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MORPHOLOGIC PECULIARITIES OF GASTROINTESTINAL TRACT ORGANS UNDER SUBCHRONIC IMPACT OF NANO-DISPERSIVE MANGANESE OXIDE (III, IV)

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The study of morphologic peculiarities of gastrointestinal tract organs of test animals under subchronic (during 30 days) intragastric infusion of water suspension of nano-dispersive manganese oxide (III, IV) in the amount of 10.4 mg/kg revealed a complex of pathomorphologic changes in the form of moderate diffusively-spread inflammatory response in mucous and submucous layer of the stomach, small intestine and large intestine expressed in the form of lympho-macrophage infiltration with considerable admixture of eosinophils and plasmocytes. In the intermuscular nervous ganglias there's been recorded changes with obvious perineural edema. While infusing a microdispersive equivalent in the same way the inflammation response in the organs studied had local character and low degree of manifestation. Perineural edema and dystrophia of the intermuscular nervous ganglias has not been detected.

Key words: *nano dispersive manganese oxide, morphologic changes, stomach, small and large intestines, subchronic influence.*

The world development and spread of nano-technologies and nano-bio-technologies demands the necessity of systemic development of works on studying the potential threats in the sphere of human activity [1]. In the developed countries of the world: Russia, the USA, Canada, Japan, China, South Korea, European Union countries, there are large-scale studies on the assessment of safety and potential risks connected with produced nano-materials [2]. Since the unique physical, chemical and biological qualities of nano-sized particles (which differentiates them from macroscopic dispersions and continuous phases) have not been studied, it becomes quite topical to study the toxic effects and patho-physiological

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mechanisms of nano-sized materials on vital organs and systems [3].

The most prospective for the use in leading production branches – nano-electronics, nano-optics, synthetic nano-chemistry, in consumer segment – are metal oxides, including manganese oxide [4]. The planned production of matrixes based on nano-sized manganese oxide for nano-magnetic and sorbents, nano-catalysts, semiconducting thermistors in the volume of up to 1,000 tons a year is regarded as "mass-produced product" [5]. In this case there is a possibility of direct exposing of the personnel taking part in the process and the population in the territory is possible.

Exponentially growing development, production and commercialization of nano-technologic products containing nano-sized manganese oxide requires a fundamental study of peculiarities of toxic influence on the organs of the target, including pathomorphologic aspects of changes when being influenced through several channels. Accumulation and classification of the information is required for forecasting and working out criteria of safety for the products containing nano particles.

Studies and assessment of parameters of acute toxicity of nano-dispersive manganese oxide (III, IV) and its chemical equivalent in micro-dispersive state, carried out in accordance with the operating MU 1.2.2520-09 "Toxicological-hygienic assessment of nano-materials safety" [6] confirms a greater danger of nano-sized manganese oxide in comparison with its micro-sized equivalent. Medium lethal dose (LD_{50}) for the rats at one-off intragastric infusion was 2,577± 669.6 mg/kg of the body weight (3^{rd} class of danger under the classification of toxicity of lethal effects, All Union State Standard (GOST) 12.1.007.76 "Classification and general requirements for safety" and 6,000±542.5 mg/kg (4^{th} class of danger) [7]. It's a known fact that when metals get into the organism perorally, the gastrointestinal tract is directly affected. With manganese the increase of acid-producing stomach function and malabsorption of intestine are detected more frequently than with other metals [8].

The goal of this work is an experimental study and assessment of morphologic peculiarities of stomach tissues, small and large intestines under subchronic peroral influence of nano-dispersive manganese oxide (III, IV). Experimental studies follow on the works carried out within the framework of implementation of a scientific direction "Hygienic assessment of safety of the materials containing nano particles" of the branch program "Hygienic justification of minimizing risks for the population health of the Russian Federation for 2011-2015" [9].

Materials and methods. In the experimental studies there's been studied water suspension of nano-dispersive manganese oxide (III, IV) which was obtained by direct interaction of ions Mn^{2+} + MnO_4^- with the presence of nanoreactors micelle cetyltrimethylammonium bromide (CTAB, $C_{16}H_{33}(CH_3)_3NBr$) [10]. The synthesis was carried out in the laboratory of multi-phase dispersive systems of the Institute of Technical Chemistry of the Ural department RAS. Usage of micelles of superficially-active substance prevented the growth of particles and allowed to synthesize stabilized particles of certain sizes that correlated to the sizes of micelles [11]. STAB was removed by multi-extraction with ethanol in acidulous medium (with the help of chlorohydric acid). The degree of extraction was not less that 98%. For the comparative analysis while assessing morphologic peculiarities there was synthesized water micro-dispersive solution of manganese oxide (III, IV) using the same methodology but without adding STAB. The assessment of the size and form of micro-dispersive particles of the substance in water suspension was effected by the method of dynamic light diffusion with the analyzer Horiba LB-550 (Horiba, Japan) and laser analyzer Microtrac S3500 (Microtrac, USA) accordingly. The unit area of the surface (S_{BET})

of the particles was determined by the method of Brunauer, Emmett and Teller [12], texture parameters – by nitrogen sorption at the temperature of -196 °C on the analyzer ASAP 2020 (Micromeritics, USA). The concentration of manganese oxide in water suspension was determined by the method of atomic-adsorption spectrometry with air-

acetylene flame on the analyzer Perkin Elmer 3110 (Perkin Elmer Inc. USA).

The assessment of morphologic peculiarities of gastrointestinal tract tissues under subchronic infusion (30 days) of tested substances was conducted on reproductive rats line Wistar, males with the weight of 190.0±20.0 grams. The tested animals were divided into 3 groups with 10 specimen in each. The animals of the 1st group (experimental group) were infused nano-dispersive manganese oxide suspension (III, IV) with concentration of 41.0 mg/dm3 once with a catheter introgastrically (dosage 10.4 mg/kg ($1/250LD_{50}$). The animals of the 2nd group (comparison group) were injected micro-dispersive manganese oxide (III, IV) in the same dosage and by the same way. Its concentration was 54.6 mg/dm^3. The animals of the 3rd group (control group) were injected distilled water in the equivalent amount (1.2 ml).

For the period of the experiment the animals were kept in the conditions of laboratory vivarium (5 specimen in polypropylene cages of a standard size T/3) with semi-synthetic diet according to the methodological recommendations of "Toxicologic-hygienic assessment of nano-materials safety" (MU 1.2.2520-09). The access to the feed-stuff and water was not limited. Temperature in the rooms during the observation period was 23.0±2.0 Cº, humidity – 60.0±5.0%. Experimental manipulations were effected in accordance with the International recommendations (code of ethics) on conducting medical and biological research using animals [13].

In 30 days after the end of the experiment by the method of decapitation, the researchers took blood, stomach, small and large intestines of the rats. The selected material (small and large intestines) was treated in 10% neutral formaldehyde, then it was dehydrated in alcohols of high concentration, saturated by chloroform and paraffine and then it was embedded by homogenized paraffine medium "Histomix". Serial sections of blocks 4 micron thick were prepared with the help of sledge microtome Leica JUNG SM 2000R and dyed according to generally accepted methodology with haematoxylin and eosin. The obtained histologic samples were studied with a light-optical microscope Axiostar (Carl Zeiss, Germany). Microphotographs were taken on the light-optical microspoce MEIJI (Japan) with the installed camera microscopy VISION (Canada) with the zoom of x100, x200, x400, x800.

Identification of nano and micro-sized particles of manganese oxide in blood samples of the tested animals was performed by the method of electronic microscopy with a scanning microscope of high resolution (3-10 nm, maximum zoom 300000X) S-3400N (HITACHI, Japan) with a detachable device for x-ray energy dispersive micro analysis (Bruker, Germany) and field-emission scanning electronic microscope Ultra 55 (Carl Zeiss, Germany) in the mode of secondary electrons under accelerating voltage in the range of 1-20 kW and zoom from 1,000 up to 50,000 times. Sample preparation consisted of applying native material onto quartz substrate in the form of a thin dash without fixation; drying in indoor temperature in desiccator; coating carbon-conducting layer up to 15 nm thick onto the surface of the sample. The evaluation of particle sizes in the samples was carried out using software Smart SEMTM. The analysis of element composition – with the help of energy dispersive spectrometer Inca Energy (Oxford Instrument, Great Britain) with the resolution of < 137 eV, combined with a scanning electron microscope Ultra 55 (Carl Zeiss, Germany).

Results. Studied in the experiment water suspension of nano-dispersive manganese oxide (III, IV) had a structure of birnessite. The size of the particles in 97.8% of cases from the general amount of particles in a dispersive solution was 34–39 nm, the form was non-spherical (Figure 1-3).

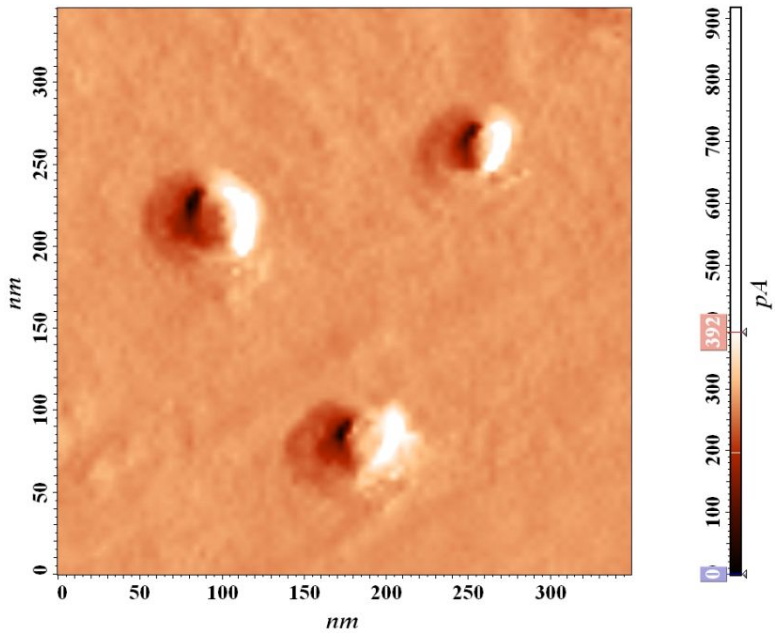


Figure 1. Image of synthesized particles of nano-dispersive manganese oxide with the help of atomic-powered microscopy

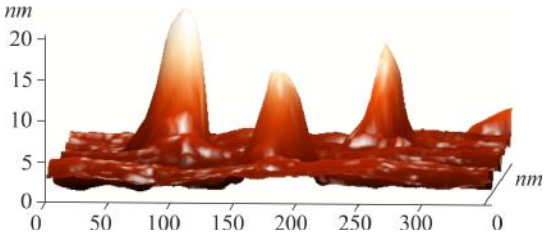


Figure 2. 3D-configuration of the surface of synthesized particles of nano-dispersive manganese oxide

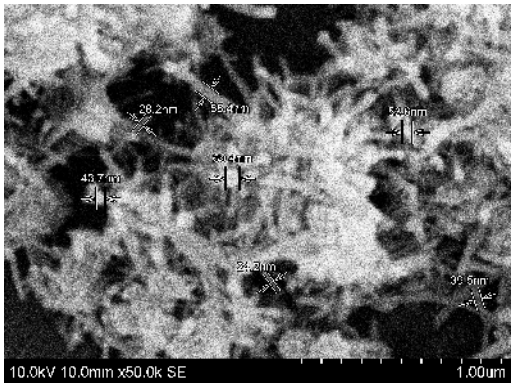
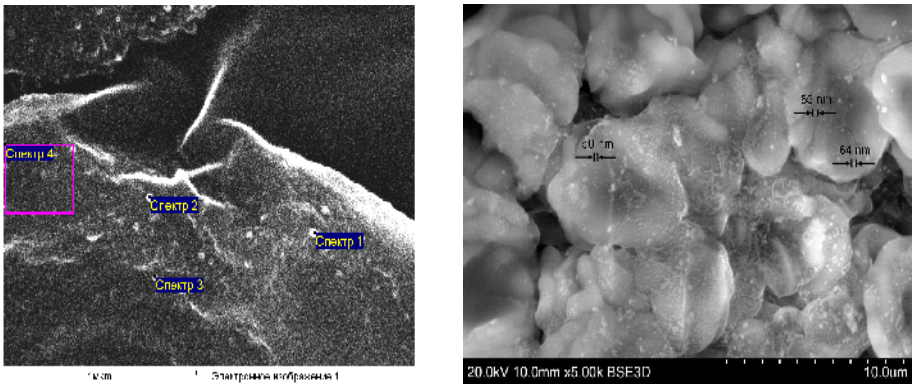


Figure 3. Electronic image of synthesized particles of nano-dispersive manganese oxide made with scanning electron microscopy, zoom 50 thousand. Visualization by the method of reverse electrons

(S-3400N «HITACHI»)

Microparticles of manganese oxide were particles of "traditional" dispersion" (1 μm and more) and in 98% of cases in the smaller axis had the particle size of 5.5 μm, particle form – non-spherical. Unit area of the surface of nano-particles of manganese oxide was 150.23 m^2/gr, which is 1.2 time higher than the unit area of the surface of micro-dispersive equivalent.

With electronic microscopy in the blood samples of the test animals (the experimental group) all over the seen area on the surface of aggregated erythrocytes there might be observed particles of non-spherical form with nano-sized range (less than 0.1 μm) (Figure 4a, 4b).



a *b*

Figure 4. Electron image of the rat blood sample from the experimental group. Dose of nano-dispersive manganese oxide is 10.4 mg/kg. Visualization by the method of scanning electron microscopy with electron recapturing: a - zoom 5 thousand; b - zoom 50 thousand.

The assessment of the elementary analysis results shows that the given particles in their chemical composition relate to manganese the share of which is 3.4%.

The analysis of electronic images of the blood from comparison group showed that at the background of single erythrocytes, the whole visible area is filled with particles corresponding to micro-size range (Figure 5). The results of the elementary analysis of the studied blood sample confirm that those particles are manganese particles. The results of electron microscopy and elementary analysis of the blood sample composition of the control group show the absence of nano- and micro-sized particles of manganese (Figure 6).

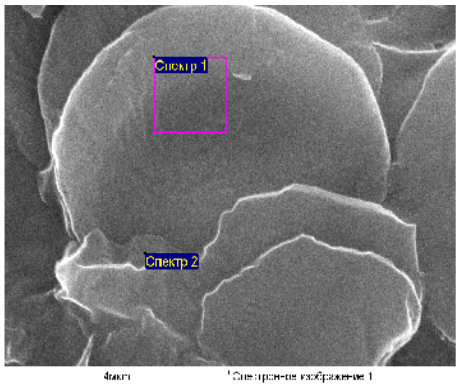


Figure 5. Electronic image of rat blood sample from the comparison group. Dose of micro-dispersive manganese oxide is 10.4 mg/kg. Visualization by method of scanning electron microscopy with electron recapturing (zoom x1000)

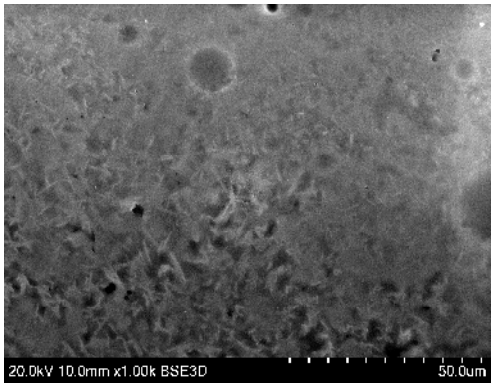
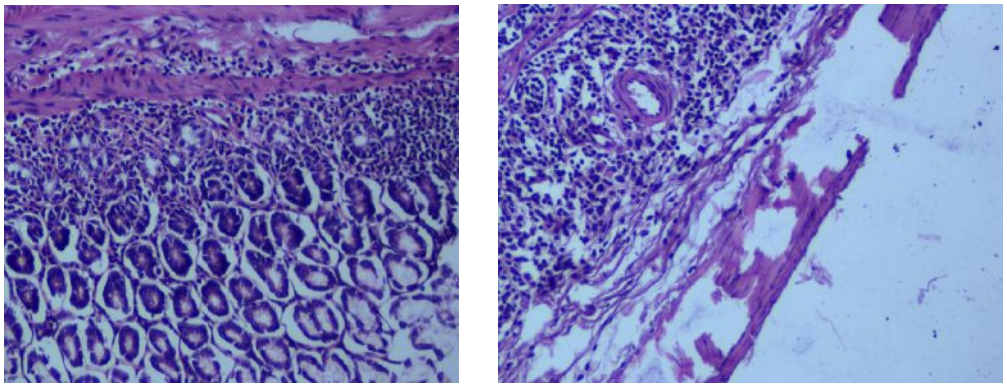


Figure 6. Electronic image of rat blood sample from the control group. Visualization by means of field-emission scanning electron microscopy (zoom x30000)

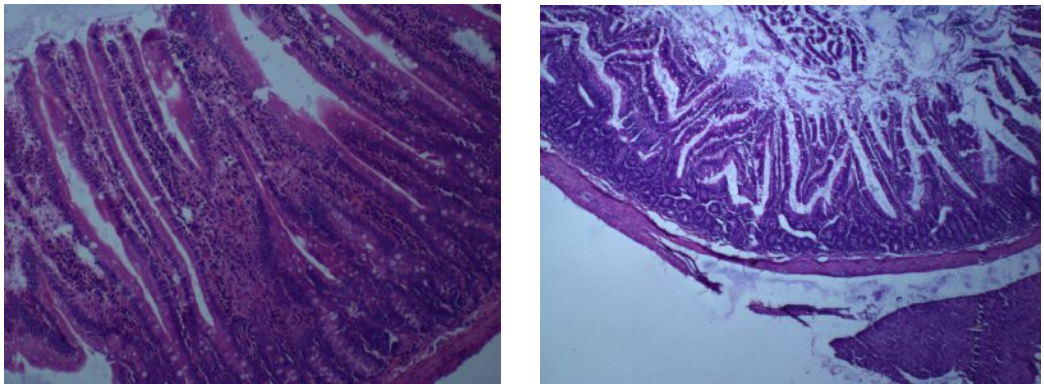
Morphologic analysis of gastrointestinal tract organs of the tested animals under subchronic intragastric infusions of water suspension of nano- and micro-dispersive manganese oxide in the dosage of 10.4 mg/kg showed the presence of inflammatory changes in mucous and submucous layer of stomach, small and large intestine. The character and degree of

manifestation of those had certain differences. The animals of the experimental group had in their lympho-macrophage infiltration a significant amount of eosinophilic and plasmic cells, moderate degree of manifestation, diffuse character of spreading (Figure 7a).



a *b*

Figure 7. Rat's stomach under subchronic catheter intragastric infusion of the tested substance in the dose of 10.4 mg/kg. The dye – haematoxylin-eosin, zoom x400: a – nano-dispersive manganese oxide; b – micro-dispersive manganese oxide



a *b*

Figure 8. Small intestine of the rat under subchronic catheter intragastric infusion of the tested substance in the dose of 10.4 mg/kg. The dye is haematoxylin-eosin: a – nano-dispersive manganese oxide, zoom x100; b – micro-dispersive manganese oxide, zoom x200

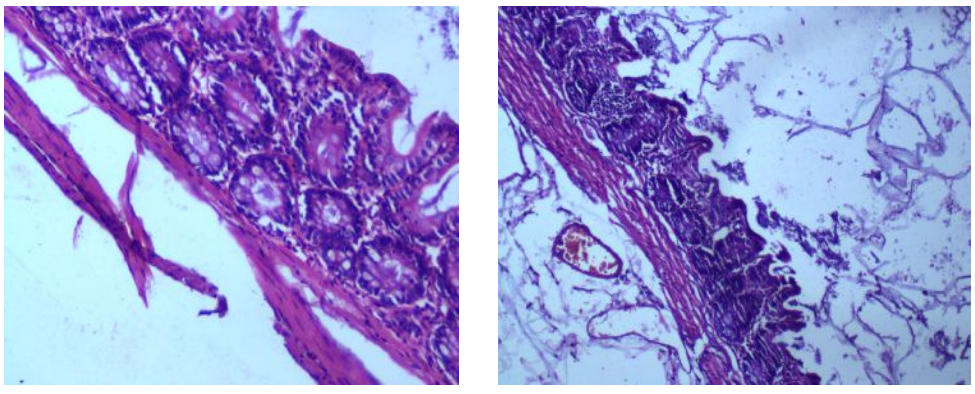


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Figure 9. Large intestine of the rat under subchronic catheter intragastric infusion of the tested substance in the dose of 10.4 mg/kg. Dye is haematoxylin-eosin: a – nano-dispersive manganese oxide, zoom x200; b – micro-dispersive manganese oxide, zoom x400

Besides, in the small and large intestines the inflammatory changes were alongside with development of significant edema of mucous and submucous layers, hyperplasia of lymphoid tissue of the intestine associated with mucous membrane and appearance of folliculars with light centers. In intermuscular nervous ganglias with eosinophilic polioplasm there've been detected dystrophic changes with manifested perineural edema (Figure 8a, 9a). The animals from the comparison group under subchronic infusion of micro-sized suspension had local obliterated inflammatory response in the studied organs characterized by lympho-macrophage infiltration with admixture of single eosinophils in mucous and submucous membranes (Figure 7b-9b).

Conclusions and recommendations. Patho-morphologic changes of the tissues in gastrointestinal tract organs of the tested animals under intragastric infusions of water suspension of nano-dispersal manganese oxide (III, IV) during 30 days in the dose of 10.4 mg/kg is characterized by diffusely-spread and moderately manifested inflammatory changes of the mucous and submucous layers of stomach, small and large intestines expressed through lympho-macrophage infiltration with a significant admixture of eosinophils and plasmocytes. Besides, in the intermuscular nervous ganglias there've been detected dystrophic changes with obvious perineural edema. Such changes haven't been detected when infusing a micro-dispersive equivalent. When infusing a micro-dispersive equivalent, an inflammatory response had local character and a low degree of manifestation.

In order to form a general concept and understanding about peculiarities of patho-morphologic changes of gastrointestinal tract organs under peroral infusion of the tested substance and in order to solve the problems of hygienic regulation, it is necessary to conduct a detailed experimental studies in the conditions of chronic access to nano-dispersive manganese oxide.

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