Acute and subchronic toxicity studies of the original drug FS-1

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Abstract

Interest in iodine complexes has increased significantly in recent years because of their wide spectrum of biological activity. The FS-1 is an ion nanostructured complex formed by proteins and/or polypeptides, carbohydrates, salts of alkali and alkaline earth metals with intercalated iodine. Patented in 2014, it is intended for the treatment of infectious diseases of bacterial origin including nosocomial infections and multidrug resistant tuberculosis. The aim of the study was to determine its acute and subchronic toxicity. The study of acute and subchronic toxicity was performed on adult Wistar rats according to OECD guidelines. The data on acute toxicity showed LD_{50} > 2,000 mg/kg after a single intragastric administration. Twenty-eight days of FS-1 administration at a dose of 500 mg/kg resulted in toxic effects. At a dose of 250 mg/kg, the toxic effects were temporary and a return to normal followed after the recovery period. Doses of 100 mg/kg had no adverse effects on the rats.

Antibacterial agent, lethal dose, iodine complexes, prolonged administration, rats

Most often used in medical practice, iodine complexes containing the ligands polyvinylpyrrolidone (PVP-I), polyvinyl alcohol and polysaccharides are called iodophors (Gottardi 1991). Iodine coordinated with organic macromolecular ligands exhibits more stable characteristics and lower toxicity than solutions of molecular iodine with potassium iodide (Gottardi 1991; Navikaite et al. 2013). Despite their diverse biological activity, the use of iodine and its complexes is limited by their relative instability and resulting toxicity (Glick et al. 1985). Based on data from clinical cases of iodine poisoning, lethal doses range between 12 and 120 mg/kg (WHO 2009). Furthermore, the presence of the internal environment of proteins reduces the biocidal activity of PVP-I (Zamora et al. 1985).

Interest in iodine complexes has increased significantly in recent years because of their wide spectrum of biological activity. Studies have been conducted on obtained samples of complexes of iodine with thioamides, selenoamides, and amides (Hadjikakou and Hadjiliadis 2006), cefotaxime sodium and iodine (El-Dien et al. 2009), atenolol and iodine (Pandeeswaran and Elango 2009), organic hyaluronan with inorganic iodine (Brenes et al. 2011), β-carotene, stearic acid, tripalmitin, lysozyme, folic acid, cytochrome C, valinomycin, gramicidin with inorganic iodine (Solanki et al. 2008), and iodine-lithium-alpha-dextrin (Yuldasheva et al. 2012).

The substance FS-1 was patented in 2014 (Ilin and Kulmanov 2014). It is an original antibacterial agent intended for the treatment of infectious bacterial diseases, including nosocomial infections and multidrug resistant tuberculosis. FS-1 is an ion nanostructured complex formed by proteins and/or polypeptides, carbohydrates, salts of alkali and alkaline earth metals with intercalated iodine. The proteins and/or polypeptides nanostructured ion complex contain at least one terminal amino acid with electron-donating functional groups (Ilin and Kulmanov 2014).

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The aim of the study was to determine acute and subchronic toxicity of the original FS-1 substance, and to determine the possibility and place of reversibility of its toxic effects in rats.

Materials and Methods

Test solution

An FS-1 working solution was prepared by dissolving FS-1 in distilled water immediately prior to its administration to animals. The amount depended on the body mass of animals involved in the experimental groups. The working solution was administered orally by gavage at a volume of 1 ml. The solvent for the test substance (distilled water) was used as a negative control.

Animals

Both sexes were used at the total amount of 92 subjects, weighing 180–200 g.

According to the OECD Guideline 2002 for the Testing of Chemicals No. 423 "Acute Oral Toxicity – Acute Toxic Class Method", the preferred rodent species is the rat, and 6 animals should be used for each dose. Females are generally slightly more sensitive than males. Thus, 12 healthy adult female Wistar rats were used in the acute toxicity experiment.

According to the OECD Guideline 2008 for the Testing of Chemicals No. 407 "Repeated Dose 28-Day Oral Toxicity Study in Rodents", the preferred rodent species is the rat. A total of 40 males and 40 females of adult Wistar rats were used in the subchronic toxicity experiment for testing of 4 groups (20 animals per group, 10 females and 10 males).

All the animals were kept in individually ventilated cages (IVCs, Tecniplast, Italy). Room temperature and humidity were maintained at 22 °C (± 3 °C) and 45–60%, respectively, with a light-dark cycle of 12 h (light from 07:00 h to 19:00 h). The animals were fed commercially available standard pellet chow (Ssniff) and water was supplied *ad libitum*. Animals were sacrificed as per rules of humane treatment of laboratory animals in a CO_2 chamber, containing 70% CO_2 at a flow rate of 30 litres per min. Animal experiments were approved by the local Animal Ethics Committee of the Scientific Center for Anti-Infectious Drugs, in accordance with the Kazakhstani law (PHT-007/1).

Acute toxicity study

The initial dose of FS-1 300 mg/kg was selected on the basis of the US Environmental Protection Agency for iodine LD_{50} (315 mg/kg) in rats when administered intragastrically (US Environmental Protection Agency 2006). Dose searching continued until a dose was found at which marked signs of toxicity in several animals were observed or the loss of no more than one animal in the group occurred. Animals were kept under observation for 14 days, for the first day every 2 h, and each following day every 12 h. Animal body weight was measured weekly, starting with the first day of the study. Macroscopic organ analysis was carried out upon completion of the study. The toxic effects of the drug were evaluated by the general state of the animals and their survival, LD_{50}.

Subchronic toxicity study

Based on the OECD, the following doses were selected for this study: the highest dose that causes observable, non-fatal toxic effects 1/4 LD_{50} (500 mg/kg), the mean dose 1/8 LD_{50} (250 mg/kg), and the lowest dose (100 mg/kg).

Experiments were carried out on four groups of rats comprised of 20 animals (10 males and 10 females). The drug was administered orally (by gavage). The volume of administration of the test substances was calculated for each animal according to its body mass. The administration was carried out once a day in the morning, six days per week. The total period of administration of the drug was 28 days, followed by a 28-day recovery period. Animal observation and body mass measurements were performed once a week. Animals were sacrificed after 28 and 56 days. The following indicators were selected as a test to characterize the state of the animals under the influence of the test substance: 1) animal death; 2) evaluation of the general condition of the animals and appetite; 3) the nature of motor activity; 4) the occurrence and nature of seizures; 5) the state of hair and skin; 6) the state and colour of the mucous membranes; 6) a change in body weight.

Biochemical variables (total bilirubin, alkaline phosphatase, albumin, total protein, urea, creatinine, aspartate aminotransferase, and alanine aminotransferase) were measured to characterize the functional state of internal organs using the BioSystem A 25 biochemical analyzer (Spain). Haematological indicators were determined using the HumaCount (Germany). Histopathological examination was conducted in all animals included in the experiment.

Statistical analysis

The mean value (x) and standard deviation (SD) were calculated for each variable measured and analyzed statistically by analysis of variance (ANOVA) to determine significant differences between groups at $P < 0.05$.

Calculation of the body weight gain (BWG) was produced by the following formula (1):

$$BWG = (P_1 - P_2) \times (100 / P_2), \quad (1)$$

where P_1 is the mean weight of the animal at the end of the experiment and P_2 is the mean weight of the animal at the beginning of the experiment.

Results

Acute oral toxicity

Animals treated with FS-1 at a dose of 300 mg/kg did not show any toxicity symptoms or mortality (n = 6). Therefore, the next dose selected was 2,000 mg/kg. After oral administration of FS-1 at a dose of 2,000 mg/kg, death was not observed in the rats (n = 6). Toxic symptoms in the first hours of the experiment were observed in the form of animals huddled in groups and a dramatically increased response to external stimuli (noise). All symptoms disappeared completely after 6 h. The study of the dynamics of body weight after FS-1 administration showed no weight loss. Macroscopic examination of the internal organs of experimental animals after necropsy did not reveal any abnormalities. Under the OECD guideline No. 423, the study was stopped at the maximum dose of 2,000 mg/kg.

Subchronic oral toxicity

In accordance with the recommendations of OECD No. 407, the study was conducted on laboratory rats for 28 days, followed by a 28-day recovery period.

No death was observed during the 28-day administration of FS-1. During observation of the animals, a satisfactory state was found in the animals treated with the test substance at a dose of 500 mg/kg (in 4 of the 20 animals the physical activity was low, there was confusion, and the animals huddled in a group). After discontinuing administration of the test substance, the appearance of experimental animals returned to that of the control group. In both groups where animals were given a dose of 250 mg/kg and 100 mg/kg, respectively, animal behaviour did not differ from the control group and no clinical signs of iodine poisoning were observed.

While analyzing the results of changes in the body weight, a slowing of the weight gain in rats treated with FS-1 at a dose of 500 mg/kg was observed. Animals in this group exhibited a significant decrease in the body weight relative to the control group ($P \leq 0.05$).

The body weight gain remained unchanged in rats treated with FS-1 at doses of 250 and 100 mg/kg. After discontinuing the FS-1 administration, the rate of weight gain remained the same and matched that of the control group of animals (Table 1).

Table 1 - Coefficient of body weight gain (BWG), %.

Indicator	Animal group			
	Control (solvent)	FS-1 500 mg/kg	FS-1 250 mg/kg	FS-1 100 mg/kg
	Administration period of 28 days			
BWG % male	42.34 ± 4.38	32.92 ± 4.77*	38.43 ± 5.51	41.57 ± 4.42
BWG % female	46.12 ± 6.46	35.68 ± 3.84*	42.11 ± 4.70	42.37 ± 4.05
	Recovery period of 28 days			
BWG % male	72.32 ± 6.51	61.05 ± 3.65*	71.32 ± 4.33	74.85 ± 2.81
BWG % female	78.43 ± 4.35	67.46 ± 3.91*	74.85 ± 5.96	75.09 ± 3.17

Each value represents the mean ± standard deviation
*Significant at $P < 0.05$

Table 2. Haematological indicators in females after FS-1 administration.

Haematological indicator	Administration period of 28 days				Recovery period of 28 days			
	Control (solvent)	FS-1 (500 mg/kg)	FS-1 (250 mg/kg)	FS-1 (100 mg/kg)	Control (solvent)	FS-1 (500 mg/kg)	FS-1 (250 mg/kg)	FS-1 (100 mg/kg)
WBC ($\times 10^9$/l)	5.45 ± 0.92	6.54 ± 0.91	6.00 ± 1.19	6.45 ± 1.43	5.68 ± 1.23	6.08 ± 1.37	6.44 ± 2.13	6.13 ± 1.87
LYM ($\times 10^9$/l)	3.24 ± 1.33	2.91 ± 1.83	3.89 ± 1.33	3.26 ± 0.75	2.66 ± 1.80	2.66 ± 1.57	3.41 ± 2.28	2.29 ± 1.65
MID ($\times 10^9$/l)	0.92 ± 0.24	1.09 ± 0.18	0.95 ± 0.20	0.87 ± 0.42	1.26 ± 0.90	1.15 ± 0.34	1.03 ± 0.62	0.92 ± 0.64
GRA ($\times 10^9$/l)	1.29 ± 0.28	1.17 ± 0.43	1.32 ± 0.56	1.34 ± 0.34	1.26 ± 1.01	1.13 ± 0.68	1.21 ± 0.95	0.93 ± 1.19
RBC ($\times 10^{12}$/l)	9.27 ± 1.02	8.70 ± 1.21	9.35 ± 0.45	8.59 ± 1.39	9.23 ± 1.31	9.05 ± 1.22	8.42 ± 0.95	8.92 ± 0.80
HGB (g/l)	137.0 ± 4.80	142.0 ± 2.35	139.40 ± 4.10	129.58 ± 9.16	138.20 ± 9.98	142.25 ± 5.95	135.20 ± 4.09	145.20 ± 7.12
HCT (%)	52.82 ± 7.35	53.42 ± 5.89	49.36 ± 4.27	48.60 ± 5.14	55.26 ± 5.91	51.51 ± 5.57	45.10 ± 4.33	49.86 ± 4.86
PLT ($\times 10^9$/l)	866.60 ± 107.74	795.40 ± 131.58	821.60 ± 147.95	827.40 ± 95.33	947.40 ± 202.64	823.47 ± 129.66	912.20 ± 114.22	872.40 ± 107.41

WBC – white blood cell count; LYM – lymphocytes; MID – mid-range absolute count; GRA – granulocytes; RBC – red blood cell count; HGB – haemoglobin; HCT – haematocrit, PLT – platelet count

At 29 and 56 days from the start of the experiment, blood sampling was conducted on the animals to further the haematological and biochemical research.

Haematology indicators in the control group of rats did not exceed the physiological range (Giknis and Clifford 2008) for this animal species (Tables 2 and 3). In male rats treated with FS-1 at a dose of 500 mg/kg for 28 days, there was a marked increase in the level of white blood cells and lymphocytes, 9.92 ± 1.32 and 8.35 ± 1.25, respectively ($P \leq 0.05$ in both cases). This change was temporary and returned to normal after the recovery period. These changes were not observed in the female group.

Peripheral blood examination clearly revealed a significant effect in rats treated with FS-1 at both doses of 250 mg/kg and 100 mg/kg. A decrease in haemoglobin ($P \leq 0.05$) only occurred among male subjects who received doses of 250 mg/kg, but these changes in the composition of the peripheral blood were within the physiological range (Giknis and Clifford 2008). Thus, FS-1 at both doses of 250 mg/kg and 100 mg/kg did not change the qualitative and quantitative composition of the peripheral blood.

Blood biochemical indicators were investigated to detect metabolic abnormalities in rats under the influence of FS-1 (Tables 4 and 5). A significant increase in hepatic indicator profiles, such as alanine aminotransferase, aspartate aminotransferase, and total bilirubin was observed under the influence of FS-1 at a dose of 500 mg/kg ($P \leq 0.05$). This rise was observed in both male and female groups. Based on the biochemical indicators of this group of animals, it can be concluded that females were more sensitive than males to the substance in the test dose. This conclusion was confirmed by the data obtained in the evaluation of renal and hepatic function in females. The renal load in females at a dose of 500 mg/kg was evaluated based on increases in creatinine and urea.

A slight decrease in albumin in the group of females treated with FS-1 at a dose of 250 mg/kg was observed. This indicator was significantly different from the control but was within the physiological range for this animal species. The changes were temporary and no significant deviations were observed in relation to the same biochemical blood indicators in the negative control group of animals after the recovery period.

Biochemical indicators of the blood serum of animals treated with FS-1 at a dose of

Table 3. Haematological indicators in males after FS-1 administration.

Haematological indicator	Administration period of 28 days				Recovery period of 28 days		
	Control (solvent)	FS-1 (500 mg/kg)	FS-1 (250 mg/kg)	FS-1 (100 mg/kg)	Control (solvent)	FS-1 (500 mg/kg)	FS-1 (250 mg/kg)	FS-1 (100 mg/kg)
WBC ($\times 10^9$/l)	6.05 ± 1.34	9.92 ± 1.32*	6.33 ± 0.98	5.88 ± 1.58	5.82 ± 1.33	6.02 ± 0.94	5.68 ± 0.90	7.89 ± 2.18
LYM ($\times 10^9$/l)	3.77 ± 1.36	8.35 ± 1.25*	3.27 ± 1.43	3.85 ± 1.13	3.09 ± 1.26	3.33 ± 0.49	3.53 ± 0.57	3.31 ± 1.46
MID ($\times 10^9$/l)	0.96 ± 0.65	1.06 ± 0.60	1.23 ± 0.30	0.82 ± 0.81	1.18 ± 1.04	1.28 ± 0.64	1.55 ± 0.28	1.35 ± 0.61
GRA ($\times 10^9$/l)	1.32 ± 0.79	1.53 ± 0.70	1.25 ± 0.63	1.30 ± 0.73	1.25 ± 0.82	1.59 ± 0.31	1.52 ± 0.10	0.93 ± 1.23
RBC ($\times 10^{12}$/l)	9.87 ± 1.20	9.61 ± 1.69	7.88 ± 1.09	8.61 ± 1.74	9.46 ± 1.53	9.25 ± 1.06	9.66 ± 0.50	9.86 ± 0.23
HGB (g/l)	149.40 ± 15.22	149.40 ± 19.05	134.20 ± 7.50*	147.60 ± 11.65	147.0 ± 8.63	151.0 ± 5.81	148.0 ± 7.81	147.20 ± 1.30
HCT (%)	50.18 ± 6.38	50.14 ± 6.84	45.72 ± 9.43	49.36 ± 7.07	60.46 ± 5.59	58.63 ± 4.08	60.66 ± 3.05	58.16 ± 0.72
PLT ($\times 10^9$/l)	908.80 ± 115.67	1000.40 ± 168.41	856.60 ± 154.19	1002.60 ± 112.91	1013.80 ± 123.84	1009.60 ± 134. 01	989.60 ± 152.64	1095.60 ± 121.20*

WBC – white blood cell count; LYM – lymphocytes; MID – mid-range absolute count; GRA – granulocytes; RBC – red blood cell count; HGB – haemoglobin; HCT – haematocrit, PLT – platelet count; Significant at $P < 0.05$

100 mg/kg did not differ from the control group for both males and females.

Stated biochemical data were further confirmed by the following histological examination of the animals.

Two types of follicles, round or oval, and irregular shapes were observed under microscopic examination of histological sections of the thyroid gland of the control group animals (Plate III, Fig. 1). The colloid was weakly eosinophilic or partially basophilic. Epithelial cells were observed with a flattened shape or slightly increased in volume.

The follicle size increased in the rats treated with FS-1 at a dose of 500 mg/kg (Plate III, Fig. 2). An irregular, reduced form of follicles was observed, along with a rounded shape to the follicle. Thyrocytes freely positioned in the cavity of the follicle lost connection with the basement membrane. There was an apical part of thyrocytes with cytoplasmic outgrowths, facing into the cavity of the follicle. Proliferative activity of thyrocytes was observed. Nuclei of thyrocytes reduced in size, and chromatin was condensed. Some atrophic follicles changed. Colloid resorption was also present. Vessels of capsules and partitions were extended. Large mast cells and interstitial oedema were observed.

The elements of the liver triad, central veins and radial liver beams were not broken in histological sections of the liver controls (Plate IV, Fig. 3). At higher magnification, focal expansion of the lumen of Disse, mainly in the periportal zone, was marked. Cell nuclei were round, some cells had two nuclei. There was a focal activation of Kupffer cells. In the group treated with FS-1 at a dose of 500 mg/kg, two types of hepatocyte nuclei were observed: one group of light-coloured nuclei with small nucleoli, other nuclei were dark-coloured. Hepatocytes with two nuclei were also observed. Hepatocytes were changed dystrophically, including fatty dystrophy in the shape of a small droplet, and also a focal-large droplet (Plate IV, Fig. 4). There was an enlargement of the space of Disse, sinusoid congestion, and activation of Kupffer cells indicating the appearance of lymphohistiocytic infiltration of focal perivascular oedema.

Histological sections of the kidneys of rats in the control group showed that the cortical was represented by renal corpuscles, urinary space was clearly visible (Plate V, Fig. 5). Dark-coloured proximal tubular epithelial cells, and some muddy cytoplasm and basal-located

Table 4. Biochemical indicators of the blood of males after FS-1 administration.

Biochemical indicator		Administration period of 28 days				Recovery period of 28 days			
		Control (solvent)	FS-1 (500 mg/kg)	FS-1 (250 mg/kg)	FS-1 (100 mg/kg)	Control (solvent)	FS-1 (500 mg/kg)	FS-1 (250 mg/kg)	FS-1 (100 mg/kg)
ALT, U/l	Hepatic profile	77.60 ± 12.73	140.90 ± 24.01*	83.94 ± 7.07	79.43 ± 25.38	77.20 ± 16.11	89.88 ± 21.38	74.56 ± 12.76	74.32 ± 13.71
AST, U/l		134.70 ± 45.94	230.40 ± 29.26*	156.58 ± 25.15	132.90 ± 39.00	160.78 ± 46.07	151.56 ± 57.81	162.32 ± 34.85	174.32 ± 34.55
Bilirubin total, mg/dl		0.33 ± 0.14	0.85 ± 0.14*	0.32 ± 0.16	0.33 ± 0.05	0.37 ± 0.16	0.48 ± 0.15	0.41 ± 0.09	0.37 ± 0.42
ALP-AMP, U/l	Protein profile	193.58 ± 43.55	206.02 ± 29.89	204.90 ± 35.80	220.12 ± 22.60	201.36 ± 43.47	200.42 ± 33.41	206.47 ± 20.75	205.64 ± 21.42
Protein total, g/l		65.78 ± 9.32	69.34 ± 3.99	67.72 ± 5.98	63.90 ± 4.84	67.64 ± 7.13	63.58 ± 5.23	65.60 ± 5.20	65.47 ± 4.15
Albumin, g/l		28.20 ± 1.92	27.14 ± 2.34	26.39 ± 3.27	28.12 ± 1.37	32.24 ± 5.44	34.70 ± 4.43	35.24 ± 3.84	33.24 ± 2.92
Creatinine, mg/dl	Renal profile	0.68 ± 0.15	0.58 ± 0.18	0.63 ± 0.12	0.57 ± 0.14	0.66 ± 0.14	0.61 ± 0.12	0.58 ± 0.17	0.59 ± 0.09
Urea, mg/dl		34.18 ± 4.41	39.16 ± 6.19	30.62 ± 7.24	35.94 ± 7.39	35.62 ± 4.45	33.02 ± 9.66	36.40 ± 6.35	33.47 ± 3.37

ALT - alanine aminotransferase; AST - aspartate aminotransferase; ALP-AMP – alkaline phosphatase 2-amino-2-methyl-1-propanol buffer
*Significant at $P < 0.05$

nucleus constitute the main share of cortical substance. Light-coloured distal tubules with a wider space were also observed. In the medulla, the thick parts of the nephron and collecting ducts were visible. Figure 6 (Plate V), shows the rats treated with FS-1 at a dose of 500 mg/kg. Vascular glomeruli were unevenly congested, thickened by plasmatic impregnation. Mesangial matrix was increased, and individual glomeruli were hypercellular. The palmate structure of glomeruli appeared in the cavity of the capsule of fibrin masses. Renal tubules exhibited signs of granular dystrophy, and proximal tubules were in most cases constricted. The focal necrosis of epithelial cells was marked. The main share of epithelial tubules was swollen, and the space of the tubule was very constricted as a result of it. There were some vessels with signs of stasis. Histological examination of animals treated with FC-1 at doses of 250 mg/kg and 100 mg/kg showed no difference from the control group of animals.

Discussion

Data on the acute toxicity of FS-1 on laboratory rats showed that $LD_{50} > 2{,}000$ mg/kg after a single intragastric administration. Thus, FS-1 can be attributed to the Class 5 toxicity, i.e. non-toxic substances.

Results of the subchronic toxicity study of FS-1 showed that prolonged exposure to the pharmacological agent at a dose of 500 mg/kg can cause toxic effects. The obtained data showed a slowdown in the body weight gain in the studied rats. Apparently, this effect can be explained by the thyroid-stimulating properties of iodine. It is known that thyroid hormones affect metabolic processes. Excessive administration of iodine in the diet leads to hyperthyroidism (Aakre et al. 2015).

Inflammation, anaemia, metabolic acidosis, hyperchloraemia and hyperkaliaemia, acute fibrinolysis, and increased cytolytic enzymes (LDH) are often observed in iodine poisoning. These changes are associated with iodaemia (Kataoka et al. 2006; Lakhal et al. 2011). Apparently, the observed changes in biochemical and haematological blood indicators are associated with high levels of iodine in blood (Glick et al. 1985). Since the changes in the biochemical and haematological blood indicators in males and females were very small and temporary, it can be concluded that no distinct sex differences were traced

Table 5. Biochemical indicators of the blood of females after FS-1 administration.

Biochemical indicator		Administration period of 28 days			Recovery period of 28 days		
		Control (solvent)	FS-1 (500 mg/kg)	FS-1 (250 mg/kg)	FS-1 (100 mg/kg)	Control (solvent)	FS-1 (500 mg/kg)	FS-1 (250 mg/kg)	FS-1 (100 mg/kg)
ALT, U/l	Hepatic profile	79.98 ± 13.26	155.96 ± 23.38*	80.10 ± 23.42	78.41 ± 31.58	78.36 ± 18.79	90.72 ± 16.68	79.33 ± 20.34	79.30 ± 22.06
AST, U/l		108.04 ± 24.51	245.16 ± 31.92*	104.63 ± 43.75	105.60 ± 26.64	135.74 ± 31.68	151.46 ± 28.47	145.26 ± 47.30	133.26 ± 35.30
Bilirubin total, mg/dl		0.52 ± 0.10	1.01 ± 0.12*	0.49 ± 0.13	0.41 ± 0.12	0.49 ± 0.15	0.48 ± 0.07	0.43 ± 0.08	0.43 ± 0.08
ALP-AMP, U/l	Protein profile	174.76 ± 14.40	235.74 ± 25.27*	177.26 ± 8.54	189.08 ± 8.63	171.18 ± 36.40	167.86 ± 27.54	162.82 ± 27.32	162.30 ± 24.12
Protein total, g/l		67.78 ± 9.32	67.83 ± 5.46	64.50 ± 7.97	69.72 ± 6.29	67.04 ± 6.10	64.0 ± 5.88	66.26 ± 6.20	66.26 ± 4.17
Albumin, g/l		28.20 ± 3.92	26.38 ± 3.20	21.52 ± 1.05*	30.76 ± 1.60	28.18 ± 4.23	30.68 ± 5.29	31.02 ± 4.86	35.02 ± 2.86
Creatinine, mg/dl	Renal profile	0.68 ± 0.15	1.23 ± 0.08*	0.78 ± 0.11	0.59 ± 0.16	0.69 ± 0.17	0.60 ± 0.10	0.69 ± 0.10	0.65 ± 0.43
Urea, mg/dl		34.18 ± 4.41	61.36 ± 7.70*	30.43 ± 5.37	32.04±5.93	36.50 ± 5.55	35.02±7.24	34.80 ± 5.63	36.80 ± 7.33

ALT - alanine aminotransferase; AST - aspartate aminotransferase; ALP-AMP – alkaline phosphatase 2-amino-2-methyl-1-propanol buffer
*Significant at $P < 0.05$

after FS-1 administration at the studied doses.

High doses of iodine have a direct cytolytic effect on the cells of the gastrointestinal tract, as well as the liver and kidneys. Lower doses do not cause clinical signs of poisoning, nor have an effect on the body through the thyroid system (Sherer et al. 1991; Kataoka et al. 2006; Tsurumaru et al. 2010).

On the basis of biochemical and histological studies, it can be argued that the liver, kidneys, and the thyroid gland were the target organs of toxic destruction at a dose of 500 mg/kg.

At a dose of 250 mg/kg, the toxic effects were temporary and returned to normal after the recovery period. The dose of 100 mg/kg had no adverse effects on the rats based on the results of clinical, haematological and biochemical studies and necropsy.

In conclusion, for FS-1 the dose of 100 mg/kg body weight of in both male and female animals is NOAEL (the highest concentration where no adverse treatment-related findings are observed) and the dose 250 mg/kg is LOAEL (the lowest concentration of a chemical used in a toxicity test that has a significant adverse effect on the exposed population of test organisms compared with the controls).

References

Aakre I, Bjøro T, Norheim I, Strand TA, Barikmo I, Henjum S 2015: Development of thyroid dysfunction among women with excessive iodine intake-A 3-year follow-up. J Trace Elem Med Biol **31**: 61-66

Brenes RA, Sobotka L, Ajemian MS, Manak J, Vyroubal P, Slemrova M, Adamkova V, Zajic J, Dudrick SJ 2011: Hyaluronate-iodine complex: a new adjunct for the management of complex sternal wounds after a cardiac operation. Arch Surg **146**: 1323-1325

El-Dien FAN, Mohamed GG, Frag EY 2009: Utility of π-acceptor reagents for spectrophotometric determination of sulphonamide drugs via charge-transfer complex formation. Chem Pap **63**: 646-653

Giknis MLA, Clifford CB 2008: Clinical laboratory parameters for CrlːWI (Han). Charles River Laboratories, 14 p.

Glick PG, Guglielmo BJ, Tranbaugh RF, Turley K 1985: Iodine Toxicity in a Patient Treated by Continuous Povidone-Iodine Mediastinal Irrigation. Ann Thorac Surg **39**: 478-480

Gottardi W 1991: Iodine and Iodine compounds. In: Block SS (Ed.): Disinfection, sterilization, and preservation. 4th ed. Lea & Febiger, Philadelphia, pp. 159-183

Hadjikakou S, Hadjiliadis N 2006: Interaction of thioamides, selenoamides, and amides with diiodine. Bioinorg Chem Appl 2006: Article ID 60291

Ilin AI, Kulmanov ME 2014: Antibacterial agent for treating infectious diseases of bacterial origin. KZ Patent № 28746. Filing date 30.12.2010; publication date 19.06.2014

Kataoka M, Tsumura H, Kaku N, Torisu T 2006: Toxic effects of povidone–iodine on synovial cell and articular cartilage. Clin Rheumatol **25**: 632-638

Lakhal K, Faidherbe J, Choukhi R, Boissier E, Capdevila X 2011: Povidone iodine: Features of critical systemic absorption. Ann Fr Anest Reanim **30**: e1-e3

Navikaite V, Danilovas PP, Klimaviciute R, Bendoraitiene J 2013: The stability of water-soluble modified starch iodophors. Chem Technol (Kaunas, Lith) **63**: 34-40

OECD, Organisation for Economic Cooperation and Development 2002: Guidelines for the testing of chemicals/ section 4: Health effects test no. 423: Acute oral toxicity - acute toxic class method.

OECD, Organisation for Economic Cooperation and Development 2008. Guidelines for the testing of chemicals/ section 4: Health effects test No. 407: Repeated dose 28-day oral toxicity study in rodents

Pandeeswaran M, Elango KP 2009: Spectroscopic studies on the molecular complex of the drug atenolol with iodine. J Solution Chem **38**: 1558-1572

Sherer TT, Thrall KD, Bull RJ 1991: Comparison of toxicity induced by iodine and iodide in male and female rats. J Toxicol Environ Health **32**: 89-101

Solanki GK, Amin A, Padhiyar A, Ray AK, Oza AT 2008: Polaron hopping in some biomolecular solids and their charge transfer complexes. Indian J Biochem Biophys **45**: 421-429

Tsurumaru D, Utsunomiya T, Matsuura S, Komori M, Kawanami S, Ishibashi T, Honda H 2010: Gastric mucosal changes caused by lugol's iodine solution spray: Endoscopic features of 64 cases on screening esophagogastroduodenoscopy. Gastroent Res Pract 2010: Article ID 494195

US Environmental Protection Agency 2006: Iodine and iodophor complexes. Registration eligibility decision for iodine and iodophor complexes, 739-R-06-010, 70 p.

Yuldasheva GA, Zhidomirov GM, Ilin AI 2012: Effect of organic ligands with conjugated π-bonds on the structure of iodine-α-dextrin complexes. Biotechnol Appl Biochem **59**: 29-34

Zamora JL, Price MF, Chuang P, Gentry LO 1985: Inhibition of povidone-iodine's bactericidal activity by common organic substances: an experimental study. Surgery **98**: 25-9 WHO 2009: Iodine and inorganic iodides: human health aspects. Concise international chemical assessment (CICA), document 72

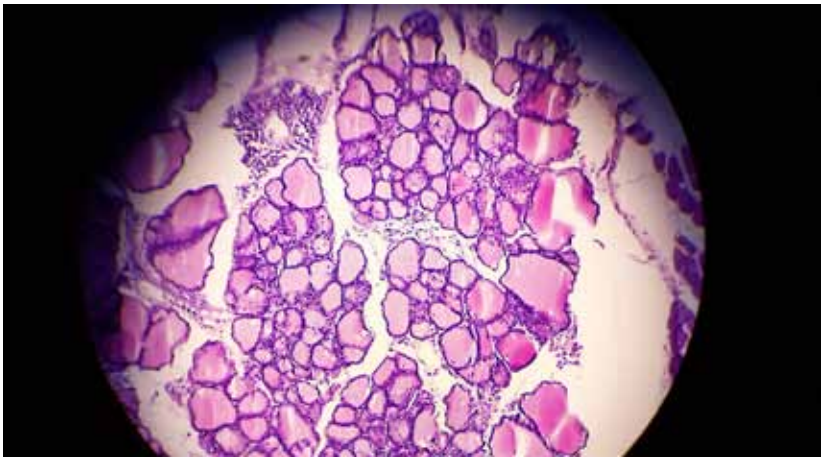

Fig. 1. Histological structure of the thyroid gland of rats in the control group
Haematoxylin-eosin stain, × 200 magnification

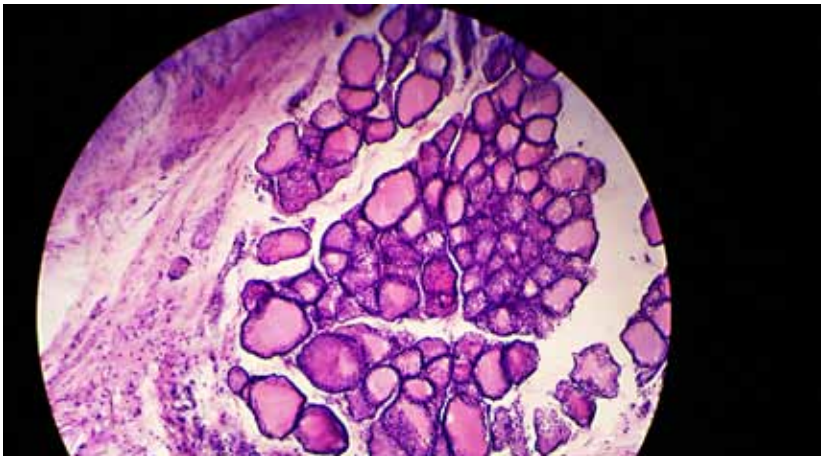

Fig. 2. Histological structure of the thyroid gland of rats administrated FS-1 at a dose of 500 mg/kg
Haematoxylin-eosin stain, × 200 magnification

Plate IV

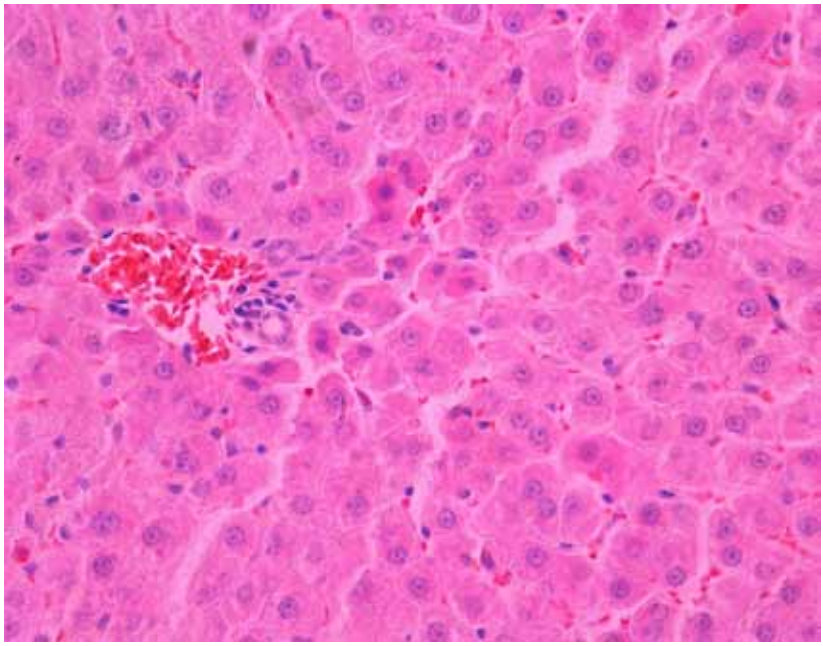

Fig. 3. Histological structure of rat liver in the control group
Haematoxylin-eosin stain, × 400 magnification

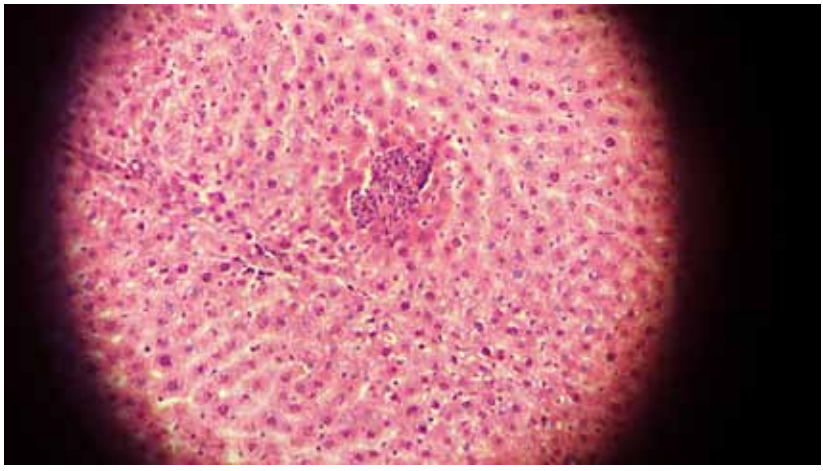

Fig. 4. Histological structure of rat liver, administered FS-1 at a dose of 500 mg/kg
Haematoxylin-eosin stain, × 200 magnification

Plate V

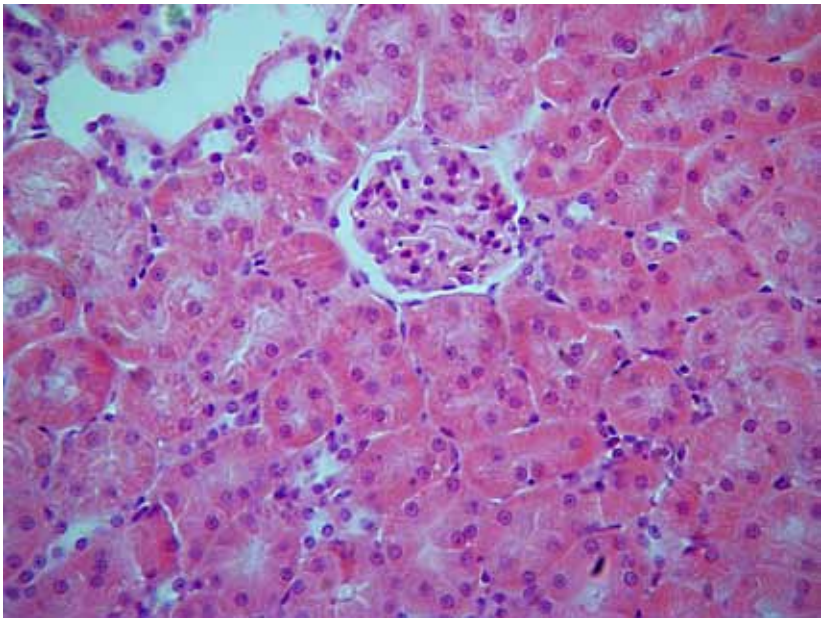

Fig. 5. Histological structure of rat kidney in the control group
Haematoxylin-eosin stain, × 200 magnification

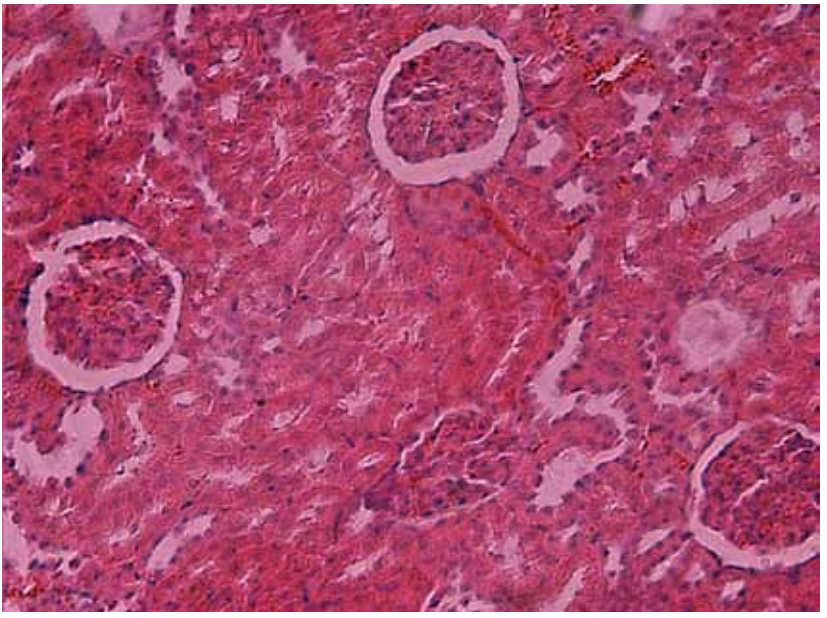

Fig. 6. Histological structure of rat kidney, administered FS-1 at a dose of 500 mg/kg
Haematoxylin-eosin stain, × 400 magnification