EXPERIMENTAL MODELS AND INSTRUMENTAL STUDIES FOR THE ASSESSMENT OF RISK IN THE HYGIENE AND EPIDEMIOLOGY

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TOXICOLOGICAL EVALUATION OF NANO-SIZED COLLOIDAL SILVER IN EXPERIMENTS ON MICE. BEHAVIORAL REACTIONS, MORPHOLOGY OF INTERNALS

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The results of toxicity studies of nano-sized colloidal silver (NCC), the most widely used in medicine, food and life, are given. When evaluating safe doses of silver NP (using commercially available NCC solution stabilized with polyvinylpyrrolidone (PVP), with the size of silver NP at the range of 5-80 nm) when orally administered to male mice, BALB/c mice at doses of 0.1; 1.0 and 10 mg/kg of body weight per silver different effects from the motor and orienting-exploratory activity were revealed, for the part of them the dependence on the dose of the NCC was typical. The following peculiarities were found: reduction in motor activity to reduce the frequency of activities requiring physical effort, reduction of the execution time of these actions; increasing anxiety in terms of frequency and duration of attacks of orienting-investigative activity and animals washing. Morphological examination revealed a series of tissue changes of internal organs (especially liver and spleen, to a lesser extent – kidney, heart and colon) with increase of the spectrum and severity of structural changes with increasing doses of the NCC. From the combination of the data the conclusion was made that maximal ineffective dose (NOAEL) of this nanomaterial at subacute oral administration is no more than 0.1 mg/kg body weight.

Key words: *silver nanoparticles, toxicity, morphology, behavior reactions*

The nanoparticles (NP) of silver are contained in many types of consumer products such as medical drugs, dressing materials, disinfectants, paint and varnish products, textiles, water filters, packing materials, cosmetics, and dietary supplements [12, 19]. The global scope of production of materials containing this type of NP as of 2011 was already more than 500 t/year in terms of silver [20], and currently it continues to increase. The waste of items and products containing silver NP are disposed, including at the waste incineration enterprises. Herewith, the major part of silver NP is concentrated in the ash and sludges of the liquid air cleaning systems [9, 20] from where it can be delivered to the fields in the composition of fertilizers, accumulated in the plants and different aquatic and soil organisms after that it is transferred to human by the trophic chains.

According to the available literature, silveer NP cn be toxic at the peroral administration; herewith the assessments of the toxic doses of silver NP at their intake to the gastrointestinal tract are contradictory [6, 10, 15, 18, 23] that can be stipulated by the differences in the sizes of particles and structure of their surface, insufficient duration of

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experiments and the limited set of the studies biological toxicity markers.

The objective of the cycle of studies performed jointly by FSBI "FNTS health-care technology risk management to public health" and FSBI "Institute of Nutrition" is the assessment of the silver NP doses safe for human during their intake through the gastrointestinal tract using the guidelines on the nanomaterials safety assessment [5]. The most of experiments in relation to the acute and subacute toxicity of nanomaterials are performed currently in rats [22]. Together with it, the justification of the safe doses of nanomaterial requires the conduction of experiments not less than in two species of laboratory animals. In this relation the subject of study in this article is the assessment of some integral and histopathological changes developed when exposed to silver NP introduced to the gastrointestinal tract of linear mice. As the object of study we selected the commercially produced nano-sized colloidal silver (NCC) stabilized with polyvinylpyrrolidone (PVP) which is the permitted food additive E1201 as well as is used safely in the composition of infusion solutions – blood substitutes.

Materials and methods. The studied NCC solution ("cluster silver") "Argovit-S" according to TU 9310-03-79044259-12 was prodived by LLC SPC Vektor-Vita, Russia, Novosibirsk[1]. The NCC was in the form of aqueous solution of brown color (in the translucent light) with greenish-grey shadow (in the reflected light) and slight opalescence. The wavelegth of the maximum absorbtion in the visible range was 403.2 nm. According to the data of analysis using the inductively coupled plasma mass spectrometry[2] (MR 1.2.2641-10), the total content of silver in the undiluted NCC solution was 10.09±0.04 mg/cm^3. The PVP stabilizer content in the product was 19 % by weight. According to the data of transmission electronic microscopy (MR 1.2.2641-10, microscope JEOL JEM-100CX; JEOL, Japan; accelerating voltage 80 kV) the composition of the studied NCC sample contined the silver NP with high electronic density, sharp outlines, mainly of rounded, ellipsoidal form and separate triagle particles, belonging to the dimensional fractions with diameter less than 5 nm; 10–20 and 50–80 nm^3. As it was demonstrated by the analysis using the dynamic laser light scattering method on the instrument Nanotrack Wave (Microtrac Inc., USA) 80% of silver NP in the product had the hydrodynamic diameter within the range of 10.6-61.8 nm.

The work with animals is performed in accordance with "Guide for the care and use of laboratory animals" (ILAR, DELS) and "Good laboratory practice" [13]. The study of hystopathologicl changes caused by silver NP in the organs and tissues is carried out on the sexually mature BALB/C male mice with initial weight of 26.0±3.0 g obtained from the nursery of laboratory animals "Andreevka" of FBIS "Scientific Biomedical Technology Center" of the Federal Medical-Biological Agency of Russia. The animals in the number of 75 animal units were divided into 5 groups, 15 animal units in one group (group No. 1 – control, groups No. 2, 3, 4, 5 – experimental). NCC and stabilizer PVP were administered daily in the doses specified in table 1 at the fixed time intragastrically through the catheter. The animals of control group received the carrier – diatilled water. The duratiton of experiment was 90 days.

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Table 1

Doses and volume of NCC and PVP srabilizer at the intragastric introduction to mice in the subacute experiment

Group	Number of animals in group	NCC as suspension in water	PVP stabilizer as solution in water	Total volume of introduced products, ml/kg of body weight/day
		Dose*, mg/kg of body weight/day		4,0
1 (control)	15	–	–	
2	15	–	200,0	
3	15	0,1	200,0	
4	15	1,0	180,0	

5	15	10,0	–	

N o t e : * – for NCC in terms of silver.

During the experiment daily we assessed the appearance, general condition of animals and appetite. The body weight was determined directly before the beginning of experiment and on the 30th, 60th and 90th day of the experiment.

Pulling out of the experiment was performed on the 90th day in accordance with existing rules [13]. After euthanasia we performed the dissection and sampling of internal organs (brain, heart, lungs, liver, lien, kidneys, genital glands) with further measurement of the absolute and calculation of the relative weight of organs. For morthological studies we sampled the heart, liver, kidneys, lien, twisted intestine, large intestine and brain. The sampled material was fixed in 10% neutral (buffered with 0.1 M sodium phosphate, pH 7.00±0.05) solution of formalin in the ratio 1:50 by weight, the fragments of organs were dehydrated in the alcohols of ascending concentrations, impregnated with chloroform and paraffin and then filled with homogenized paraffin medium Histomix. The cuts with thickness of 4 μm were performed on sliding microtome JUNG SM 2000R (Leica, Germany) and painted using the common methods with hematoxylin and eosine. The microphotos were obtained on the light-optical microscope MEIJI (Techno, Japan) equipped with camera "microscopy VISION" (VISION, Canada) at the magnification of ×200, ×400, ×1000. In total, 550 of histological preparations were studied.

The impact of intragastric NCC introduction on the motional and orientative-research activity of animals was studied using the methods of Ya.I. Buresh et al. [1] and in accordance with MU 1.2. 2635-10. The testing was performed at the plant "Otkrytoe pole» TS0501 (LLC "NPC Otkrytaya Nauka", Russia), the behavior was fixed during minutes using the high resolution video camera installed at the height of 2 m. УчетThe accounting, analysis and statistical processing of data were performed in program RealTimer v.1.2.1. The mathematical processing of the results of study was carried out using the parametric methods of statistics, with prior assessment of compliance of the obtained results with the normal distribution law. We calculated the sqampling mean (M) and standard error (m), checked the supposition on the coincidence of the sampling means using the Student's t-criterion. The differences of obtained results were considered as statistically significant at $p \leq 0.05$. The calculations were performed using the set of programs Statistica 6.0.

Results and their discussion. When assessing the integral indicators of the body of animals during the whole experiment in all the groups we did not reveal the disorders or changes in the condition of the skin of animals, intensity and character of motion activity, reaction to the tactile, sound and light stimuli. No decrease of appetite, signs of intoxication and death of animals was observed during the whole term of monitoring. The average body weight in the animals of experimental group No. 5 obtained the maximum NCC dose starting from the 60th day of experiment is credibly ($p<0.05$) reduced by 16-19% in relation to this indicator in the control group. The average body weight of animals in the other experimental groups did not differ from the control (table 2).

We observed the certain dose-dependent increase in the relative weight of lien in the mice of experimental groups No. 4 by 1.75 times and No. 5 – by 2.25 times ($p<0.05$) compared to the same indicator in the control group (table 3).

Table 2

BALB/C mice body weight dynamics during the subacute experiment

Group	Average body weight in group, g ($M \pm m$)			
	before the beginning of experiment	30th day	60th day	90th day
1 (control)	28,1±2,1	30,7±3,3	32,1±2,1*	34,6 ±5,5*
2	27,1±2,0	29,1±3,2	31,2±3,0*	32,4±2,0*
3	26,9±2,8	29,0±4,1	31,1±5,0*	31,6±2,8*
4	26,1±2,5	28,7±3,1	30,3±3,1*	30,4±2,5*
5	26,5±1,5	26,2±2,4	27,0±2,0^	28,1±2,6^

N o t e : ^$p<0.05$ compared to the value of the control group indicator; * $p<0.05$ compared to the value of indicator before the beginning of experiment.

Table 3

Average ($M\pm m$) body weight and relative weight of the organs of mice on the 90th day of subacute experiment

Indicator		Group				
		1 (control)	2	3	4	5
Body weight, g		34,2±3,65	32,4±2,0	31,6±2,8	30,4±2,5	28,1±3,56
Relative weight of internal organs, % of body weight	brain	1,32±0,10	1,4±0,2	1,7±0,3	1,5±0,2	1,4±0,2
	heart	0,5±0,1	0,5±0,1	0,6±0,1	0,5±0,2	0,5±0,1
	lungs	0,8±0,2	0,9±0,3	1,2±0,5	0,9±0,2	0,8±0,2
	liver	5,0±0,8	4,9±1,1	5,8±1,65	4,9±0,9	4,6±0,6
	lien	0,4±0,1	0,5±0,1	0,9±0,6	**0,7±0,2***	**0,9±0,3***
	kidneys	1,5±0, 1	1,6±0,2	1,6±0,1	1,4±0,3	1,4±0,2
	genital glands	0,9±0,3	0,7±0,2	0,8±0,3	0,6±0,2	0,8±0,2

N o t e : * $p<0.05$ compared to the value of the control group indicator.

No credible changes in the relative weight of the other examined organs in the experimental groups in relation to the control indicators are established.

When assessing the motional and orientative-research activity on the 90th day of the experiment (table 4) it was established that the mice of experimental group No. 2 obtained PVP by 2 times rarely compared to control stood up without the keeping at the wall of arena ($p<0.001$). The same, not having the clear dependence on the dose, changes of this indicator are observed also in the animals of groups No. 3-5 that can be considered as the non-specific effect of the introduced nanomaterial carrier (PVP). Herewith, the average duration of standings is credibly reduced compared to control only in the animals of group No. 3 obtained NCC in the lowest of doses.

The frequency of fadings in the animals of group No. 2 was reduced by 1.8 times compared to the indicator in control ($p<0.001$). The changes of this indicator observed in groups No. 3 and 4 were comparable with such changes in group No. 2 and did not demonstrate the monotonous dependence on the dose. However, in the mice of group No. 5, obtained NCC in the dose of 10 mg/kg of body weight, the frequency of fadings was reduced compared both to the indicator in control (by 6.2 times, $p<0.001$) and the indicator in group No. 2 (by 3.5 times, $p<0.001$), that cannot be explained by the impact of introduced PVP. No dependence of the average duration of fdings on the NCC dose is detected. The assessment of frequency of behavioral acts under the duration of washings demonstrated the credible and dose-dependent decrease of this indicator in groups No. 3-5 ompared to control. Herewith, in group No. 2 the frequency of washings credibly did not differ from such in control, and the average duration of washing acts (in contrast to the animals of groups No. 3-5) was credibly decreased. The anxiety indicator characterized by the frequency of looking into the holes was not affected by the introduction of PVP to the mice of group No. 2, since in groups No. 3-5 obtained NCC this indicator was credibly decreased, though its certain dependence on the dose of nanomaterial was absent. The duration of lookings into the hole is credibly increased in groups No. 3 and 4 that is not detected in group No. 5. No credible difference under the indicator of crossing the squares of field in the experimental animals of groups No. 3-5 is established (except for small under the value of decrease in group No. 4 for peripheral squares). The value of integral indicator of general activity was credibly changed compared tto control in groups No. 3 and 4 at the absence of credible changes in group No. 5.

Table 4

Indicator of motional and orinetative-research activity in mice on the 90th day of experiment

Indicator ($M\pm m$)	Groups of animals				
	1 (control)	2	3	4	5
Number of events for 5 min					
Standing without keeping at the wall	10,8±1,6	5,5±2,0**	2,4±0,6**	4,7±3,8*	3,5±3,5**
Washing	1,3±0,2	1,3±0,6	0,5±0,5*	0,5±0,3**	0,25±0,2**
Looking into the holes	27,5±2,0	28,0±1,2	15,0±3,2*	18,1±4,5**	23,0±3,5*
Crossing of the central square	5,0±1,0	6,3±1,1	5,1±1,4	4,3±1,9	8,1±4,5
Crossing of peripheral square	46,5±2,9	40,3±8,9	35,4±11,4	31,4±10,4*	41,7±7,9
Fading	6,8±1,1	3,8±1,1**	5,3±1,8	3,3±1,3**	1,1±0,4**
General activity	91,0±6,3	81,3±10,9	58,4±13,8*	59,1±15,5**	76,6±16,0
Duration of events (sec) for 5 min					

	1 (control)	2	3	4	5
Standing without keeping at the wall	0,7±0,03	0,8±0,2	0,35±0,2*	0,7±0,3	0,8±0,4
Washing	1,3±0,3	0,5±0,2**	3,7±4,9	3,2±2,2	0,9±0,8
Looking into the holes	1,7±0,1	1,9±0,1	2,6±0,4*	2,7±0,7*	1,9±0,4
Fading	0,5±0,2	0,3±0,2	3,4±1,7*	2,1±1,0*	1,0±0,4*
General activity	3,7±0,4	3,2±0,4	6,7±4,9	6,7±2,2*	3,6±1,2

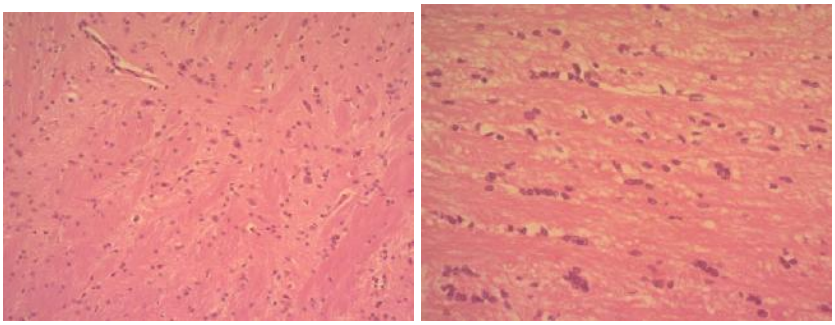
N o t e : * $p<0.05$; ** $p<0.001$ compared to the value of control group indicator.

The obtained results evidence the presence of different effects in relation to the motional and orientative-research activity of mice in groups No. 3-5 a part of which can be explained by NCC impact. Herewith, in a number of cases no clear dependence on its dose is observed. However, in relation to the frequency of washings and fadings of animals we can trace the certain dependence on the dose, herewith, if in group No. 3 (dose 0.1 mg/kg of body weight NCC) the observed changes are relatively small, for groups No. 4 and 5 these changes differ significantly and credibly from control.

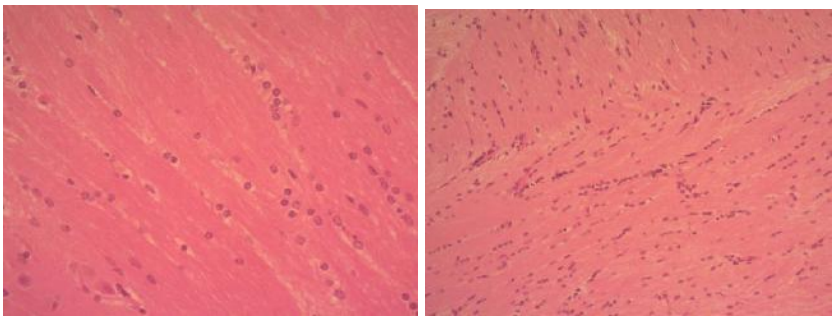
The results of morphological study of internal organs (brain, heart (cardiac muscle), liver, lien, kidney, large intestine, twisted intestine) for mice of groups No. 1 and 3-5 are presened at the representative light-optical microphotos (fig. 1-7). When assessing the morphology of the internal organs of mice of control group (fig. 1-7 (a)) no significant morphological changes are established in the structure of brain, kidney, liver and large intestine tissue. The single eosinophils are found in the part of liver portal tracts. The hyperplasia of lymphoid tissue is observed in the structure of the lien tissue with increase in the volume of white pulp up to 35-40% and formation of reactive follicles. The structure of lymphoid tissue, associated with mucous membrane of twisted intestine, has the hyperplasia with formation of reactive follicles, eosinophilia of reactive infiltrate, and the Paneth cells hyperplasia. The observed effects of the immune body reaction can be considered as not exceeding the limits of normal age-specific changes for the animals of this age.

When assessing the structure of tissues of the examined internal organs of mice in group No. 2 no morphological changes in relation to the control are detected.

The mice of group No. 3 obtained NCC during 3 months in he dose of 0.1 mg/kg of body weight (fig. 1-7 (b)) did not have any morphological changes in the strucuture of the tissue of brain, heart, kidney, twisted and large intestine. The structure of the tissue of liver had the morphological changes which included the focal albuminous degeneration of hepatocytes and expressed infiltrate eosinophilia. The lien of mice of the considered group had the close under the degree of manifestation to control group hyperplasia of lymphoid tissue with increase in the volume of white pulp up to 35-40% as well as eosinophilia and accumulations of large multinucleated cells.

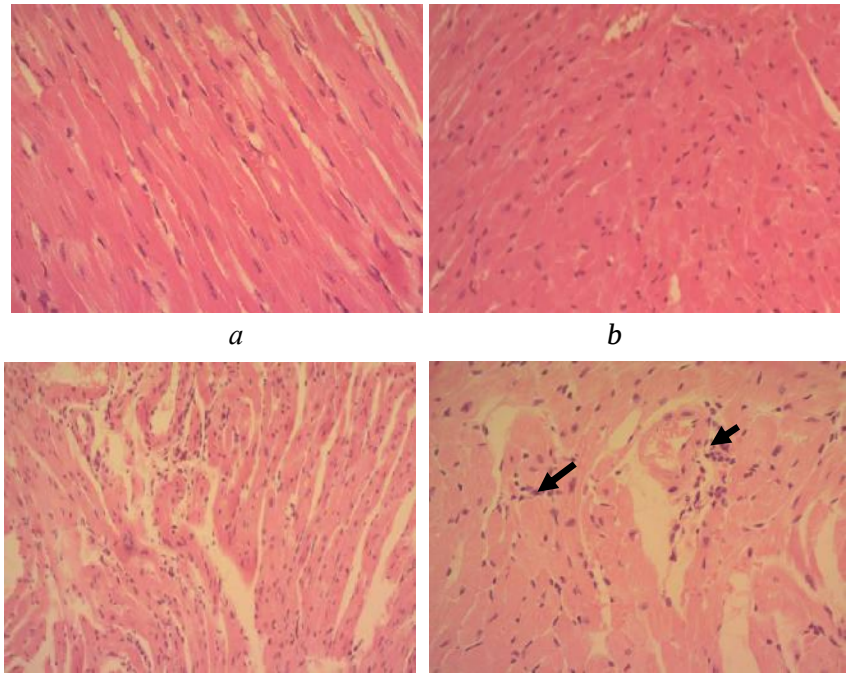


a *b*



c *d*

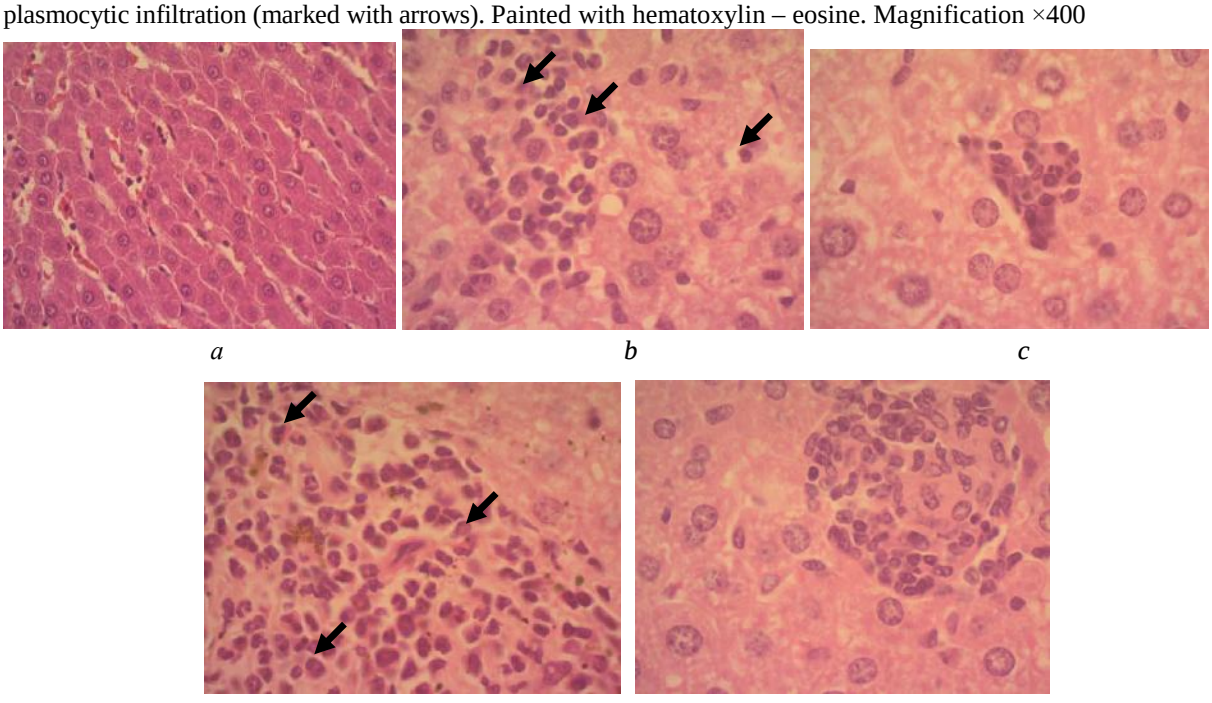
Fig. 1. Mice brain microphotos: *a* - *d* - animals from groups No. 1, 3–5 respectively. Painted with hematoxylin – eosine. Magnification ×200



a *b*

c *d*

Fig. 2. Microphotos of the cardiac muscle of mice: *a* – animal from group No. 1 (control); *b* – animal from group No. 3; *c* – animal from group No. 4: slight edema, perivascular accumulations of lymphocytes and plasmatic cells are observed; *d* – animal from group No.5: morphologival changes in the focal slightly manifested periarterial lymphocyto-plasmocytic infiltration (marked with arrows). Painted with hematoxylin – eosine. Magnification ×400



a *b* *c*

d *e*

Fig. 3. Representative light-optical mice liver picture: *a* – animal from group No. 1 (control); *b* – animal from group No. 3: hepatocytes in the condition of focal albuminous degeneration; in the gaps of sinusoids – the accumulations of eosinophils (marked with arrows), lymphocytes and plasmatic cells; *c*, *d* – animal from group No. 4: hepatocytes in the condition of distibuted hydrapic and hyaline-drip dystrophy; eosinophilic infiltration of portal tracts (marked

with arrows); *e* – animal from group No. 5: signs of granulomatous inflammation with moderately expressed eosinophilia of infiltrate. Painted with hematoxylin – eosine. Magnification ×400 (*a*, *d*), ×1000 (*b*, *c*, *e*)

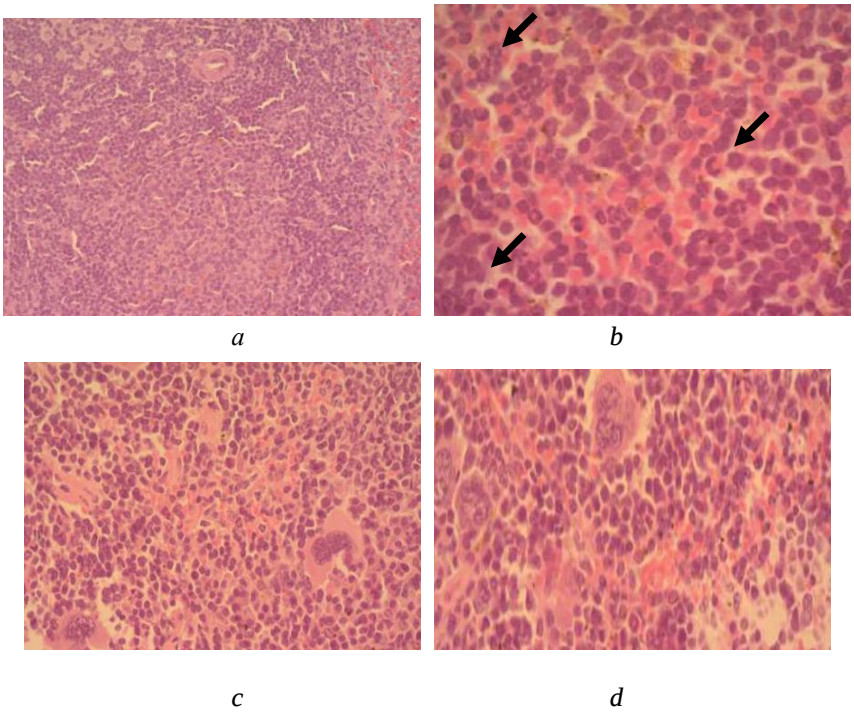


Fig. 4. Representative light-optical mice lien picture: *a* - *d* - animals from groups No. 1, 3–5 respectively. The eosinophilic infiltration is marked with arrows. Painted with hematoxylin – eosine. Magnification ×200 (*a*, *c*, *d*), ×1000 (*b*)

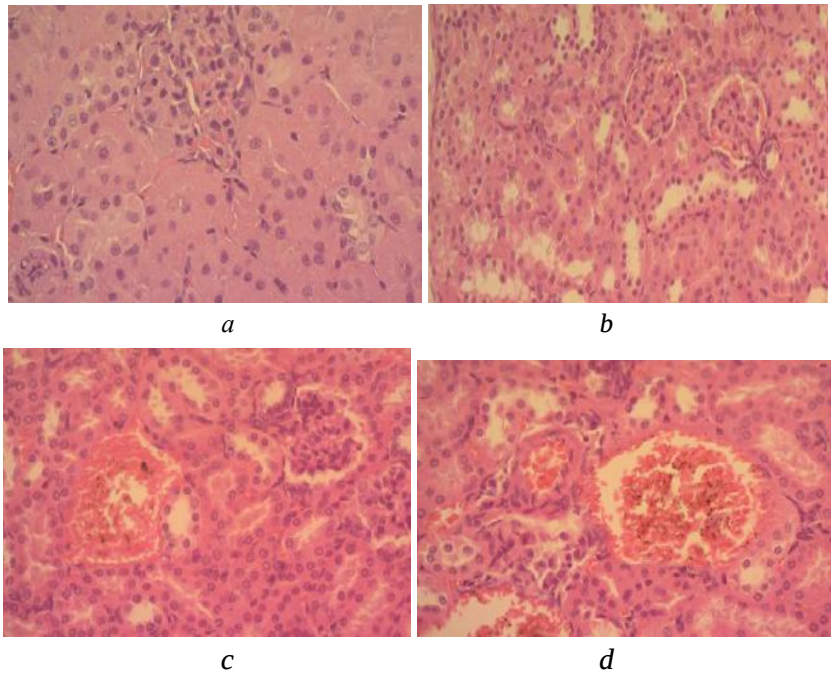


Fig. 5. Representative light-optical mice kidney picture: *a* – animal from group No. 1; *b* – animal from group No. 3; *c* – animal from group No. 4: in the glomerules – proliferation of mesangial cells up to 8 in the segment; *d* – animal from group No. 5. Painted with hematoxylin – eosine. Magnification ×400

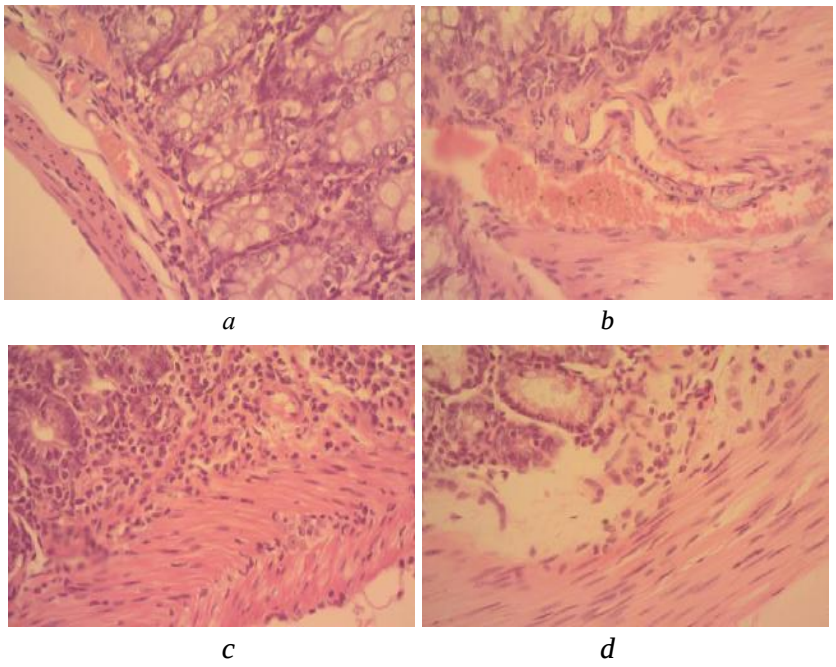


Fig. 6. Representative light-optical large intestine picture: *a* – animal from group No. 1; *b* – animal from group No. 3; *c* – animal from group No. 4; *d* – animal from group No. 5. Cut of villi: axial (*a*, *b*), lateral (*c*, *d*). The eosinophilic infiltration is marked with arrows. Painted with hematoxylin – eosine. Magnification ×400

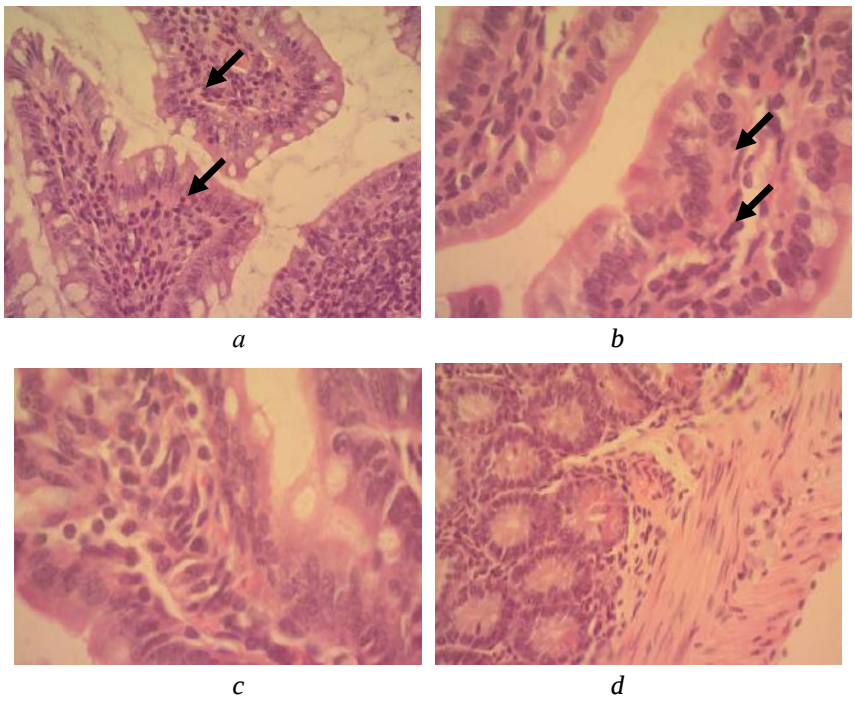


Fig. 7. Representative light-optical twisted intestine picture: *a* – animal from group No. 1; *b* – animal from group No. 3; *c* – animal from group No. 4; *d* – animal from group No. 5. Cut of villi: axial (*a*, *b*, *c*), lateral (*d*). The Paneth cells are marked with arrows. Magnification ×400 (*a*, *d*), ×1000 (*b*, *c*)

The mice of group No. 4, obtained NCC in the dose of 1.0 mg/kg of body weight (fig. 1, 2 (*c*), 3 (*c*, *d*), 4–7 (*c*)), the structure of the tissue of brain and twisted intestine had no morphological changes in relation to the control group. Insignificant perivascular accumulations of lymphocytes and plasmatic cells are detected in the heart tissueThe liver had morphological changes in relation to control group in the form of eosinophilic infiltration, localization of Kupffer's cells in the peri-

portal area, distributed hydrapic and hyaline-drip dystrophia, more expressed than in the animals of group No. 3. The structure of the lien tissue had the morphological changes also similar to the changes observed in group No. 3 in the form of eosinophilia and proliferation of large multinucleated cells, hyperplasia of lymphoid tissue with the volume of white pulp in the range of 35 to 45%. The kidney compared to the data obtained from the animals of control group had the proliferation of mesangial cells and expressed dystrophical changes in the nephron capsule external leaf cells. The large intestine walls had the morphological changes in relation to the control group in the form of distributed colitis with edema of walls, proliferation of lymphocytes, macrophagocytes, eosinophils and plasmatic cells in the own plate.

The mice of group No. 5 obtained NCC in the dose of 10 mg/kg of body weight/day as for the animals of other experimental groups the structure of the tissue of brain and twisted intestine had no morphological changes in relation to the control group indicators. The heart tissue had morphological changes in the form of focal slightly expressed periarterial lymphocytoplasmocytic infiltration. The liver of animals was characterized by the morphological changes in relation to the control group in the form of granulomatous inflammation with moderately expressed infiltrate eosinophilia. The structure of the tissue of lien had the morphological changes manifested in the furher increase of the volume of lymphoid tissue with increase in the share of white pulp up to 45%, eosinophilia and proliferation of large multinucleated cells. The kidneys were characterized by the focal slightly epressed proliferation of mesangial cells, dystrophical changes in the nephron capsule external leaf cells with periarterial lymphocytoplasmocytic infiltrates. The structure of the tissue of large intestine was dominated by the morphological changes in relation to the control group in the form of distributed colitis with eosinophilia of inflammatory infiltrate and reactive hyperplasia of lymphoid tissue associated with mucous membrane.

The obtained results demonstrated that NCC is characterized by the different manifestations of acute toxicity at the 90-day introduction to the gastrointestinal tract of mice. In particular, we detected the credible decceleration on animals in the increase of body weight on the 60^{th} and 90^{th} days of the experiment and decrease in the relative weight of lien, which is the most visible at the use of maximum of the studied doses of nanomaterial – 10 mg/kg of body weight. Studying the behavioral reactions demonstrated that NCC in all the studied doses stipulates the decrease of motional activity that is evidenced by the decrease in the frequency of actions requiring the physical forces and reduction of time for the performance of this actions, results in the increase of anxiety under the indicators of frequency and duration of the acts of orientative-research activity and washing of animals. When the specified effects had the specific for NCC and dose-dependent character their manifestation was unambiguously expressed in terms of silver 10 mg-kg of body weight. As it can be understood from the analysis of available literature the obtained results are the first evidence of the availability in NCC of the signs of neurotoxic effect at the peroral administration. Previously in the study [21] the performed single introduction of silver NP to the pregnant female rats in the very high doses (10-1000 mg/kg of body weight) was not accompanied by any unfavorable changes in the behavioral reactions. The reason of these differences in addition to the apparent factors (type of animals, administration scheme) can be characterized by the ability of silver contained in NCC to pass anysotropically through the blood-brain barrier and accumulate selectively in brain at the multiple administration in the subacute experiment [7, 8]. Therefore, the impact of silver NP on the brain though not confirmed in this work by the data of morphological amalysis requires the further special study.

According to the provided results of the light-optical morphological studies NCC when introduced to the gastrointestinal tract of mice during 90 days causes the series of changes in the tissues of internal organs (first of all – liver and lien; to a lesser degree – kidney, large intestine and heart) with the rise of spectrum and degree of manifestation of the structural changes at the increase of dose. In the liver the specified changes are similar to the picture of granulomatous inflammation with focal albuminous degeneration of hepatocytes, expressed eosinophilia of infiltrate; in the lien the changes were manifested as the hyperplasia of lymphoid tissue with increase in the volume of white pulp up to 45 %, eosinophilia, accumulations of large multinucleated cells; in the heart – in the form of focal slightly expressed periarterial lymphocytoplasmocytic infiltration; in the large intestine – in the form of distributed colitis with eosinophilia of inflammatory infiltrate and reactive hyperplasia of lymphoid tissue associated with mucous membrane. The brain and twisted intestine did not have the morphological changes at the use of the specified doses in relation to the control group.

Comparative manifestation of the strucutural changes in the internal organs of animals obtained NCC correlates with the known from literature data on the biological distribution of silver NP introduced to the gastrointestinal tract. Thus, it was demonstrated that the organ accumulating the biggest amount of these NP is the liver; it is followed by the lien, and in kidneys the accumulation of silver NP (in particular, as opposed to gold NP) is less significant [3, 11, 23]. The works [2, 11] prove that the silver NPs are able to penetrate through the intestinal wall to the blood, circulate and accumulate in a number of internal organs. According to the available data [22] the cells can capture the silver NP; after that under the impact of different oxidants (including endogenic) the silver is gradually emitted from them in the ionic form which, as it is known, has the ability to inhibit permanently the major of enzymes and membrane transport systems being bound with thyolic groups of active proteins [14]. The numerous studies in vitro of the cell cultures demonstrated that the threshold concentration of the toxic action of silver NP in the incubation medium is not less than 3 μkg/cm^3. Herewith, according to the computer simulation of the silver Np biokinetics this concentration in the tissue of liver and lien can be developed at the single or repeated intragastric introduction in the dose of about 5-10 mg/kg of body weight [16]. These assessments correlate with the obtained results characterizing the expressed morphological chanages in the liver and lien (and, in particular, in kidney) of mice when using the doses in the range of 1–10 mg/kg of body weight that evidences on the development of toxic effect, then at the dose of 0.1 mg/kg the changes are marginal. It is representative that the large intestine being the first barrier at the way of silver NP from the gap of gastrointestinal tract to the body is not the target of their toxic action. This correlates with the results of studies in which no significant ultrastructural changes in the enterocytes under the data of electronic microscopy (as opposed to the lien and liver) at the acute intraintestinal introduction of the high doses of NCC [2], as well as with the absence of NCC impact on the permeability of intestinal barrier in rats in the age of 4 months for protein macromolecules. One of the explanations of this paradoxial fact can be that the intestinal absorption of silver from the studied product occurs mainly in the form of zerovalent silver NPs which probably have lower toxicity compared to this metal in the cationic form [17]. The target organs for the toxic action of silver at its introduction in the form of NP are the liver and lien in the cells of which probably the release of sliver ions occurs in the high local concentrations under the impact of oxidants produced endogenically by the heterophilic leukocytes, macrophagocytes and (in case of liver) Kupffer's cells [4].

In aggregate of the morphological data obtained in mice concumed NCC for 3 months it can be concluded that the maximal ineffective dose (NOAEL) of this nanomaterial is according to the examination of these organs not more than 0.1 mg/kg of body weight.

References

1. Buresh Ja., Bureshova O., Hyuston D.P. Metodiki i osnovnye jeksperimenty po izucheniju mozga i povedenija [Techniques and basic experiments on the brain and behavior]. Moscow: Vysshaja Shkola, 1991, 268 p. (in Russian)

3. Buzulukov Ju.P., Gmoshinskiy I.V., Raspopov R.V., Demin V.F., Solovyev V.Ju., Kuzmin P.G., Shafeev G.A., Hotimchenko S.A. Izuchenie absorbcii i bioraspredelenija nanochastic nekotoryh neorganicheskih veshhestv, vvodimyh v zheludochno-kishechnyj trakt krys, s ispol'zovaniem metoda radioaktivnyh indikatorov [Study of biodistribution of nanoparticles and absorption of some inorganic substances introduced into the gastrointestinal tract of rats using the method of radiotracer]. *Medicinskaja radiologija i radiacionnaja bezopasnost'*, 2012, vol. 57, mo. 3, pp. 5–12. (in Russian)

4. Mayanskiy A.N., Mayanskiy D.N. Ocherki o nejtrofile i makrofage [Essays on neutrophils and macrophages]. Edit by V.P. Kaznacheev. Novosibirsk: Nauka. Sib. otdelenie, 1983, 256 p. (in Russian)

5. Onishhenko G.G., Tutel'jan V.A., Gmoshinskij I.V., Hotimchenko S.A. Razvitie sistemy ocenki bezopasnosti i kontrolja nanomaterialov i nanotehnologij v Rossijskoj Federacii [Development of the safety assessment and control of nanomaterials and nanotechnology in the Russian Federation]. *Gigiena i sanitarija*, 2013, no. 1, pp. 4–11. (in Russian)

6. Shumakova A.A., Smirnova V.V., Tananova O.N., Trushina Je.N., Kravchenko L.V., Aksenov I.V., Selifanov A.V., Soto H.S., Kuznecova G.G., Bulahov A.V., Safenkova I.V., Gmoshinskij I.V., Hotimchenko S.A. Toksikologo-gigienicheskaja harakteristika nanochastic serebra, vvodimyh v zheludochno-kishechnyj trakt krys

[Toxicological-hygienic characteristic of silver nanoparticles introduced into the gastrointestinal tract of rats]. *Voprosy pitanija,* 2011, vol. 80, no. 6, pp. 9–18. (in Russian)

7. Buzulukov Yu.P., Arianova E.A., Demin V.F., Safenkova I.V., Gmoshinski I.V., Tutelyan V.A. Bioaccumulation of silver and gold nanoparticles in organs and tissues of rats studied by neutron activation analysis [Bioaccumulation of silver and gold nanoparticles in organs and tissues of rats studied by neutron activation analysis]. *Biology Bulletin,* 2014, vol. 41, no. 3, pp. 255–263. (in Russian)

8. Lee J.H., Kim Y.S., Song K.S. and etc. Biopersistence of silver nanoparticles in tissues from Sprague–Dawley rats. *Part. Fibre Toxicol,* 2013, vol. 10, no. 36. Available at: http://www.particleandfibretoxicology.com/content/10/1/36.

9. Vejerano E.P, Leon E.C., Holder A.L., Marr L.C. Characterization of particle emissions and fate of nanomaterials during incineration. *Environ. Sci.: Nano,* 2014, vol. 1, no. 2, pp. 133–143.

10. Hong J.S., Kim S., Lee S.H. and etc. Combined repeated-dose toxicity study of silver nanoparticles with the reproduction/developmental toxicity screening test. *Nanotoxicology,* 2014, vol. 8, no. 4, pp. 349–362.

11. Van der Zande M., Vandebriel R.J., Doren E.V. and etc. Distribution, elimination, and toxicity of silver nanoparticles and silver ions in rats after 28-day oral exposure. *ACS Nano,* 2012, vol. 6, no. 8, pp. 7427–7442.

12. Blaser S.A., Scheringer M., MacLeod M., Hungerbühler K. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles [Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles]. *Sci. Total Environ,* 2008, vol. 390, no. 2–3, pp. 396–409. (in Russian)

13. *Guide for the care and use of laboratory animals.* Eighth Edition. Committee for the Update of the Guide for the Care and Use of Laboratory Animals; Institute for Laboratory Animal Research (ILAR); Division on Earth and Life Studies (DELS); National Research Council of the national academies. Washington: The national academies press, 2011.

14. Lapresta-Fernández A., Fernández A., Blasco J. Nanoecotoxicity effects of engineered silver and gold nanoparticles in aquatic organisms. *Trends Anal.Chem.*, 2012, vol. 32, no. 2, pp. 40–59.

15. Marambio-Jones C., Hoek E.M.V. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J. Nanopart. Res.* 2010, vol. 12, no. 5, pp. 1531–1551.

16. Demin V.A., Gmoshinsky I.V., Demin V.F., Anciferova A.A., Buzulukov Yu.P., Khotimchenko S.A., Tutelyan V.A. Modeling interorgan distribution and bioaccumulation of engineered nanoparticles (using the example of silver nanoparticles) [Modeling interorgan distribution and bioaccumulation of engineered nanoparticles (using the example of silver nanoparticles)]. *Nanotechnologies in Russia,* 2015, vol. 10, no. 3–4, pp. 288–296. (in Russian)

17. Xiu Z.M., Zhang Q.B., Puppala H.L., Colvin V.L., Alvarez P.J.J. Negligible Particle-Specific Antibacterial Activity of Silver Nanoparticles. *Nano Lett.*, 2012, vol. 12, no. 8, pp. 4271–4275.

18. Park E.J., Bae E., Yi J. and etc. Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. *Environ. Toxicol. Pharmacol.,* 2010, vol. 30, no. 2, pp. 162–168.

19. Savage N., Diallo M.S. Nanomaterials and water purification: opportunities and challenges. *J. Nanopart. Res.*, 2005, vol. 7, no. 4–5, pp. 331–342.

2. Platonova T.A., Pridvorova S.M., Zherdev A.V., Vasilevskaja L.S., Arianova E.A., Gmoshinskij I.V., Hotimchenko S.A., Dzantiev B.B., Popov V.O., Tutel'jan V.A. Identifikacija nanochastic serebra v tkanjah slizistoj obolochki tonkoj kishki, pecheni i selezenki krys metodom prosvechivajushhej jelektronnoj mikroskopii [Identification of silver nanoparticles in the mucosal tissues of the small intestine, liver and spleen of rats by transmission electron microscopy]. *Bjulleten' jeksperimental'noj biologii i mediciny,* 2013, vol. 155, no. 2, pp. 204–209. (in Russian)

20. Fabrega J., Luoma S.N., Tyler C.R., Galloway T.S., Lead JR. Silver nanoparticles: behaviour and effects in the aquatic environment . *Environ. Int.*, 2011, vol. 37, no. 2, pp. 517–531.

21. Philbrook N.A., Winn L.M, Afrooz A.R., Saleh N.B., Walker V.K. The effect of TiO(2) and Ag nanoparticles on reproduction and development of Drosophila melanogaster and CD-1 mice. *Toxicol. Appl. Pharmacol,* 2011, vol. 257, no. 3, pp. 429–436.

22. Stensberg M.C., Wei Q., McLamore E.S., Porterfield D.M., Wei A., Sepúlveda M.S. Toxicological studies on silver nanoparticles: challenges and opportunities in assessment, monitoring and imaging. *Nanomedicine (Lond),* 2011, vol. 6, no. 5, pp. 879–898.

23. Kim Y.S., Kim J.S., Cho H.S. and etc. Twenty-eight-day oral toxicity, genotoxici-ty, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. *Inhal. Toxicol,* 2008, vol. 20, no. 6, pp. 575–583.