents exhibit noteworthy biological activity, low toxicity, and heightened reactivity (Gao et al., 2019; Wen et al., 2020; Pachuta-Stec, 2022).

A well-known fact is the high biological activity of compounds formed by the combination of the 1,2,4-triazole nucleus and various functional substituents. Low toxicity, reactivity, and high biological activity of 1,2,4-triazole derivatives make this class of compounds very attractive. Over the past years, a whole series of new drugs and fertilizers have been registered in Ukraine, the active substances of which are derivatives of 1,2,4-triazole. All this in the complex indicates that further studies of new substituted 1,2,4-triazole are promising both theoretically and practically (Küçükgüzel & Çıkla-Süzgün, 2015; Cao & Lu, 2017; Li & Zhang, 2022; Nasri et al., 2022).

Over the last five years, a series of newly registered veterinary drugs and fertilizers in Ukraine have featured active substances derived from 1,2,4-triazole (e.g., Avistem RP AB-05365-01-14, Trifuzol RP AB-05486-01-14, "Trifuzol-Neo", RP AV-07793-01-18, "Fortis Combi", RP A 06016). This collective evidence underscores the promising nature of further research into new substituted 1,2,4-triazoles, aligning with both theoretical and practical interests. It is noteworthy that specific substituted 1,2,4-triazoles possess the capacity to impede the growth of mycobacteria (Bihdan et al., 2019; Gotsulya et al., 2020).

In connection with the increase in the number of medicines and their high activity, which can be accompanied by the occurrence of adverse reactions that differ in manifestation and degree of severity, the problem of safety research arises in potential drugs. One of the requirements for the potential of medicines is the ratio between their effectiveness and toxicity. Most unwanted manifestations are possible to predict, based on experimental studies, which largely allow one to guarantee safety of clinical trials and subsequent medical use of new drugs. Studying the safety of potential drugs, particularly acute toxicity, is one of the most important and necessary stages in developing new drugs (Katsaraba et al., 2022; Martyshuk et al., 2022; Sameliuk et al., 2022).

Based on the conducted studies, it was established that when the dose of the compound was increased to 80 mg/kg of the animal's weight, changes in internal organs were noted in guinea pigs, which indicates their functional stress with signs of the onset of exhaustion and endothelial dysfunction. The given examples represent the results of a comparative analysis of the study of chronic toxicity based on histological studies of signs of non-specific changes in the organs of guinea pigs during long-term administration of the compound 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine (Karpenko et al., 2022).

It was established that subcutaneous administration of the compound 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine in doses of 20 and 40 mg/kg of animal weight (two- and four-fold dose) leads to the absence of nonspecific manifestations of inflammation in the lungs, liver, kidneys, and myocardium. Thus, 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine can be used as a tuberculocide with a level of safety up to 4 times the dose.

It has been proven that the use of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine on guinea pigs to 4-fold administration affected biochemical indicators and mass coefficients of internal organs. The specified indicators in the animals were within the physiological control of the intact group.

Conclusions

We have determined the safety of the studied compound in guinea pigs to 4-fold injection. The biochemical parameters and mass coefficients of internal organs in animals were within the physiological control of the intact group. Thus, 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-(1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine) can be recommended for further in-depth studies as a compound with a safety level of up to 4 times the dose.

References

Bihdan, O., Parchenko, V., Zazharskyi, V., Fotina, T., & Davydenko, P. (2019). Studying of physico-chemical properties of 5-(2-,3-fluorophenyl)-4-((arylgeteryl)yliden)amino-1,2,4-triazole-3-thiols and any of their retrievalpro-

ducts. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 10(1), 464–474.
Cao, Y., & Lu, H. (2017). Advances in the application of 1,2,4-triazole-containing hybrids as anti-tuberculosis agents. Future Medicinal Chemistry, 13(23), 2107–2124.
Doherty, W., Adler, N., Knox, A., Nolan, D., McGouran, J., Nikalje, A. P., & Evans, P. (2017). Synthesis and evaluation of 1,2,3-triazole-containing vinyl and allyl sulfones as anti-trypanosomal agents. European Journal of Organic Chemistry, 2017(1), 175–185.
Gao, F., Wang, T., Xiao, J., & Huang, G. (2019). Antibacterial activity study of 1,2,4-triazole derivatives. European Journal of Medicinal Chemistry, 173, 274–281.
Gotsulya, A. S., Zazhzharskiy, V. V., Davidenko, P. O., Zazhzharskaya, N. M., Kulishenko, O. M., Panasenko, O. I., Gutyj, B. V., Pryima, O. B., Mazur, I. Y., Pritsak, V. V., Drachuk, U. R., Sobolta, A. G., & Riy, M. B. (2020). Features of experimental modeling of tuberculosis in guinea pig with the participation of N'-(2-(5-((thephylline-7'-yl)methyl)-4-R-1,2,4-triazole-3-ylthio) acethyl)isonicotinohydrazide. Ukrainian Journal of Ecology, 10(4), 191–194.
Karpenko, Y., Hunchak, Y., Gutyj, B., Hunchak, A., Parchenko, M., & Parchenko, V. (2022). Advanced research for physico-chemical properties and parameters of toxicity piperazinium 2-((5-(furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetate. ScienceRise: Pharmaceutical Science, 36, 18–25.
Katsaraba, O. A., Sachuk, R. M., Gutyj, B. V., Velesyk, T. A., Radzykhovskyi, M. L., Sharandak, P. V., & Pepko, V. O. (2022). Pharmacological studies of the veterinary medicinal product "Dibutalastin Ointment". Ukrainian Journal of Veterinary and Agricultural Sciences, 5(2), 43–48.
Khanage, S. G., Mohite, P. B., Pandhare, R. B., & Raju, A. S. (2012). Synthesis and antimicrobial activity evaluation of some substituted N'[arylidene]-2-(3-methyl-5-pyridin-4-yl-1H-1,2,4-triazol-1-yl)acetohydrazide compounds. Biointerface Research in Applied Chemistry, 2(5), 417–423.
Khanapurmath, N., Kulkarni, M. V., Joshi, S. D., & Kumar, G. N. A. (2019). A click chemistry approach for the synthesis of cyclic ureido tethered coumarinyl and 1-aza coumarinyl 1,2,3-triazoles as inhibitors of *Mycobacterium tuberculosis* H37Rv and their *in silico* studies. Bioorganic and Medicinal Chemistry, 27(20), 115054.
Küçükgüzel, Ş. G., & Çıkla-Süzgün, P. (2015). Recent advances bioactive 1,2,4-triazole-3-thiones. European Journal of Medicinal Chemistry, 97, 830–870.
Li, J., & Zhang, J. (2022). The antibacterial activity of 1,2,3-triazole- and 1,2,4-triazole-containing hybrids against *Staphylococcus aureus*: An updated review (2020 – Present). Current Topics in Medicinal Chemistry, 22(1), 41–63.
Martyshuk, T., Gutyj, B., Vyshchur, O., Paterega, I., Kushnir, V., Bigdan, O., Bushueva I., Parchenko, V., Mykhailiuk, E., Aleksieiev, O., & Tkachenko, N. (2022). Study of acute and chronic toxicity of "Butaselmevit" on laboratory animals. Archives of Pharmacy Practice, 13(3), 70–75.
Nasri, S., Bayat, M., & Kochia, K. (2022). Strategies for synthesis of 1,2,4-triazole-containing scaffolds using 3-amino-1,2,4-triazole. Molecular Diversity, 26(1), 717–739.
Neofytos, D., Avdic, E., & Magiorakos, A. P. (2010). Clinical safety and tolerability issues in use of triazole derivatives in management of fungal infections. Drug, Healthcare and Patient Safety, 2, 27–38.
Pachuta-Stec, A. (2022). Antioxidant activity of 1,2,4-triazole and its derivatives: A mini-review. Mini-Reviews in Medicinal Chemistry, 22(7), 1081–1094.
Palchykov, V. A., Zazharskyi, V. V., Brygadyrenko, V. V., Davydenko, P. O., Kulishenko, O. M., & Borovik, I. V. (2020). Chemical composition and antibacterial effect of ethanolic extract of *Buxus sempervirens* on cryogenic strains of microorganisms *in vitro*. Chemical Data Collections, 25, 100323.
Sameliuk, Y., Kaplaushenko, A., Nedorezanıuk, N., Ostretsova, L., Diakova, F., & Gutyj, B. (2022). Prospects for the search for new biologically active compounds among the derivatives of the heterocyclic system of 1,2,4-triazole. Hacettepe University Journal of the Faculty of Pharmacy, 42(3), 175–186.
Stephanov, O. V. (2001). Preclinical studies of drugs [Preclinical studies of medicines]. Avicenna, Kyiv (in Ukrainian).
Tan, Z., Deng, J., Ye, Q., & Zhang, Z. (2021). Triazole-containing hybrids with anti-*Mycobacterium tuberculosis* potential – Part I: 1,2,3-triazole. Future Medicinal Chemistry, 13(7), 643–662.
Wen, X., Zhou, Y., Zeng, J., & Liu, X. (2020). Recent development of 1,2,4-triazole-containing compounds as anticancer agents. Current Topics in Medicinal Chemistry, 20(16), 1441–1460.
Xu, Z., Song, X. F., Qiang, M., & Lv, Z. S. (2017). 1H-1,2,3-triazole-tethered 8-OMe ciprofloxacin and isatin hybrids: Design, synthesis and *in vitro* anti-mycobacterial activities. Journal of Heterocyclic Chemistry, 54, 3735–3761.
Zazharskyi, V. V., Davydenko, P. O., Gavrilina, O. G., Parchenko, V. V., & Gotsulya, A. S. (2018). Experimental modeling of tuberculosis and its features in guinea pigs with the introduction of isoniazid and GKP-305. Scientific and technical bulletin of the State Research Control Institute of Veterinary Drugs and Feed Additives and the Institute of Animal Biology, 19, 227–239.
Zazharskyi, V., Davydenko, P., Kulishenko, O., Borovik, I., & Brygadyrenko, V. (2019). Antimicrobial activity of 50 plant extracts. Biosystems Diversity, 27(2), 163–169.