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Subchronic Toxicity of Ivermectin and Butaphosphan in Layer Chickens

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ABSTRACT

The development of new veterinary drugs to treat and prevent poultry parasitic infections, as well as the study of their safety is a hot topic for modern parasitology. The purpose of this research was to study the subchronic toxicity of the ivermectin and butaphosphan-based drugs at a therapeutic and threefold therapeutic dose during a seven-day oral administration to the Hisex White chickens. The provisional name of the drug is Iverbutan. The chickens from the first experimental group were given the drug at a threefold therapeutic dose of 3 mL of the drug per one liter of drinking water. The chickens from the second experimental group were given the drug at a therapeutic dose of 1 mL of Iverbutan per one liter of drinking water. The chickens from the control group received water without the drug. The chickens were weighed, and then the body temperature and blood samples from the axillary vein were measured on days 1, 8, and 17 of the experiment before the morning feeding. On day 8 of the study, the chickens from the first experimental group showed a 7.4% decrease in body weight and increase in body temperature by 0.8%, an increase in alanine aminotransferase activity by 2.1 times, aspartate aminotransferase activity by 1.6 times, and bile acids by 1.4 times. Moreover, there was a 4.6% decrease in glucose concentration, a 3.5% increase in lactate dehydrogenase activity, a 7.3% decrease in triglycerides, as well as a decrease in hemoglobin by 3.2% and erythrocytes by 10.6% in the first experimental group, compared to the control group. On day 17 of the experiment, the above blood parameters in the chickens from the first experimental group did not significantly differ from the control group, indicating the reversibility of the hepatotoxic effect. In this regard, a three-fold therapeutic dose can be considered a threshold. The chickens from the second experimental group showed no changes in their physiological status as compared to the control. Thus, the study results confirm the safety of the drug in the recommended dosage regimen.

Keywords: Blood, Butaphosphan, Chickens, Ivermectin, Metabolism, Subchronic Toxicity

INTRODUCTION

The development of new combined veterinary drugs to treat and prevent poultry parasitic infections, as well as the study of their safety is a hot topic for modern parasitology. Combined and safe drugs introduced into veterinary practice minimize the number of injections of various drugs and the time spent by veterinarians for additional treatments (Arisov et al., 2020a). This is especially important in the industrial sector.

Various pharmacological class drugs affect physiological functions and physiological processes in the animal's body. The presented work has studied the tolerability of combined Iverbutan (the provisional name of the drug), which contains 0.4% ivermectin and 10% butaphosphan as active ingredients. Iverbutan is administered to broiler chickens, breeding poultry, replacement of chickens or poultry during the molt period. One of the means to control chicken parasites is broad-spectrum antiparasitic drugs including those based on ivermectin (Moreno et al., 2015; Mestorino et al., 2017). This compound is a semi-synthetic derivative of avermectins (Campbell and Benz, 1984).

Causative agents of parasitic diseases are known to cause pronounced permanent changes in the body of animals, particularly, metabolism, morphophysiology of blood, hormonal status, etc. Furthermore, parasitic diseases are accompanied by stress reactions that develop in the body of animals (Samadieh et al., 2017; Indyuhova et al., 2021a; Indyuhova et al., 2021b). Therefore, biostimulants are necessary to improve animals' physiological status in the course of treating parasitosis. Thus, butaphosphan is an organic source of phosphorus,

which plays a major role in phosphorylation processes and gluconeogenesis processes in the liver. This organophosphorus compound is well tolerated (Fisinin et al., 2016) and does not accumulate in the body; it supports carbohydrate, energy, protein, and lipid metabolisms in the body, activates the immune system, and fastens recovery after pathologies of various origins (Rollin et al., 2010; Lima et al., 2017).

This work continues a series of studies to investigate the pharmaco-toxicological properties of new antiparasitic Iverbutan (Arisov et al., 2018; Indyuhova et al., 2021c). This work evaluates the study results of subchronic toxicity of the combined antiparasitic drug in poultry. It should be noted that these studies are necessary to assess a dose range, a route of drug delivery to the body, and its safety (Arisov et al., 2020b)

Based on the foregoing, the work aimed to study the subchronic toxicity of the drug based on ivermectin and butaphosphan in therapeutic and three-fold therapeutic oral dosages for seven days in layer chickens of Hisex White Cross.

MATERIALS AND METHODS

Ethical approval

The study of subchronic toxicity in chickens was approved at the meeting of the Scientific Council of the Federal Science Center (No. 2019/05/FSC VIEV; Russia). All manipulations were carried out in compliance with international requirements (Anonymous, 1986; Anonymous, 2010).

Feeding and housing conditions

The experimental part of the work was conducted at the Podolsk Base of the VNIIP-FSC VIEV (All-Russian Scientific Research Institute for Fundamental and Applied Parasitology of Animals and Plant, a branch of the Federal State Budget Scientific Institution "Federal Scientific Center VIEV", Russia). To study the subchronic toxicity, 15 replacement layer chickens of Hisex White Cross were selected. The chickens were selected according to the principle of analogs taking into account age, body weight, feeding, and housing conditions. The average weight of the chickens was 190 g before the experiment. The chickens were 20 days old at the beginning of the experiment. The chickens were then divided into three equal groups (first experimental, second experimental, and control), five chickens each. The chickens were kept in groups, in double-deck cages (5 chickens each). The first and second experimental groups were kept on the upper tier. Each group was in a separate cage module. The chickens from the control group were kept on the lower tier. Overall dimensions of one cage module were 0.7 m^2 which contained 5 chickens. Each cage was equipped with an autonomous drinking system. The access to water was unlimited. The feeding and housing conditions corresponded to the zootechnical requirements.

One day old chickens were vaccinated with a polyvalent vaccine (Kursk Biofactory, Russia) against Marek's disease. The chickens were vaccinated with a live vaccine (ARRIAH, Russia) against Gumboro disease on day 10 and also were vaccinated with a live vaccine (Kursk Biofactory, Russia) against virulent Newcastle disease on day 15. Prior to the experiment, the chickens received no other veterinary medicinal products.

Experiment design

The dosage and the frequency of administration were selected to identify potential negative effects on the chickens when the drug is used for a long time or when it is overdosed (GPSMP, 2012).

According to the draft instruction, the veterinary drug Iverbutan is administered orally at a dose of 1 mL/L of drinking water. The prepared solution is given once to treat against nematodes of the poultry and three times in case of arachnoentomiasis (treat twice with a 24-hour interval and then once after 14 days). The tolerance was studied on the layer chickens of Hisex White Cross which were treated with the combined Iverbutan (the provisional name of the drug) containing 0.4% ivermectin and 10% butaphosphan. The chickens were given Iverbutan by a group method with drinking water for seven days. The research was carried out for 17 days. The chickens from the first experimental group were given the drug at a threefold therapeutic dose of 3 mL of the drug per one liter of drinking water. The chickens from the second experimental group were given the drug at a therapeutic dose of 1 mL of Iverbutan per one liter of drinking water. The chickens from the control group received water without the drug.

Iverbutan was diluted at ¼ of the daily intake of drinking water. To ensure that the chicken received the required dose of the drug, the water supply was stopped 2 hours before the solution was given. A new solution was prepared daily.

Sampling collection

During the experiment, the physiological status of the chickens was monitored in each group and their ethologic status was determined. Methods for determining the

ethologic status in the chickens include follow-up with a recording of symptomatic motor activity, and assessment of chickens' responses to various stimuli (Maximov and Lysov, 2006). The chickens were weighed, and then the body temperature and blood samples from the axillary vein were measured on days 1, 8, and 17 of the experiment before the morning feeding.

Hematological and biochemical parameters

The following hematological parameters of chicken's blood are presented in the study: hemoglobin concentration, red blood cell count and white blood cell count, and white blood cell differential count (pseudoeosinophiles, basophiles, eosinophiles, monocytes, lymphocytes). The studied blood biochemical parameters in the chickens included aspartate aminotransferase, alanine aminotransferase, bile acids, creatinine, total protein, alkaline phosphatase, α-amylase, glucose, lactate dehydrogenase, triglycerides, lipase.

Hematological and biochemical parameters of the blood were determined according to the generally accepted method of Kondrakhin (2004) and Nebyltsova et al. (2011).

Erythrocytes and leukocytes were counted in a Goryaev chamber by a generally accepted method. Hemoglobin was determined by the colorimetric method using sodium lauryl sulfate. A visual microscopic assessment of Romanovsky-Giemsa-stained blood smears was carried out with differential leukocyte count.

A biochemical blood assay was performed on a Cobas 6000 analyzer (c 501 module, Roche Diagnostics GmbH, Germany); test systems were Roche Diagnostics (Switzerland). A colorimetric method was used to determine total protein, bile acids, and alkaline phosphatase. An enzymatic colorimetric method was used to determine triglycerides, alpha-amylase, and lipase. A kinetic method was used to determine alanine aminotransferase, aspartate aminotransferase, creatinine, and lactate dehydrogenase. A hexokinase method was used to determine glucose.

Statistical analysis

The obtained digital data were processed statistically using the Student's t-test. The Microsoft Excel 2016 software was utilized for the statistical analysis. The following calculations were carried out using it. To compare the average values of hematological and biochemical parameters, the arithmetic mean values with a quadratic deviation were first calculated for each group. Also in the software, the formula calculated a substantial difference t score, that is, a number showing how many times the difference between the arithmetic means is greater than the square root of the sum of mean squared errors. Further, the significance of the obtained results was manually assessed in the Student's table using the t score and the joint degree of freedom. The results were considered significant at $p \leq 0.05$ (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

RESULTS AND DISCUSSION

The chickens from the experimental groups actively moved in their cages for seven days, and their behavior did not differ from the controls. The assessment results of the chickens' weight during the experiment are summarized in Table 1.

The chickens from the first experimental group were found to have a significant decrease in body weight. Thus, the values of their body weight were 0.25 ± 0.004 kg versus 0.27 ± 0.001 kg ($p < 0.01$) in the control group after the drug administration on day 8 of the experiment. In the chickens from the second experimental group, a 2.9% increase in the body weight was recorded with Iverbutan in the therapeutic dose on the seventeenth day of the study compared with the control data. The chickens' temperature status is shown in Table 2.

Table 1. The body weight of the Hisex White cross chickens on days 1, 8, and 17 of the experiment at the VNIIP – FSC VIEV Podolsk Base, Russia

Group	Study period (Day)		
	Day 1 (kg)	Day 8 (kg)	Day 17 (kg)
First Experimental Group	0.19±0.001	0.25±0.004**	0.35±0.003
Second Experimental Group	0.19±0.001	0.27±0.002	0.36±0.002
Control Group	0.19±0.001	0.27±0.001	0.35±0.002

**: Means in a column differ significantly from the controls ($p < 0.01$)

Table 2. The temperature status of the Hisex White cross chickens on days 1, 8, and 17 of the experiment at the VNIIP – FSC VIEV Podolsk Base, Russia

Group	Study period (Day)		
	1 (°C)	8 (°C)	17 (°C)
First Experimental Group	41.52±0.73	41.76±0.38	41.48±0.38
Second Experimental Group	41.10±0.65	41.10±0.50	41.24±0.53
Control Group	41.46±0.52	41.44±0.59	41.26±0.55

The body temperature of the chickens from two experimental and one control groups corresponded to the physiological standard for this animal species. On the eighth day of the study, the chickens from the first experimental group showed a 0.8% increase in the body temperature (41.76 ± 0.38°C versus 41.44 ± 0.59°C control). This value was comparable with the upper threshold of the physiological standard for chickens. The stated above is obviously associated with hepatotoxicity (Altuntas et al., 2003) during the drug administration in an increased dose (Table 3). The chickens from the second experimental group showed no rising tendency in body temperature on the eighth day of the study compared to the control.

The biochemical and hematological parameters of the Hisex White chickens are summarized in tables 3 and 4.

Table 3. Biochemical parameters of the Hisex White cross chickens' blood on days 1, 8, and 17 of the experiment at the VNIIP - FSC VIEV Podolsk Base, Russia

Parameter		Control group	First experimental group	Second experimental group
Day 1				
	Aspartate Aminotransferase (U/L)	258.20 ± 12.39	260.20 ± 29.52	243.60 ± 19.28
	Alanine Aminotransferase (U/L)	11.20 ± 3.66	10.80 ± 5.07	11.40 ± 3.68
	Bile Acids (µmol/L)	50.40 ± 4.69	49.60 ± 2.86	51.20 ± 5.15
	Creatinine (µmol/L)	24.00 ± 3.16	26.00 ± 3.62	24.60 ± 4.78
	Total Protein (g/L)	31.40 ± 2.08	31.60 ± 2.08	33.20 ± 3.33
	Alkaline Phosphatase (U/L)	1030.0 ± 31.73	1071.4 ± 40.36	1070.2 ± 26.83
	α-Amylase (U/L)	394.60 ± 81.05	450.80 ± 111.53	421.80 ± 129.03
	Glucose (mmol/L)	12.80 ± 0.14	12.64 ± 0.14	12.84 ± 0.16
	Lactate Dehydrogenase (U/L)	508.20 ± 7.68	518.20 ± 10.84	515.60 ± 11.27
	Triglycerides (mmol/L)	4.68 ± 0.18	4.82 ± 0.22	4.98 ± 0.17
	Lipase (U/L)	8.50 ± 0.32	8.68 ± 0.29	8.58 ± 0.32
Day 8				
	Aspartate Aminotransferase (U/L)	271.60 ± 12.05	427.60 ± 54.73*	261.20 ± 30.77
	Alanine Aminotransferase (U/L)	12.20 ± 1.84	25.80 ± 4.76*	11.60 ± 2.99
	Bile Acids (µmol/L)	48.40 ± 4.17	67.00 ± 3.62*	49.20 ± 1.62
	Creatinine (µmol/L)	25.00 ± 4.56	22.60 ± 2.57	24.60 ± 4.69
	Total Protein (g/L)	31.40 ± 3.79	32.00 ± 1.96	31.80 ± 2.22
	Alkaline Phosphatase (U/L)	1062.2 ± 41.24	1089.8 ± 41.04	1072.8 ± 26.12
	α-Amylase (U/L)	441.40 ± 84.87	465.80 ± 118.81	484.60 ± 53.86
	Glucose (mmol/L)	12.66 ± 0.16	12.08 ± 0.22	12.96 ± 0.12
	Lactate Dehydrogenase (U/L)	526.0 ± 8.60	544.60 ± 5.09	519.60 ± 7.19
	Triglycerides (mmol/L)	4.66 ± 0.18	4.32 ± 0.06	4.54 ± 0.13
	Lipase (U/L)	8.58 ± 0.21	8.84 ± 0.16	8.64 ± 0.23
Day 17				
	Aspartate Aminotransferase (U/L)	250.60 ± 37.80	259.80 ± 32.87	250.40 ± 33.30
	Alanine Aminotransferase (U/L)	13.60 ± 2.57	12.00 ± 3.16	12.80 ± 3.87
	Bile Acids (µmol/L)	50.60 ± 3.68	49.20 ± 3.09	49.40 ± 3.24
	Creatinine (µmol/L)	24.20 ± 3.76	23.00 ± 4.89	27.00 ± 2.63
	Total Protein (g/L)	30.20 ± 2.83	31.40 ± 3.35	32.20 ± 2.04
	Alkaline Phosphatase (U/L)	1087.0 ± 40.28	1063.8 ± 40.57	1097.8 ± 28.36
	α-Amylase (U/L)	410.40 ± 69.44	441.20 ± 97.07	465.60 ± 35.77
	Glucose (mmol/L)	12.94 ± 0.16	12.84 ± 0.16	13.04 ± 0.17
	Lactate Dehydrogenase (U/L)	517.20 ± 7.28	522.60 ± 7.70	524.60 ± 5.67
	Triglycerides (mmol/L)	4.84 ± 0.17	4.96 ± 0.21	4.82 ± 0.20
	Lipase (U/L)	8.56 ± 0.28	8.58 ± 0.24	8.66 ± 0.25

*: Means in a row differ significantly from the controls ($p < 0.05$)

Table 4. Hematological parameters of the Hisex White cross chickens on days 1, 8, and 17 of the experiment at the VNIIP - FSC VIEV Podolsk Base, Russia

Parameter	Control group	First experimental group	Second experimental group
Day 1			
Hemoglobin (g/L)	149.2 ± 1.93	148.8 ± 2.29	149.4 ± 2.25
Erythrocytes ($\times 10^{12}$/L)	3.04 ± 0.11	2.96 ± 0.11	3.0 ± 0.11
Leukocytes ($\times 10^{9}$/L)	21.08 ± 0.56	20.78 ± 0.58	21.28 ± 0.54
Pseudoeosinophiles (%)	27.8 ± 0.37	28.4 ± 0.24	28.2 ± 0.58
Basophiles (%)	2.2 ± 0.2	1.6 ± 0.24	1.8 ± 0.37
Eosinophiles (%)	6.0 ± 0.32	6.6 ± 0.24	6.6 ± 0.24
Monocytes (%)	4.6 ± 0.4	5.4 ± 0.51	5.6 ± 0.51
Lymphocytes (%)	59.4 ± 0.4	58.0 ± 0.63	57.8 ± 0.58
Day 8			
Hemoglobin (g/L)	146.0 ± 1.3	141.4 ± 1.44	150.4 ± 1.69
Erythrocytes ($\times 10^{12}$/L)	3.02 ± 0.07	2.7 ± 0.11	3.14 ± 0.09
Leukocytes ($\times 10^{9}$/L)	21.42 ± 0.35	20.40 ± 0.34	21.08 ± 0.37
Pseudoeosinophiles (%)	27.0 ± 0.55	28.6 ± 0.51	27.8 ± 0.37
Basophiles (%)	1.6 ± 0.24	1.8 ± 0.37	1.6 ± 0.24
Eosinophiles (%)	6.8 ± 0.37	6.8 ± 0.37	6.8 ± 0.37
Monocytes (%)	5.8 ± 0.58	5.6 ± 0.4	6.6 ± 0.51
Lymphocytes (%)	58.8 ± 0.58	57.2 ± 0.73	57.2 ± 1.2
Day 17			
Hemoglobin (g/L)	148.0 ± 1.67	148.8 ±2.35	149.8 ± 2.13
Erythrocytes ($\times 10^{12}$/L)	3.06 ± 0.13	3.1 ± 0.10	3.18 ± 0.10
Leukocytes ($\times 10^{9}$/L)	21.4 ± 0.29	21.56 ± 0.35	21.36 ± 0.39
Pseudoeosinophiles (%)	28.6 ± 0.4	28.8 ± 0.37	28.4 ± 0.51
Basophiles (%)	1.8 ± 0.2	2.0 ± 0.32	1.8 ± 0.37
Eosinophiles (%)	7.4 ± 0.24	7.2 ± 0.37	7.2 ± 0.37
Monocytes (%)	6.6 ± 0.51	6.4 ± 0.68	5.8 ± 0.58
Lymphocytes (%)	55.6 ± 0.93	55.6 ± 1.21	56.8 ± 1.07

The main mechanism of the cytotoxic action of various drugs is damage to cell plasmalemma and its cytoskeleton. The described process is accompanied by the release of cytosolic enzymes into the blood, namely, alanine aminotransferase and aspartate aminotransferase (Floyd et al., 2006; Yin et al., 2020). The liver function in chickens was assessed by a combination of biochemical parameters (Vinogradova et al., 1989; Altuntas et al., 2003; Varga et al., 2017). Thus, on the eighth day, the blood of the chickens from the first experimental group showed a significant increase in the activity of alanine aminotransferase by 2.1 ($p < 0.05$), aspartate aminotransferase by 1.6 ($p < 0.05$), and bile acids by 1.4 ($p < 0.05$) compared to the control. This work detected an increase predominantly in the alanine aminotransferase activity, a marker of hepatocyte cytolysis. (Donkova, 2003).

The AST to ALT ratio is known to decrease with toxic liver damage (Ushakova et al., 2021), which was also found in the chickens from the first experimental group on day 8 of the study. According to Donkova (2003), the stated ratio ranges in chickens from 20 up to 24 units. Thus, on day 8, the de Ritis ratio in chickens from the first experimental group was 16.6 units (22.3 units in the control). The chickens from the second experimental group had the de Ritis ratio value of 22.5 units (22.3 units in the control). The toxic liver damage developed in chickens was also observed in the pharmaco-toxicological studies during the administration of various drugs in high therapeutic doses (Niyogi and Bhowmik, 2003; Kamel et al., 2010).

The chickens from the first experimental group also showed a number of physiological and biochemical changes which indicate a liver function abnormality during the drug administration in the three-fold therapeutic dose. Thus, on the eighth day of the study, a 4.6% decrease in glucose and a 3.5% increase in lactate dehydrogenase activity were found in the chickens from the first experimental group compared to the control. It is evident that the described changes cause a decrease in carbohydrate metabolism and energy metabolism in the chickens from the first experimental group. This is possibly associated with abnormality in hepatic glycogen synthesis (Chudov and Ismagilova, 2012). The increased lactate dehydrogenase activity may indicate the increased proportion of oxygen-free glycolysis amid decreased hemoglobin by 3.2% and erythrocytes by 10.6% in the chickens from the first experimental group, which contributes to a potential decrease in oxygen delivery to their cells and tissues. These hematological parameters were within the reference constant values (Buyko et al., 2014). This may in part have influenced a decrease in carbohydrate and energy metabolisms in the study chickens. It should be noted that the erythrocyte system is impaired in hepatopathies of various origins (Sysueva, 2008). The stated above causes changes in red blood cells. Moreover, a 7.3% decrease in triglycerides in the chickens from the first experimental group was detected on the eighth day of the study, compared to the control. On the seventeenth day of the study, the above blood parameters in chickens from the first experimental group did not significantly differ statistically from the control group, which attests to the reversibility of hepatotoxic effects (Janakat and Al-Merie, 2002). At the same time, on the eighth day of the study, the chickens from the first experimental group showed no changes in hepatic protein synthesis, which may be due to the stable rate of protein metabolism with butaphosphan (Rollin et al., 2010), which is part of the drug.

The study by Arkhipov et al. (2014) demonstrated a significant increase in the alanine aminotransferase activity in the blood. These data were obtained in the study on the tolerability of an antiparasitic drug containing ivermectin for oral administration, which was given to broilers at doses of 1.2 and 2 mg/kg (by active ingredient). Moreover, they reported no significant changes in the values of the alanine aminotransferase activity in the chickens' blood after 10 days, compared to values of the control group. The stated above is consistent with the results presented in the current study on reversible hepatotoxic effect.

The tendency for an increase in the percentage of eosinophils in the chickens from the first experimental group was observed. Thus, the percentage of their eosinophils ranged from 6.6 ± 0.24% to 7.2 ± 0.37% during the experiment. At this time, a decrease was determined in the percentage of lymphocytes from 58.0 ± 0.63% to 55.6 ± 1.21%. On this basis, it can be assumed that there is a tendency for possible initiation of allergic processes and immunosuppression (Wakenell, 2010). However, all hematological parameters in the chickens from the first experimental group were within the reference values.

The chickens from the second experimental group showed an increase in the α-amylase activity by 9.8 and 13.5% on the eighth and seventeenth days of the study, respectively, compared with the control. On the eighth day of the study, a 2.4% increase in glucose was found in the blood of the chickens from the second experimental group related to the control. Obviously, the chickens from the second experimental group showed a rising tendency in carbohydrate and energy metabolisms during the administration of the combined drug in the therapeutic dose. On day 17 of the study, a 6.6% increase should be noted in total protein in chickens from the second experimental group related to the control. At the same time, they showed an increase in hemoglobin by 3.0 and 1.2%, as well as erythrocytes by 4.0 and 3.9% on the eighth and seventeenth days of the study, respectively, compared to the control values. Biochemical and hematological parameters of the chickens (Tables 3 and 4) show that the drug in the therapeutic dose does not have a negative effect on the physiological state of the chickens. All blood parameters were within the reference values (Buyko et al., 2014). Furthermore, the increased body weight of the chickens on the seventeenth day of the study confirms the previously stated.

CONCLUSION

Iverbutan administered for seven days in the therapeutic dose and three-fold therapeutic dose did not cause any change in the ethologic status of the Hisex White chickens. There were no negative changes in the physiological or biochemical status of the chickens after the drug was orally administered in the therapeutic dose for seven days, compared to the control chickens. The three-fold therapeutic dose can be considered as a threshold due to the rendered reversible hepatotoxic effect. Thus, the obtained study results confirm the safety of the drug in the recommended dosage. The obtained data on

studying the Iverbutan tolerability on target animals (chicken breeds for producing eggs) allow us to study its therapeutic efficacy in the industrial sector, which requires further research in this field.

DECLARATIONS

Authors' contribution

Evgenia Indyuhova developed the study design, conducted research work, collected and analyzed the data, and prepared the Article. Mikhail Arisov participated in the development of the study design and analyzed the study results. Vladimir Maximov analyzed the obtained data. Tatiana Azarnova analyzed and interpreted the obtained data.

Consent to publish

All authors agree to publish this manuscript.

Competing interests

The authors have declared that no competing interest exists.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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