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ORIGINAL ARTICLE

Experimental simulation of tuberculosis and its features in rabbits under conditions of isoniazid and *N*'-(2-(5-((theophyline-7-yl) methyl)-4-ethyl)-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrozide

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The results of morpho-biochemical studies of tuberculosis inflammation in rabbits in an experimental model with comparative isoniazid treatment and N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide. It was found that subcutaneous administration of N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide at a dose of 10 mg/kg body weight leads to the absence of specific and nonspecific manifestations of inflammation in the lungs, liver, kidneys and spleen. The calculated value of the drug indicates a high degree of safety N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotino-hydrazide and its prospects for veterinary practice as an effective and safe tuberculocidal drug
Keywords: tuberculosis, experimental model, rabbits, treatment, 1,2,4-triazole.

Introduction

About one-third of the world's population is infected with tuberculosis bacteria. The disease develops only in a small proportion of infected people. People with a weakened immune system are at increased risk of developing tuberculosis. The likelihood of active tuberculosis in a person with HIV is approximately 26-31 times higher (Bhandari & Thoen, 2014; Gotsulya et al., 2020; Zazharskyi et al., 2020).

About a quarter of the world's population has latent tuberculosis. This means that people are infected with tuberculosis bacteria but have not (yet) contracted the disease and cannot transmit it. The risk that people infected with tuberculosis bacteria will develop tuberculosis during their lifetime is 5-15 %. A person with tuberculosis can infect up to 10-15 other people with whom he has close contact during a year. Without proper treatment, an average of 45 % of HIV-negative people with tuberculosis and almost all HIV-positive people with tuberculosis will die (Leveri et al., 2019).

One of the goals set by Ukraine for the period up to 2030 is to eradicate the tuberculosis epidemic. "Tuberculosis eradication strategy", developed by WHO and approved by the World Health Assembly in 2014, calls for reducing TB rates by 80 % and mortality rates by 90 % by 2030 compared to 2015 levels (Alihalassa, 2018; Zazharskyi et al., 2019; Bihdan, 2019).

Tuberculosis is present all over the world. In 2017, the highest number of new tuberculosis cases occurred in the Southeast Asian and Western Pacific regions, accounting for 62 % of new cases. In the African region, 25 % of new cases were reported. Thus, 87 % of new cases of tuberculosis occurred in 30 countries with severe tuberculosis. Eight countries - India, China, Indonesia, Philippines, Pakistan, Nigeria, Bangladesh, and South Africa - have accounted for two-thirds of new tuberculosis cases (Mozaffari, 2018).

Anti-TB drugs have been used for many decades. In each country where the study is conducted, registered strains resistant to one or more drugs. Drug resistance occurs with improper use of anti-TB drugs, misuse by health care providers, and poor quality of drugs or early discontinuation of treatment by patients (Prasad & Gupta, 2015).

Multidrug-resistant tuberculosis (MDR-TB) is a form of tuberculosis in which the mycobacterium does not respond at least to isoniazid and rifampicin, the two most potent first-line anti-TB drugs. MLS-TB can be treated and cured using second-line drugs. However, such treatment options are limited and require extensive chemotherapy (treatment lasting up to two years) with the high cost and toxicity of drugs.

In some cases, more severe drug resistance may develop. Extensively drug-resistant tuberculosis (XDR-TB) is a more complicated form of MDR-TB. Bacteria that do not respond to the most effective second-line anti-TB drugs cause this form (Palchykov et al., 2020).

Today, chemoresistant tuberculosis has become widespread in the epidemic. Aggressive properties, high viability, and drug resistance characterize its pathogen. The virulence factors of *Mycobacterium bovis* are unique properties that allow it to infect, survive, multiply, and cause disease in host animals. Most of the virulence factors of *M. bovis* are the same as those of the classic human tuberculosis microorganism, *Mycobacterium tuberculosis*, since both can cause identical clinical diseases in humans and are genetically very similar (Collins, 2001).

Materials and methods

The tuberculocidal action of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide was determined in 12 rabbits with an average weight of 2.2 kg. 4 groups of 3 animals in each were formed:

-I group: subcutaneous administration of isoniazid at a dose of 10 mg/kg body weight;

-II group: subcutaneous administration of N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide at a dose of 10 mg/kg animal weight;

-III group: a control group of animals that were artificially infected with the pathogenic strain of *M. bovis* without further treatment;

-IV group: control group (clinically healthy animals) injected subcutaneously with isotonic sodium chloride solution (6 ml/kg).

Evaluation of the effect of the substance under study was carried out on the following indicators:

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

b) 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 for 1, 7, 14, 30, 60, 90 days after administration);

d) macroscopic examination and mass coefficients of internal organs of rabbits (90 days).

All animals were maintained under standard laboratory 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).

The study of blood biochemical parameters was performed using photometers "Microlab-200" and "Vitalab Eclipse" (Merck, Germany) with software after reaction using the appropriate diagnostic test kit "Lachema" (Czech Republic).

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 residual nitrogen content by the color reaction with Nessler's reagent, the nitrogen concentration of free amino acids by the method of G. A. Uzbekova in the modification of Z. S. Chulkova, urea content - according to Marsh, creatinine concentration - according to Popper's method, the activity of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) - according to Reitman and Frankel. The state of hematopoiesis was assessed by the total number of erythrocytes, leukocytes (*in vitro* method) - in a chamber with a Goryaev grid. The content of hemoglobin in the blood was determined by the hemoglobin cyanide method. The data in the tables are presented as $x \pm 1.96 \times SD$.

Results and discussion

The method of studying the therapeutic efficacy of the effects of drugs on the body of mammals studied the dynamics of the body weight of rabbits of all groups (table 1).

Table 1. Dynamics of body weight of animals (rabbits)

Group of animals	Observation days / Body weight, kg					
	1	7	14	30	60	90
I	2.2±0.1	2.4±0.15	2.6±0.13	2.8±0.12	3.25±0.21	3.6±0.3***
II	2.15±0.14	2.5±0.1	2.7±0.1	2.9±0.14	3.5±0.19	4.0±0.24***
III	2.3±0.16	2.2±0.23	2.2±0.23	2.4±0.21	1.9±0.25	-
IV	2.1±0.14	2.3±0.24	2.45±0.21	2.6±0.31	2.9±0.23	3.5±0.29***

* (P<0.05); ** (P<0.01); *** (P<0.001).

Note that body weight exceeds rabbits of the I and intact groups in animals of the II experimental group during the entire observation period. This indicator was higher on day 7 (4.2 %; 8.7 %), 14 (3.8 %; 10.2 %), 30 (3.6 %; 11.5 %), 60 (7.7 %; 20.7 %) and 90 days (11.1 %; 14.3 %). This, in our opinion, can be caused by the ability of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide to participate in the redox processes of the macroorganism during the disease. On day 90 of the experiment, the body weight of group II rabbits exceeded the intact control by 0.5 kg ($P < 0.01$). The body weight of the control groups, artificially infected with the pathogenic strain of *M. bovis* for 30 days, ranged from 2.3 to 2.4 kg, with a sharp decrease by 60 days to 1.9 kg ($P < 0.05$) and subsequent death of animals.

The study of morpho-biochemical parameters of the blood of rabbits with long-term administration of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)-isonicotinohydrazide are shown in table 2 and table 3.

Table 2. Biochemical parameters of rabbit blood under conditions of long administration of *N*'-(2-(5-((theophyllin-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide

Parameter	Group of animals/days of observation											
	I			II			III			IV		
	1	60	90	1	60	90	1	60	90	1	60	90
Total protein, g/l	70.4±8.9	79.3±9.4	65.1±7.5	72.4±7.9	83.5±11.3	67.5±8.8	73.2±9.2**	33.1±5.3	-	70.1±6.9	69.0±8.3	64.2±7.3
Albumins, g/l	43.2±4.6	43.2±5.3	44.3±4.7	43.1±6.2	47.5±5.8	44.5±4.9	43.1±6.1**	22.2±4.1	-	42.6±7.8	40.2±6.8	43.1±7.3
Globulins, g/l	27.2±3.6	36.1±4.2**	20.8±1.9	29.4±2.2	36.0±2.9**	23.0±1.8	30.2±2.5***	11.3±0.7	-	27.5±3.1	28.8±2.8	21.1±1.9
Protein coefficient, units	1.6±0.12	1.2±0.11	2.1±0.21	1.5±0.13	1.3±0.1	1.9±0.22	1.4±0.14	2.1±0.31	-	1.5±0.19	1.4±0.14	2.1±0.17
Urea, mmol/l	3.6±0.65	3.6±0.54	4.5±0.78	3.6±0.94	3.8± 0.79	3.8±0.64	3.6±0.87	5.4±0.89	-	3.6±0.85	3.5±0.77	3.7± 0.84
Urea nitrogen, mg%	6.9±1.1	6.9±0.98	8.6±1.3	7.1±1.2	7.4±1.3	8.1±1.2	6.5±1.7	10.3±1.4	-	7.1±0.88	6.9±1.3	7.1± 1.2
Creatinine, μmol/l	110.1±16.3	138.5±15.9	101.5±12.7	151.2±18.7	185.1±21.3***	102.5±16.8	117.3±16.4	104.3±12.9	-	112.1±13.7	110.4±12.6	102.4±14.1
AST, units/l	190.3±18.7**	121.2±12.9	11.8±1.8***	180.2±24.7***	104.5±15.3***	43.1±14.8	187.3±21.7***	21.1±2.7	-	156.2±21.8***	58.4±5.9***	88.3± 8.6***
ALT, units /l	78.2±18.6	70.5±19.1	45.2±8.7	80.4±23.8	71.7±22.8	48.5±15.9	90.2±19.7***	23.2±1.8	-	80.1±17.5	71.3±14.7	68.1±13.8
De Ritis ratio, units	2.4±0.12***	1.7±0.35	0.3±0.12	2.2± 0.22***	1.5±0.19	0.9±0.1	2.1±0.16***	0.9±0.12	-	2.0±0.32	0.8±0.1	1.3±0.12
Alkaline phosphatase, units/l	220.1±28.5***	207.4±24.6	84.2±15.8	214.5±19.7***	215.5±18.6	69.9±14.8	207.9±19.8***	52.8±8.7	-	208.6±21.3***	220.5±19.7	38.6± 4.9
Total bilirubin, μmol/l	8.3±3.2***	20.8±2.5	0.9±0.13	8.5±1.2***	17.1±1.9	1.7±0.21***	12.6±1.4	0.9±0.15	-	8.2±1.8***	8.3±2.1	0.6±0.13
Glucose, mmol/l	7.5±0.76	5.2±0.95	6.5±0.56	7.1±0.88	9.4±1.2	7.7±1.6	7.7±1.5	6.7±0.98	-	7.1±1.4	7.2±1.7	10.7±2.1
Calcium, mmol/l	3.1±0.4	3.2±0.3	3.1±0.6	3.3±0.4	3.1±0.6	3.5±0.4	3.1±0.3	2.7±0.2	-	3.1±0.3	3.1±0.2	2.1±0.4
Inorganic phosphorus, mmol/l	2.2±0.12	2.3±0.21	1.2±0.16	2.3±0.32	2.4±0.25	1.3±0.24	2.2±0.45	1.1±0.12	-	2.2±0.33	2.6±0.52	1.6±0.15
Ca/P, units	1.4±0.16	1.4±0.23	2.6±0.43	1.3±0.31	1.3±0.33	2.7±0.42	1.3±0.21	2.5±0.43	-	1.4±0.37	1.2±0.54	1.3±0.44
Cholesterol, mmol/l	2.3±0.14	2.1±0.18	2.1±0.21	2.3±0.32	2.7±0.11	3.4±0.54	2.5±0.34***	0.8±0.15	-	2.5±0.17	2.5±0.16	3.2±0.42

*P<0.05, **P<0.01, ***P<0.001.

We note an increase in total protein in the second experimental group after application of the drug on the 60th day of observation compared with group I and an intact group of animals by 5.3 % and 21.0 % ($P < 0.05$) due to globulins by 25,0% ($P < 0.05$). On day 90 of the experiment, the level of total protein in the group of rabbits treated with *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide remained at the level of the physiological norm, exceeding the level in animals of group I and clinical control by 3.7 % and 5.1 %, respectively. Moreover, we observed at the time of recovery (90 days) decrease in the concentration of urea in the blood plasma of rabbits of group II and intact group compared with animals receiving isoniazid by 5.8 % and 17.1 % ($P < 0.05$). In our opinion, a group I have the final effects of reduced protein catabolism and mild dehydration.

The enzymes AST and ALT are indicative of the liver. These enzymes transfer amino groups from aspartic acid and alanine to α-ketoglutaric acid (Gutyj et al., 2019; Bashchenko et al., 2020; Brezvyn et al., 2021). They are localized in the hyaloplasm of cells and mitochondria. Transferases are quite sensitive and informative indicators of liver damage. In the blood of rabbits of group I on the 60th and 90th day of observation, there was a sharp decrease in the level of AST by 69.1 U/l ($P < 0.01$) and 178.5 ($P < 0.001$) U/l. Moreover, this indicator was 90 days lower than the analogs of the II and intact groups by 31.3 U/l ($P < 0.05$) and 76.5 ($P < 0.01$) U/l, and the De Ritis ratio decreased by 3.0 ($P < 0.01$) and 4.3 ($P < 0.001$) times, respectively.

Plasma creatinine concentration is an essential indicator of renal function. The kidneys accumulate the end product of creatine metabolism - creatinine, which is synthesized in these organs from such amino acids as arginine, glycine, and methionine. Due to the significant destruction of renal cells, creatinine secretion is disrupted by glomerular filtration and its accumulation in the serum (Vasylyev et al., 2021; Slivinska et al., 2021). We found in animals of the II experimental group on the 30th day of treatment a sharp increase in this indicator compared with the I and intact groups by 33.6 % ($P < 0.01$) and 67.8 % ($P < 0.001$), respectively, which on the 90th day of observation came to the level of the physiological norm.

Less specific for determining the functional state of the liver are indicators of alkaline phosphatase activity. Alkaline phosphatase is a zinc-containing metalloprotein that is involved in mineral metabolism. It breaks down esters of orthophosphoric acid to form inorganic phosphorus. The enzyme is located in the cell in a state bound to the plasma membrane. Alkaline phosphatase consists of various isoenzymes localized mainly in the epithelium of the biliary tract, plasma membranes of hepatocytes and neurons, bones, intestines, placenta, kidneys. In animals of the second experimental group, alkaline phosphatase recovered faster on the 90th day of treatment, reached the physiological norm, and was 17 % higher than

in group I rabbits. Its intensive synthesis can explain the increase in alkaline phosphatase activity due to irritation of the epithelium of the biliary tract by toxins.

Bone tissue responds very quickly to changes that occur both internally and externally. It should also be noted that this ability is inherent not only in the structure but also in the chemical composition of the bone. This exceptional lability is because bone tissue is constantly involved in such an integral function as the general metabolism. Due to the constant flow of mineral and organic components from bone to blood, the homeostasis of the internal environment is ensured. From the blood to create new bone structures is a constant flow of relevant substances. With calcium deficiency, this macronutrient can enter the blood, serum, and tissue, thus ensuring the ionic stability of the body's internal environment. Calcium metabolism is inextricably linked to phosphorus, and their normal state requires a proper quantitative ratio of these elements.

Analysis of serum calcium levels in experimental animals showed that in rabbits treated with *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotino-hydrazide on day 90 of TB treatment, the calcium level is higher and is 3.5 ± 0.4 mmol/l than in animals treated with isoniazid (3.1 ± 0.6 mmol/l). The calcium-phosphorus ratio in rabbits of group II was equal to that of the intact group (1.3 ± 0.21 mmol/l), while in group I, it was two times higher and was 2.6 ± 0.43 mmol/l ($P < 0.05$).

Morphological parameters of the blood of rabbits with long-term administration of the drugs are shown in Table 3.

Table 3. Morphological parameters of rabbit blood with long-term administration of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide

Parameter	Group of animals/days of observation											
	I			II			III			IV		
	1	60	90	1	60	90	1	60	90	1	60	90
Hemoglobin, g/l	108.1±17.5	99.1±12.8	113.0±114.7	109.2±13.7	101.5±12.5	125.5±15.4	107.5±13.5	98.0±10.9	-	103.7±9.7	103.2±14.8	121.0±17.8
Hematocrit, %	27.0±7.6	23.8±5.7	36.3±7.4	26.4±4.2	23.0±3.6	39.7±5.3	29.1±6.4	25.1±4.3	-	29.5±7.3	30.6±5.6	39.3±7.6
Erythrocytes, T/l	3.5±0.34	3.4±0.42	4.1±0.33	3.6±0.25	3.4±0.56	4.3±0.31	3.6±0.24	2.9±0.43	-	3.4±0.41	3.4±0.38	4.3±0.29
ESR, mm/g	1.1±0.1	3.2±0.24	3.1±0.31***	1.1±0.1	2.1±0.18***	1.1±0.13	1.1±0.1	4.1±0.38***	-	1.1±0.1	1.1±0.1	1.0±0.1
Platelets, G/l	390.1±23.6	480.6±31.2***	271.7±27.4	367.9±32.7	348.6±35.6	376.5±33.8	348.2±29.6*	258.4±26.5	-	321.0±28.6	294.5±29.8	280.7±27.5
Leukocytes, G/l	5.9±2.1	4.3±1.6	5.1±1.4	5.7±1.5	3.8±1.3	7.3±1.7	6.2±1.8	8.2±1.7	-	6.3±1.6	7.6±2.1	5.9±1.7
Leukocyte formula, %												
Basophils	0	0	0	0	0	0	0	0	-	0	0	0
Eosinophils	1.0±0.1	2.0±0.12	2.1±0.14***	1.0±0.1	2.0±0.21	2.0±0.18	1.0±0.1	4.0±0.58***	-	1.0±0.1	1.0±0.1	3.0±0.32***
Neutrophils									-			
Myelocytes	0	0	0	0	0	0	0	0	-	0	0	0
Metamyelocytes	0	0	0	0	0	0	0	0	-	0	0	0
Band	1.0±0.1	3.0±0.23***	2.0±0.12	0	2.0±0.24***	1.0±0.11	0	3.0±0.18	-	1.0±0.15	0	0
Segmented	42.0±10.2***	44.2±13.7	15.0±5.6	33.2±11.5	27.5±9.8	20.5±7.6	38.1±9.7**	11.1±3.6	-	35.3±12.4	39.2±14.6	21.2±5.4
Lymphocytes	51.3±15.8	49.5±16.8	59.2±15.8	48.2±14.6	67.5±21.1	56.5±18.7	51.2±16.7	46.2±18.5	-	52.0±17.6	53.0±16.4	51.6±18.6
Monocytes	4.7±0.3	1.5±0.22	22.2±5.5***	18.3±4.7***	1.0±0.1	20.2±6.4	10.2±3.6	35.8±10.7**	-	11.2±3.1	7.2±1.6	24.4±11.6

Hemoglobin (Hb) is a red pigment, a chromoprotein found in red blood cells and carries oxygen. We note a decrease in Hb in both experimental groups on day 60 of treatment by 8.3 % and 7.1 %. However, on day 90 of observation in group I, this indicator did not reach the level of clinically healthy animals, amounting to 113.0 ± 114.7 g/l, whereas in the treatment of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide Hb even exceeded the intact group by 3.7 % (125.5 ± 15.4 g/l).

In a study of rabbit blood, it was found that mature erythrocytes are large with a small area of enlightenment in the center. Their number in the intact group during the whole period ranged from 103.7 ± 9.7 g/l to 121.0 ± 17.8 g/l. The introduction of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide had a positive effect on the state of hematopoiesis in rabbits than isoniazid: on day 90 of treatment, the number of erythrocytes and platelets exceeded 4.9 % (4.3 ± 0.31 T/l) and 38.6 % (376.5 ± 33.8 T/l, $P < 0.05$), respectively. Carrying out hemostatic and proinflammatory effects, platelets, in our opinion, can play an essential role in the development of diseases of the respiratory system.

The last indicators of leukopoiesis in rabbits on the 60th day of caution depended on the efficiency of the blood test: the number of leukocytes in blood in the first and second groups decreased to 4.3 ± 1.6 G/l (izoniazid) and 3.8 ± 1.6 G/l *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide) with a probable increase in these values in rabbits of the control group, individually infected with the pathogenic strain of *M. bovis* without treatment (III) up to 8.2 ± 1.7 G/l ($P < 0.05$), which is indicative of the presence of a pathological process in the body of the rabbits.

In rabbits, the leukogram profile is lymphocytic. Neutrophils in rabbits are very peculiar, they have a much larger grain size than other animal species, so they are called pseudoeosinophils. Neutrophils contain lysosomal enzymes that kill bacteria and enzymes that produce active antimicrobials in the blood. The level of rod-shaped neutrophils is 60 days higher than in group II and is 3.0 ± 0.23%, which indicates a degenerative shift of neutrophils - inhibition of hematopoietic function. The characteristics of the leukogram indicate that during the experiment, in groups I and II, the number of basophils and eosinophils were within the clinically healthy group, which means the absence of allergic reactions in rabbits. Activation of the hematopoietic system in the direction of intensification of hematopoiesis and resistance are adaptive reactions of the body that ensure the implementation of the protective function of the blood and contribute to the formation of the immune status of animals.

N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotino-hydrazide enhances the body's ability to stimulate functional activity of immune system and increases the resistance of animals to tuberculosis. The highest intensity of the immune system was found on day 60 of treatment with the highest lymphocyte counts - up to 67.5 ± 21.1 %, in clinically healthy animals 53.0 ± 16.4 %, and 90 days in the number of monocytes - up to 20.2 ± 6.4 %, indicating increased production of phagocytosis.

Mass coefficient (MC) - the percentage of organ mass to body weight, an integral indicator used to assess the condition of internal organs (Table 4).

The calculation of mass coefficients was determined by the formula:

$$MC = \text{organ mass (g)} / \text{body weight (g)} * 100\%$$

Table 4. Mass coefficients of the rabbits internal organs with prolonged administration *of N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide,* g, M ± m

Parameter	Group of animals							
	I		II		III		IV	
	The mass of the internal organ	Mass ratio	The mass of the internal organ	Mass ratio	The mass of the internal organ	Mass ratio	The mass of the internal organ	Mass ratio
Bodyweight	3632.0±45.6	-	4002.4±61.5	-	1902.8±63.6***	-	3545.4±52.7**	-
Heart	7.2±0.2	0.198±0.002	8.6±0.2	0.215±0.004	4.8±0.15	0.250±0.002	8.5±0.3	0.240±0.007
Lungs	16.2±0.3	0.446±0.003	17.1±0.4	0.427±0.003	14.2±0.4**	0.75±0.06	16.1±0.3	0.454±0.002
Liver	102.1±2.1	2.81±0.38	108.4±3.2	2.71±0.20	48.1±2.6	2.53±0.37	97.8±2.9	2.76±0.21
Spleen	1.8±0.1	0.050±0.007	1.9±0.17	0.047±0.004	1.2±0.1*	0.063±0.002*	1.6±0.2	0.045±0.005
Kidneys	19.0±0.7	0.52±0.02	21.2±0.5	0.53±0.03	11.1±0.4	0.58±0.05	19.6±0.6	0.55±0.02

* P<0.05; **P<0.01 ***P<0.001

We determined that the indicators of the internal organs of rabbits in the treatment of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide are somewhat superior to isoniazid and are within the intact group. Mass coefficients of the heart are higher by 8.6 % (0.215 ± 0.004), kidneys - 1.9 % (0.53 ± 0.03). A slight increase in the mass coefficient of the liver in group I indicates the remnants of intoxication in rabbits using isoniazid.

Prolonged tuberculosis intoxication in a group of animals artificially infected with the pathogenic strain of *M. bovis* without treatment (group III) led to an increase in the mass coefficients of the heart, lungs, spleen, and kidneys compared with clinically healthy rabbits by 4.0 % (0.250 ± 0.002), 39.5 % (0.75 ± 0.06; P < 0.01), 28.6 % (0.063 ± 0.002; P < 0.05) and 5.2 % (0.58 ± 0.05), respectively. This indicates a progressive inflammatory process throughout the body, functional disorders of the cardiovascular, respiratory, and digestive systems.

Positive results were obtained after the use of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide. *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotino-hydrazide, when administered subcutaneously, has a more significant tuberculocidal effect compared to isoniazid, as evidenced by the absence of pathological changes in the lungs, liver, spleen, and kidneys.

Conclusion

These examples represent the results of a comparative analysis of isoniazid and *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide treatment based on clinical, morpho-biochemical, and pathological studies of signs of tuberculous inflammation and nonspecific changes in the organs of rabbits with an experimental model of tuberculosis. It was found that subcutaneous administration of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide at a dose of 10 mg/kg per body weight leads to the absence of specific and nonspecific manifestations of inflammation in the lungs, liver, kidneys, and spleen. The calculated value of the drug indicates a high degree of safety *for N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotino-hydrazide* and its prospects for veterinary practice as an effective and safe tuberculocidal drug

Conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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