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Study of the Biological Effect of Iron Nanoparticles

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Abstract—The biological effect of iron nanoparticles with a size of 20–40nm obtained by the plasmochemical method are studied. An antibacterial effect on antibiotic resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* is found at concentrations of iron nanoparticles in a range of 0.1–10 mg/mL. The toxic effect of iron nanoparticles administered this way is revealed in male mice, which is manifested in a change in biochemical parameters of carbohydrate, lipid, and protein metabolisms.

Keywords: iron nanoparticles, metabolism, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

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INTRODUCTION

The medical application of nanotechnologies has resulted in a new field of study, nanomedicine, which has been effectively developed in recent decades [1]. In medicine, capabilities of nanotechnologies are directed toward the control of physical, chemical, and biological processes occurring in living organisms at the molecular level using nanomaterials and nanoparticles [2].

One of the main reasons for a change in physical and chemical properties of small particles with a decrease in their size is an increase in a relative percentage of surface atoms. From the point of view of energy, a decrease in particle sizes leads to an increase in the role of surface energy. A decrease in particle sizes to the nanometer scale results in the appearance of the so-called quantum dimensional effect. At present, the unique physical properties of nanoparticles appearing due to surface or quantum dimensional effects are objects of intensive studies [3, 4].

Today, nanodevices based on nanotechnologies are designed and put into practice that make it possible to perform all necessary operations from diagnostics and monitoring to the destruction of pathogenic microorganisms, restoration of damaged organs, and supplying necessary substances to the organism [5, 6].

Nanoparticles could be used as stimulating drugs, since they have pronounced biological activity. A high antimicrobial activity of metal nanoparticles was found; elements of filtering devices for purifying drinking water were developed based on such materials [7]. The application of nanoparticles could be useful in treating diseases such as trophic ulcers, the duration of wound healing being reduced several times [8]; purulent osteomyelitis; different burning wounds; and ENT diseases in childhood [8–12].

It should be noted that using nanoparticles it is necessary to painstakingly study the effect of nanoparticles on healthy cells of the organism and to control the absence of any toxic effect of nanoparticles, which is difficult in itself [1]. For some nanomaterials, toxic as well as useful effects were found [13, 14].

The data on toxic properties of some materials indicate that toxicity depends not only on the physical origin, production method, size, and structure of nanoclusters and nanoparticles, but also on the biological model used for the tests. Target organs and mechanisms of development of the toxic effect are diverse. Properties of some nanosized materials are manifested in the capability of induction of reactive oxygen species [15, 16], while other nanoparticles could penetrate through tissue barriers into cells and interact with intracellular components [17]. This is a precondition for modeling environmental pressure of metals on the organism [13].

The present study considers some aspects of the biological effect of iron nanoparticles on the organism.

MATERIALS AND METHODS

Finely dispersed iron nanopowders synthesized in the plasmochemical complex of the State Research Institute of Organic Chemistry and Technology of Elementoorganic Compounds (GNIIKhTEOS, Moscow, laboratory 33) were used in the study. The iron nanopowders were obtained from coarse powders using a plasma technology based on the evaporation of materials (coarse powder or bar) in a plasma stream at a temperature of 5000–6000 K and condensing the vapor to ultrafine particles of a desired size (particle dispersion of 30 nm). The main element of the plasmochemical device is a reactor–an electric arc evaporator with vortex stabilization of a high aspect ratio plasma stream; beside it, it includes a dispenser of dispersed material; a piston apparatus with liquefaction of the material supplied; a tube-in-tube cooler of dispersed material with high rates of an aerosol stream; powder classifiers, an inertial and vortical cyclones with a ratio $d_{out}\backslash D \leqslant 0.33$; and nanopowder sleeve-type trapping filter. Conventional instruments (process gas compressors, gas circuit receivers, gas and water dispensers, a water pump, and a vacuum pump) were also used. Nanopowder purity was no less than 99.8%. The average particle size was within 20–40 nm.

THE STRUCTURE OF IRON NANOPARTICLES

The study of nanoparticles and solutions on their basis was performed using X-ray absorption spectroscopy based on the analysis of fine structure of spectral lines near the absorption edge (XANES, X-ray absorption near edge spectroscopy). X-ray K-edge absorption spectra (XANES) of iron in nanoparticles and reference samples (iron foil and iron oxides) were measured using an R-XAS Looper laboratory X-ray absorption spectrometer (Rigaku, Japan) established in the Nanoscale Structure of Matter Center for Collective Use of the Southern Federal University.

Measurement of the Fe K edge in nanoparticles was carried out using a Ge(311) crystal monochromator in a fluorescent mode using a I0 Ar-300 detector and an I SSD semiconductor detector with an amplifier. The voltage on an X-ray tube was $U = 25$ kV, and the current was $I = 70$ mA. Nanoparticle samples studied were measured in a dry phase and in a 0.9% aqueous solution of sodium chloride.

The absorption spectra of nanoparticles were compared with the reference samples, and a conclusion on the structure of the nanoparticles was made by the shape and energy position of peculiarities of the absorption spectra.

BACTERIAL CELLS AND MEDIA

Studies were conducted on 20 strains of *Pseudomonas aeruginosa* and 20 strains of *S. aureus* resistant to five or more specialized antibiotics isolated from patients with suppurative complications that were under treatment in the orthopedic trauma hospital of the Saratov Research and Development Institute of Traumatic and Orthopedic Surgery (SarNIITO). Vials with solutions of nanopowders in saline at different concentrations (0.1 mg/mL, 1 mg/mL, 10 mg/mL) were added with 100 μL of a final suspension (50000 CFU/mL) of microorganisms for 30, 60, 120, and 180 min at room temperature. A bacterial suspension diluted in similar proportions with saline and incubated during the same time was used as a control. Then, seeding onto meat-peptone agar was performed from each of the dilutions, 100 μL being used for each of the Petri dishes. All the dishes were put in a thermostat for 24 h at 37°C. The colonies were counted on the next day.

BIOCHEMICAL METHODS

Studies were performed on white outbred mice. Control and experimental groups were formed from 2-month-old males with a weight of 20 ± 3 g. The mice were under standard conditions with a natural change of lighting and general vivarium diet. All the animals have ad libitum access to food and water.

Mice were divided into one control group (ten mice) with vegetable oil of 10 μL without metal nanopowders being administered, a reference group, and seven experimental groups (70 mice).

The experimental part of the study was performed in accordance with protocols of the Geneva Convention and in accordance with principles of good laboratory practice (National standard of the Russian Federation, GOST R 53434-2009) [18–20].

Experimental groups were formed depending on a daily concentration of the nanopowder administered. The first group was administered with 50 μg/kg of iron nanopowder, the second one was administered with 100 μg/kg, the third one with 1 μg/kg, the fourth one with 1.25 mg/kg, the fifth one with 2.5 mg/kg, the sixth one with 5 mg/kg, and the seventh one with 12.5 mg/kg.

The nanoparticle concentrations chosen do not exceed maximum endurable doses for the given metal. All the concentrations of the nanopowder were introduced for 6 days in the form of freshly prepared suspensions in vegetable oil (refined Milora oil) with a volume of 10 μg once a day per os using a probe. The suspensions were prepared by combining samples of the nanoparticles (amount of 1000 doses) with vegetable oil with a volume of 10 mL and stirred on a magnetic stirrer for 30 min to prevent sedimentation of the particles and formation of conglomerates.

The animals were not killed in the experiment. Blood for biochemical studies was taken from the caudal vein. The study was performed in accordance with principles of humane treatment of animals given in guidelines by N.N. Karkischenko and practical guid-

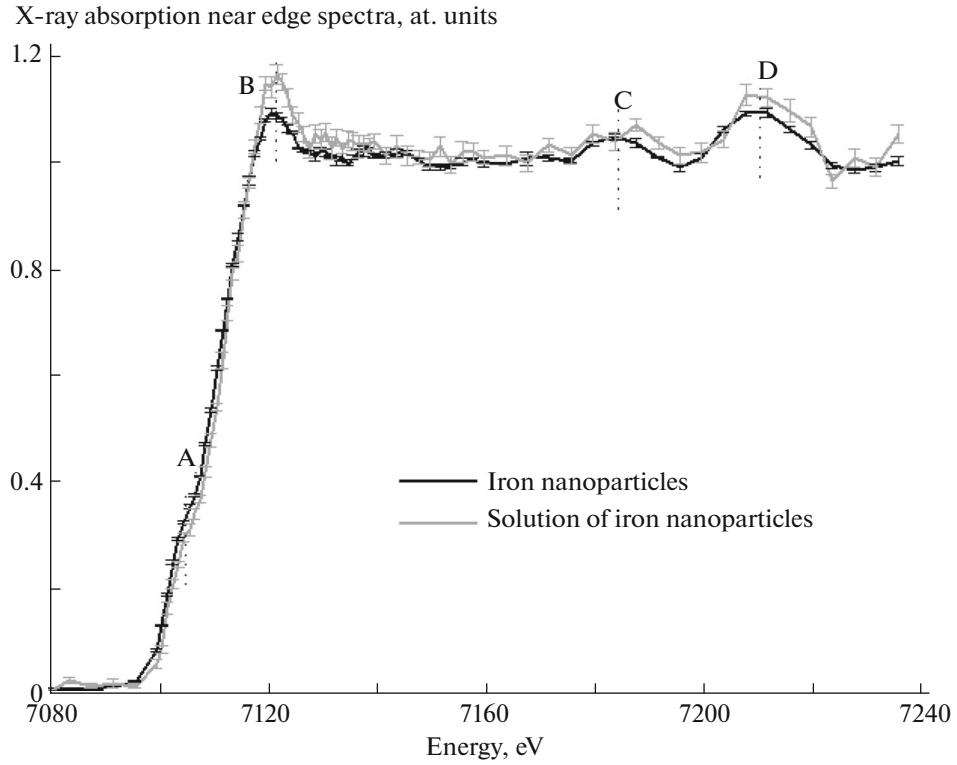

Fig. 1. Comparison of experimental Fe *K*-XANES spectra of powder of iron nanoparticles and their solution.

ance for biological safety under laboratory conditions [18, 19].

Assessment of biochemical indices of blood was performed using a HOSPITEX Screen master semiautomatic biochemical analyzer equipped with a thermostat, photometer, and microprocessor. Working on the analyzer, standard kits of reagents (ZAO Diakon-DC) were used. The blood serum had concentrations of the following metabolites determined that describe

(i) carbohydrate metabolism: glucose (glucose oxidase method), lactate (lactate oxidase method), and pyruvic acid (Umbreit method);

(ii) protein metabolism: total protein (biuret test), albumin (bromocresol green reaction), urea (urease glutamate dehydrogenase method), and creatinine (Jaffe reaction);

(iii) lipid metabolism: total cholesterol (cholesterol oxidase method);

(iv) pigmental metabolism: total bilirubin (dichloroaniline reaction) and direct bilirubin (diazotized sulfanilic acid reaction);

(v) activity of indicator enzymes: AST, ALT, lactate dehydrogenase (LDH), CPK, and GGT (colorimetric method according to Seitz/Persin) by uniform methods developed according to recommendations of the International Federation of Clinical Chemistry.

According to guidelines on laboratory animals and alternative models in biomedical research, animal models could be used to study changes in biochemical indices, to interpret them, and apply them in regards to indicators of human health [19].

RESULTS AND DISCUSSION
THE STRUCTURE OF IRON NANOPARTICLES

Upon investigation of the structure of nanomaterials with characteristic dimensions of 10–100 nm, conventional X-ray methods could not be used. To determine the structure of such materials and phase composition of their components, X-ray absorption spectroscopy is used. XANES is a method that, in comparison with other X-ray techniques, could give more detailed information on the geometry of objects studied. The fine structure of X-ray absorption includes an oscillating X-ray absorption coefficient with energy higher than the absorption edge. The fine structure of X-ray absorption is a characteristic feature of the given material and depends on its atomic structure and electron and vibrational properties. Due to this reason, this method contains important information on the material, e.g., its local atomic structure (types of atoms, their coordinates). Studying the structure of the edge of X-ray absorption, information on a charge state of atoms in the substances studied, peculiarities of chemical bonds between atoms, and geometrical parameters of the substance could be obtained. Based on a comparison of the spectra with

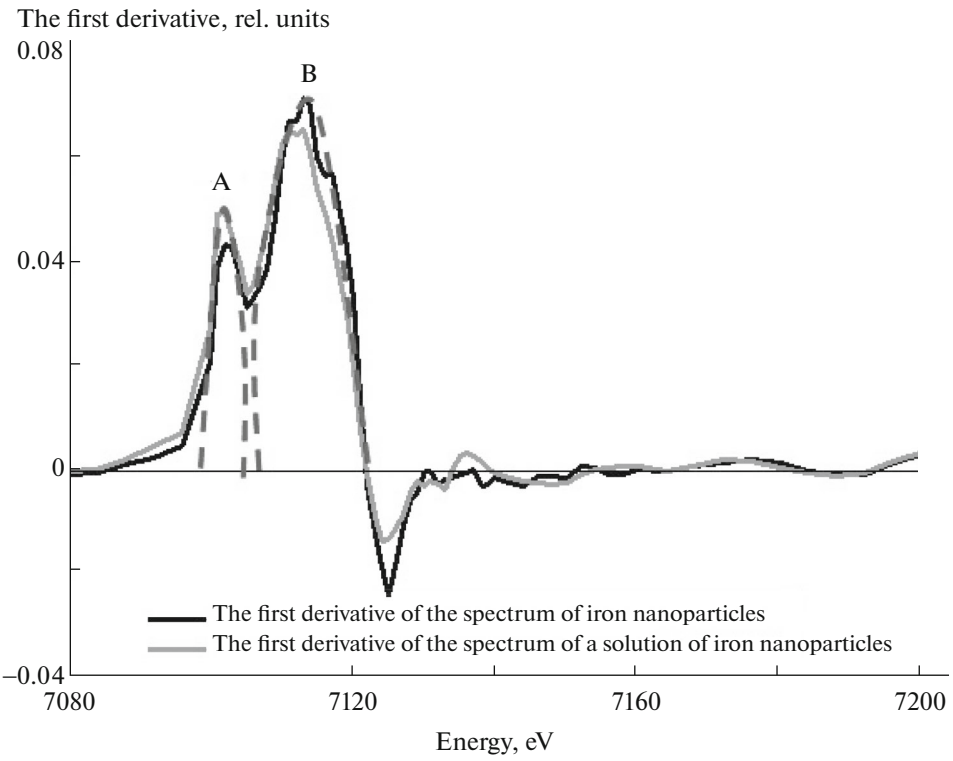

Fig. 2. Comparison of curves of the first derivatives of experimental Fe K-XANES spectra of powder of iron nanoparticles and their solution.

those of the reference samples, a conclusion on the presence in the compound of one or another phase could be made.

Figure 1 demonstrates Fe *K*-XANES spectra of the powder of iron nanoparticles and its solution.

Figure 1 shows that the nanoparticle structure in the solution has not changed and represents particles of metal iron. An increase in the intensities of peculiarities of the solution spectrum indicates that the solution contains particles smaller than those in the powder, since larger particles have settled to the bottom of the cuvette with the solution upon measurements of the absorption spectra. No change in the spectrum shape or shift in the preedge of the spectrum of iron nanoparticles in the solution with respect to the spectrum of the powder are observed, which is evidence that the iron nanoparticles have not been oxidized in the solution.

A shift in the edge of the absorption spectra could be clearly seen in curves of the first derivatives in the pre-edge region (Fig. 2). The size of the particles with the same degree of oxidation significantly affects intensities of the spectrum peculiarities, the spectrum having insignificant shifts of the absorption edge; however the positions of inflection points of the absorption edge should remain the same. Thus, to demonstrate that the particles have the same degree of oxidation and do not differ, it is necessary to study the positions of inflection points of the spectra in the absorption edge. The position of an inflection point of absorption edge is determined by the maximum of the first derivative in this point.

All extremums of curves of the first derivatives of two spectra in Fig. 2 are under each other, an insignificant shift in positions of the peculiarities A and B without doubt being connected with a measurement error. A blue dotted line denotes shapes of maxima for the energy position of the maxima to be determined. The intensities of peculiarities of the spectra could differ significantly, but the positions of maxima remained almost the same. Therefore, metal iron nanoparticles were not oxidized in the solution.

ANTIBACTERIAL ACTIVITY OF IRON NANOPARTICLES

It was found that antibacterial activity of iron nanoparticles varied within a wide range of concentrations from 0.1 mg/mL to 10 mg/mL. The use of a concentration of 0.1 mg/mL led to a decrease in the number of cells surviving by 52% ($p < 0.001$) at maximum exposure. The use of the maximum concentration of 10 mg/mL for 180 min resulted in a decrease in the number of colonies by 65%. The results of counting colonies of *Pseudomonas aeruginosa* having grown on solid media after exposure to iron nanoparticles of different concentrations and the results of counting in the

Table 1. Number of colonies of *Pseudomonas aeruginosa* grown on solid media after exposure to iron nanoparticles

Time of exposure, min	Experimental groups			
	1st 0.1 mg/mL, $n = 20$ ($M \pm m$)	2nd 1 mg/mL, $n = 20$ ($M \pm m$)	3rd 10 mg/mL, $n = 20$ (M $\pm m$)	Control $n = 20$ ($M \pm m$)
30	618 ± 25.3**	584 ± 26.2***	503 ± 28.7***	686 ± 25.8
60	508 ± 21.8***	486 ± 29.7***	415 ± 33.9***	694 ± 27.3
120	355 ± 20.4***	326 ± 28.4***	296 ± 27.8***	722 ± 23.8
180	358 ± 31.3***	303 ± 24.8***	266 ± 39.1***	759 ± 29.2

** $p < 0.01$; *** $p < 0.001$

Table 2. Number of colonies of *St. aureus* grown on solid media after exposure to iron nanoparticles

Time of exposure, min	Experimental groups			
	1st 0.1 mg/mL, $n = 20$ ($M \pm m$)	2nd 1 mg/mL, $n = 20$ ($M \pm m$)	3rd 10 mg/mL, $n = 20$ ($M \pm m$)	Control $n = 20$ ($M \pm m$)
30	1107.5 ± 73.7***	1098.7 ± 72.5***	1056.3 ± 89.5***	1234.2 ± 46.7
60	1186.8 ± 44.7***	1159.2 ± 60.1***	968.4 ± 64.2***	1380.7 ± 31.3
120	849.1 ± 38.8***	629.6 ± 58.5***	596.4 ± 41.3***	1454.1 ± 23.5
180	824.3 ± 41.7***	728.6 ± 48.1***	685.7 ± 47.8***	1556.0 ± 68.0

** $p < 0.01$; *** $p < 0.001$

control group, which has not been subjected to the effect of superdispersed powders, are given in Table 1.

The results of counting colonies of *Staphylococcus aureus* having grown on solid media after exposure to iron nanoparticles of different concentrations for 30–180 min and the results of counting in the control group, which has not been subjected to the effect of superdispersed powders, are given in Table 2.

After exposure to iron nanoparticles at concentrations of 0.1, 1, and 10 mg/mL, a statistically significant decrease in the number of bacterial cells surviving from 54 to 46% was observed with an increase in the exposure and concentration of the preparation ($p < 0.001$). At lower concentrations, no antibacterial effect of the iron nanoparticles was found.

Thus, iron nanoparticles at concentrations of 0.1–10 mg/mL have an antibacterial effect in regards to clinical strains of *Staphylococcus aureus*.

A CHANGE IN BIOCHEMICAL INDICES IN BLOOD SERUM OF WHITE OUTBREAD MICE

The main emphasis studying the activity of iron nanopowders is made on the development and testing of food supplements and biologically active drugs for iron deficiency in the organism. To solve problems related to the introduction of iron powders into the organism, it is necessary above all to have clear ideas on the biotransformation of iron powder and its effect on the organism as a whole.

The studies performed in mice on their metabolism upon per os administration of the iron nanopowders revealed significant changes in indices describing carbohydrate, lipid, and protein metabolisms, and enzyme activities (Tables 3–6).

An analysis of biochemical indices revealed that the introduction of the iron nanopowder to the animals lead to a reliable increase in the glucose level in the blood serum.

Glucose is the main index of carbohydrate metabolism and is present in most organs and tissues. Its concentration in blood is determined by the activity of glycogenolysis, glyconeogenesis, and glycolysis. The maintenance of a constant glucose concentration in blood is a necessary condition for the normal functioning of the organism, since glucose is a main—and for some tissues the only—source of energy in the cell.

Upon the administration of iron nanoparticles, a significant increase ($p < 0.001$) in the glucose content in the blood serum was observed when compared to the control (7.41 ± 0.36 mmol/L).

An increase in the glucose concentration under the effect of iron nanoparticles was linear with the attainment of saturation: the maximum glucose concentrations were found in the blood serum upon the introduction of the nanoparticles at a concentration of 1.25–12.5 mg/kg (an increase in the concentration by a factor of 1.5–1.6) when compared to the control.

Table 3. Change in metabolite concentrations in blood serum under the effect of iron nanoparticles

Biochemical index	Control	Concentration of iron nanoparticles		
		50 μg/kg	100 μg/kg	1 mg/kg
	$M \pm m$	$M \pm m$	$M \pm m$	$M \pm m$
glucose, mmol/L	7.41 ± 0.36	9.09 ± 0.67***	9.27 ± 0.37***	10.24 ± 0.29***
lactate, mmol/L	20.37 ± 1.54	6.20 ± 0,63***	8.40 ± 0.33***	12.3 ± 0.31***
pyruvic acid, mmol/L	0.12 ± 0.02	0.71 ± 0.01***	0.76 ± 0.11***	0.79 ± 0.21***
total protein, g/L	121.44 ± 11.00	143.80 ± 0.66***	147.0 ± 6.86***	150.50 ± 9.31***
albumin, g/L	65.05 ± 2.37	67.20 ± 0.59	84.0 ± 0.69***	115.00 ± 5.45***
urea, mmol/L	3.73 ± 0.16	9.14 ± 0.14***	10.3 ± 1.24***	8.7 ± 0.72***
creatinine, μmol/L	155.20 ± 11.8	292.20 ± 5,74***	305.20 ± 11.7***	317.0 ± 9.44***
cholesterol, mmol/L	5.05 ± 0.45	4.20 ± 0,21***	4.10 ± 0.31***	3.89 ± 0.19***
total bilirubin, mg/dL	1.4 ± 0.12	3.93 ± 0.06***	4.01 ± 0.08***	4.40 ± 0.09***
direct bilirubin, mg/dL	1.13 ± 0.06	1.85 ± 0.01***	1.94 ± 0.03***	1.73 ± 0.05***
indirect bilirubin, mg/dL	0.56 ± 0.03	2.09 ± 0.05***	2.17 ± 0.06***	2.44 ± 0.08***

** $p < 0.01$; *** $p < 0.001$

Table 4. Change in metabolite concentrations in blood serum under the effect of iron nanoparticles (continuation)

Biochemical index	Control	Concentration of iron nanoparticles			
		1.25 mg/kg	2.5 mg/kg	5 mg/kg	12.5 mg/kg
	$M \pm m$	$M \pm m$	$M \pm m$	$M \pm m$	$M \pm m$
glucose, mmol/L	7.41 ± 0.36	11.74 ± 0.69***	11.84 ± 1.03***	11.34 ± 0.18***	12.14 ± 0.25***
lactate, mmol/L	20.37 ± 1.54	15.20 ± 0.29**	14.84 ± 0.69**	13.73 ± 0.29**	14.34 ± 0.19**
pyruvic acid, mmol/L	0.12 ± 0.02	0.84 ± 0.01***	0.99 ± 0.01***	1.39 ± 0.08***	1.33 ± 0.23***
total protein, g/L	121.44 ± 11.00	153.80 ± 8.11***	157.00 ± 5.38***	167.00 ± 7.38***	195.50 ± 8.60***
albumin, g/L	65.05 ± 2.37	144.00 ± 2.65***	70.70 ± 1.40**	74.70 ± 1.50**	83.2 ± 6.13**
urea, mmol/L	3.73 ± 0.16	6.07 ± 0.32***	5.81 ± 0.29***	5.90 ± 0.39***	7.01 ± 0.16***
creatinine, μmol/L	155.20 ± 11.8	338.00 ± 9.98***	274.50 ± 10.66***	284.50 ± 11.6***	240.60 ± 8.78***
cholesterol, mmol/L	5.05 ± 0.45	3.34 ± 0.09**	5.49 ± 0.36	5.58 ± 0.56	4.70 ± 0.24
total bilirubin, mg/dL	1.54 ± 0.12	4.20 ± 0.07***	3.94 ± 0.07***	3.89 ± 0.17***	4.09 ± 0.13***
direct bilirubin, mg/dL	1.13 ± 0.06	1.66 ± 0.01**	1.19 ± 0.01**	1.59 ± 0.08**	1.61 ± 0.03**
indirect bilirubin, mg/dL	0.56 ± 0.03	2.54 ± 0.06***	2.75 ± 0.06***	2.65 ± 0.09***	2.36 ± 0.04***

** $p < 0.01$; *** $p < 0.001$.

Table 5. Change in enzyme activity in blood serum under the effect of iron nanoparticles

Biochemical index	Control	Concentration of iron nanoparticles		
		50 μg/kg	100 μg/kg	1 mg/kg
	$M \pm m$	$M \pm m$	$M \pm m$	$M \pm m$
AST, IU	124.00 ± 9.25	327.00 ± 12.81***	330.00 ± 11.8***	338.30 ± 51.9***
ALT, IU	56.00 ± 2.06	82.40 ± 3.62**	73.30 ± 5.52**	75.10 ± 4.9**
LDH, IU	2125.00 ± 53.28	5312.50 ± 72.88***	6700.60 ± 82.8***	8900.00 ± 286.00***
GGT, IU	112.22 ± 16.66	115.70 ± 2.95	121.80 ± 5.95	127.60 ± 6.5
CPK, IU	1390.00 ± 60.88	4636.00 ± 181.00***	4900.00 ± 281.00***	3500.00 ± 219.0***

** $p < 0.01$; *** $p < 0.001$

Table 6. Change in enzyme activity in blood serum under the effect of iron nanoparticles (continuation)

Biochemical index	Control	Concentration of iron nanoparticles			
		1.25 mg/kg	2.5 mg/kg	5 mg/kg	1.25 mg/kg
	$M \pm m$	$M \pm m$	$M \pm m$	$M \pm m$	$M \pm m$
AST, IU	124.00 ± 9.25	342.50 ± 11.91***	372.50 ± 15.33***	362.50 ± 14.3***	620.60 ± 16.3***
ALT, IU	56.00 ± 2.06	68.20 ± 3.39**	77.90 ± 2.77**	79.90 ± 3.7**	75.20 ± 2.6**
LDH, IU	2125.00 ± 53.28	12887.5 ± 280.10***	13786.5 ± 303.6***	14786.50 ± 312.6***	15100.0 ± 152.8***
GGT, IU	112.22 ± 16.6	131.40 ± 2.99**	140.50 ± 1.17**	150.50 ± 11.7**	147.00 ± 12.9**
CPK, IU	1390.00 ± 60.8	2981.00 ± 139.07***	3474.00 ± 46.05***	3574.00 ± 86.5***	3300.00 ± 77.1***

** $p < 0.01$; *** $p < 0.001$

Lactate (lactic acid) is a product of anaerobic metabolism of glucose (glycolysis), which is formed from pyruvic acid under the effect of LDH. The largest amount of lactic acid enters the blood from the skeletal muscles, brain, and red blood cells. The lactate clearance is mainly related to its metabolism in the liver and kidneys.

The introduction of iron nanopowders at chosen concentrations induced a significant decrease ($p < 0.001$) in the lactate content in the blood when compared to the control (20.37 ± 1.54 mmol/L). The lowest lactate concentrations were observed in the experiments with nanoparticle dosage of 50 μg/kg (the lactate level decreased by a factor of 3.5) and 100 μg/kg (the lactate content decreased by a factor of 2.5).

Upon the effect of iron nanoparticles, the content of pyruvic acid increased in the blood serum, an increase in pyruvic acid being dose-dependent. For high concentrations of the nanoparticles of 1.25–12.5 mg/kg, a tenfold increase in the level of pyruvic acid in the blood was noted.

Summarizing the results obtained on the effect of the iron nanoparticles within a concentration range of 50 μg/kg–12.5 mg/kg on the main indices of carbohydrate metabolism, the following conclusions could be made.

One possible reason for the increase in the glucose blood level could be a decrease in the activity of insulin due to the interaction of metal nanoparticles with sulfhydryl groups in the insulin molecule. The impairment of glucose uptake into the cell and its further involvement in metabolic processes to obtain energy results in the activation of compensatory mechanisms, in particular, glyconeogenesis, which uses lactate as a source for glucose synthesis, lactate under the effect of lactate dehydrogenase (LDH) being transformed into pyruvic acid, the main source of glucose synthesis (the content of which increased in the blood of the experimental animals).

To find the biological effect of the nanoparticles on protein metabolism, the blood serum contents of total protein, albumin, urea, and creatinine were determined.

Blood plasma proteins exert numerous functions in the organism, and the protein level is one of the most important laboratory indices. The functions of blood plasma proteins are maintaining colloid-osmotic pressure, blood clotting, involvement in immune reactions, the creation of protein reserve (when starvation proteins break down into amino acids), the maintenance of the cation blood level, and other metabolic processes.

The concentration of the total protein in blood serum mainly depends on the synthesis and breakdown of two main fractions: albumin and globulins. The evaluation of the total protein makes it possible to assess the severity of impairment of protein metabolism.

In the study it was found that introduction of the iron nanopowder led to a change in the content of the total protein when compared to the control (121.44 ± 11.00 g/L), in direct proportion with an increase in the exposure a dose-response regularity being observed.

Upon the introduction of low doses of the particles, an insignificant increase in the concentration of the total protein and its albumin fraction ($p = 0.05$) was found.

An increase in the concentration up to 1.25 and 2.5 mg/kg resulted in a dose-dependent increase in the concentration of the total protein by 30% when compared to the control. The administration of higher concentrations (100 μg/kg–12.5 mg/kg) promoted an increase in the blood protein level by 70%.

The albumin content upon introduction of the iron nanopowder within a concentration range of 12.5 mg/kg increased, on average, by 30–35%, and upon the effect of concentrations of 100 μg/kg–1.25 mg/kg it reliably changed by a factor of 2–2.4.

Since blood proteins exert a transport function transferring different compounds including metals, the results could be related to the organism adapting to metal excess.

The main product of protein decomposition is urea, the biosynthesis of which is performed in the liver from ammonia (ornithine cycle). Urea as a final

product of the protein catabolism is filtered in glomerules and removed with urine; thus, the urea blood concentration reflects the processes of its formation and excretion, i.e., liver and kidney functioning.

The administration of different doses of iron lead to an increase in the blood urea when compared to the control (3.73 ± 0.16 mmol/L).

The most significant changes were induced by the introduction of the lowest particle dose of 50–100 μg/kg (an increase in the urea concentration by a factor of 2.5–3) and by that of the highest dose of 12.5 mg/kg (an increase in the urea concentration by a factor of 2). Doses of the iron nanoparticles of 1–2.5 mg/kg induce a twofold increase in the urea level.

Another product of protein metabolism is creatinine, which upon entry to the muscle tissue from the liver is transformed into phosphocreatine (energy source). In the phosphocreatine metabolism, energy is released and creatinine is formed. Its serum concentration is relatively constant and depends on the equilibrium between the synthesis and excretion.

The studies demonstrated that introducing the iron nanopowder induced an increase in the creatinine concentration by a factor of 1.5–2, which could be also connected with impairment in the excretion function of the kidneys.

An increase in the blood concentration of indices of protein metabolism could be related to the damage of membranes under the effect of metal nanopowders capable of adsorbing on cell membranes.

To assess the state of biological membranes, the cholesterol content in the serum was determined.

Investigations revealed that a reliable decrease in the cholesterol level under the effect of the iron nanoparticles was found upon the introduction of the nanopowder at a concentration of 50 μg/kg–1 mg/kg, other doses inducing insignificant shifts in the cholesterol concentration.

The apoptosis of red blood cells promotes a hemoglobin release, which is transferred into the cells of the reticuloendothelial system of the spleen, where choleverdin is formed. Choleverdin is enzymatically reduced to bilirubin, the main red-yellow bile pigment. Upon hemoglobin breakup, first toxic indirect bilirubin is formed, which in the complex with albumin is transferred in the bloodstream to the liver, where under the effect of glucuronyl transferase it binds to glucuronic acid forming bilirubin monoglucuronide and diglucuronide (direct bilirubin). Herewith, the toxicity inherent in bilirubin is significantly reduced. Conjugated bilirubin is actively excreted against a concentration gradient in the bile ducts and from there into the small intestine.

The study of pigmental metabolism demonstrated that the exposure to the iron nanopowder at all doses used induced an increase in total bilirubin by a factor of 2.5–3 when compared to the control (1.4 ± 0.12 mg/dL), the increase in the bilirubin level occurring mainly due to an increase in the fraction of indirect bilirubin.

The concentration of direct bilirubin upon the effect of the iron nanoparticles increased with the introduction of low concentrations of the particles and remained persistently high upon the administration of high doses of the particles.

Upon introduction of the iron nanoparticles at a dose of 50 μg/kg, the concentration of direct bilirubin increased by 70%; upon introduction of the powder at a dose of 1.25 mg/kg, it increased by 60%; and upon introduction of the nanoparticles at a dose of 12.5 mg/kg, it increased by 55%.

Under the effect of iron nanoparticles, an increase in the concentration of indirect bilirubin by a factor of 3–4.5 was found.

The set of indices of pigmental metabolism indicates a negative effect of the iron nanopowders on the stability of the red blood cells inducing hemolysis. An increase in the bilirubin concentration due to the fraction of indirect bilirubin might be evidence of impairment of the liver function and its inability to bind toxic bilirubin with the formation of bilirubin diglucuronide.

Such changes in pigmental metabolism are similar to manifestations of hemolytic jaundice. An increase in direct bilirubin might indicate a decrease in the hepatocyte capability of metabolism and transport of bilirubin against a concentration gradient to the bile. Such a phenomenon could also be connected with the mechanical obstruction of the biliary excretion due to biliary tract abnormalities caused by the nanoparticles.

In the study, the activity of the key indicator enzymes was studied in the serum under the effect of the iron nanopowder within a range of concentrations of 50 μg/kg–12.5 mg/kg.

The activity of transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), is widely used in biochemical practice as a test measuring the permeability of hepatocyte membranes and severity of cytolytic syndrome, since the greatest specific activity of these enzymes is found in the liver and that of AST in the cardiac muscle.

The activity of AST increased in direct proportion to an increase in the concentration of the nanopowder. In the control group, the AST activity was 124.00 ± 9.25 units/L. Upon introduction of the iron particles at a concentration of 50 μg/kg, the AST activity with respect to the control increased by a factor of 2.7; upon administration of the particles at concentrations of 1, 1.25, and 2.5 mg/kg, it increased by a factor of 3, and increasing the dose up to 5 mg/kg, it increased by a factor of 4.

The ALT activity in the control group was 56.00 ± 2.06 IU. All the concentrations of the iron nanopowder studied induced an increase in the activity of this enzyme ($p < 0.05$), the enzyme activity increasing

inversely to the nanopowder concentration by 13–15% at 12.5 mg/kg and 2.5 mg/kg of the nanopowder, respectively, and by 36% at 1.25 mg/kg and 64% at 50 μg/kg.

Carrying out the research, the effect of the nanoparticles on the activity of gamma glutamine transferase (GGT) was analyzed, its high activity being found in kidney, liver, and pancreas tissues. GGT is contained mainly in the membranes of cells with high secretory and adsorption activity, i.e., epithelial cells lining the bile duct, hepatic ducts, pancreatic exocrine tissue, and excretory ducts.

In the control group, the activity of this enzyme was 112.22 ± 16.66 IU. Upon the effect of iron nanoparticles, an increase in the activity of this enzyme was observed, but a significant increase in the activity of this enzyme ($p < 0.05$) was found under the effect of the nanoparticles starting with concentration of 2.5 mg/kg (an increase in the enzyme activity by 40% when compared to the control).

It is necessary to note that the activity of another indicator enzyme, LDH, also has significantly increased with an increase in the nanopowder concentration by a factor of 2 upon the introduction of 50 μg/kg of the nanopowder and by a factor of 6–7 at a dose of 2.5–12.5 mg/kg. Such a significant increase in the enzyme activity could be related to enhancement in gluconeogenesis (LDH catalyzes lactate transformation into pyruvate) and, therefore, high enzyme clearance and could be a consequence of the development of destructive processes in the liver and myocardium cells.

Creatine phosphokinase (CPK) is an enzyme functioning in cells of numerous tissues. Its greatest content and activity are noted in the myocardium and skeletal muscles, and its lowest concentration is observed in the kidneys, lungs, and liver.

The CPK activity in the control group was 1390.00 ± 60.88 IU. All the concentrations of the nanoparticles studied induced CPK hyperenzymemia, a persistent increase in the activity of this enzyme being noted even at low concentrations of particles of 50 μg/kg (an increase in the enzyme activity by a factor of 4.3) and being preserved upon the introduction of particles at large doses of 1.25–12.5 mg/kg (an increase in the enzyme activity by a factor of 2.5–3).

Investigations of the activity of the indicator enzymes in the mouse serum upon the effect of iron nanoparticles make it possible to conclude that these particles have hepatotoxic action upon per os administration resulting in the destruction of cell membranes and enzymes entering the bloodstream, the maximum damaging action being observed at concentrations of 50 μg/kg–1.25 mg/kg.

The overall analysis of the changes in the biochemical indices of the blood serum under the effect of the nanoparticles administered per os makes it possible to make a conclusion on the biological effect of the iron nanoparticles. The introduction of the iron nanopowders induced an increase in the level of glucose, total protein, urea, creatinine, and total and indirect bilirubin and promoted the increase in the activity of a number of indicator enzymes (AST, ALT, LDH, GGT, and CPK) in the blood, pronounced changes being noted upon introduction even of the lowest particle concentrations of 50 μg/kg and being preserved upon an increase in the particle concentration.

Summarizing the above, it could be concluded that the spherical iron nanoparticles obtained by the plasmochemical method with a diameter of 20–40 nm have a pronounced biological effect manifesting in the antibacterial effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and exert a toxic effect on the organism of laboratory animals. Dose-dependent effects of the iron nanoparticles have been found, which makes it possible to determine precautions working with these particles. The data obtained are of interest for assessing the development of possible pathologies upon the application of metal nanoparticles, in particular, iron in food supplements and drugs, and make it possible to develop prevention techniques for intoxication with these nanoparticles.

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