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THE EFFECTS OF SUBCHRONIC EXPOSURE TO MANGANESE OXIDE NANO-PARTICLES ON THE CENTRAL NERVOUS SYSTEM, PEROLIPID XIDATION, AND ANTIOXIDANT ENZYMES IN RATS

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Manganese oxide nanoparticles (MnO), due to their high reactivity, are widely used as an active substance in production of sorbents and catalysts used in liquid waste disposal. The entry of MnO with waste water into the surface waterbodies served as a source of the local drinking water supply presents a hazard to public health. However, the health effects associated with peroral consumption of MnO with drinking water are not well studied. Some studies have assessed the changes in the processes of lipid peroxidation and antioxidant protection, the balance of CNS transmitters in blood serum, after peroral administration of MnO nanoparticles sized 15-38 μm in an aqueous suspension to Wistar rats via a probe. MnO doses totaled 260, 50, 10 and 5 mg/kg body mass/day, every day for 7 days, in the period of 90 days. It was observed that MnO catalyzes lipid peroxidation (based on increased levels of lipid hydroperoxides and malondialdehyde in blood serum) and inhibits the activity of antioxidant system (based on lower total antioxidant status and Cu/Zn- superoxide dismutase in blood serum). The study revealed a distortion of the neurotransmitter ratio based on higher levels of glutamate and lower levels of γ- aminobutyric acid in blood serum. At a dose of 5 mg/kg per day, nanodispersed MnO did not cause the above effects. The identified negative effects are confirmed by morphological changes in the brain tissues (in cerebral cortex and cerebellum).

***Keywords**: manganese oxide nanoparticles brain neurotoxicity, oxidative stress, antioxidant activity*

Introduction. Unique physical and chemical properties of manganese oxide nanoparticles make them applicable for a wide range of uses in nanotechnology industry: electronics, optics, and syn-

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thetic nanochemistry. In particular, manganese oxide nanoparticles are used as an active substance in production of sensor electrodes, nanomagnetic and sorbing materials, nanocatalysts, and semiconducting thermistors [8]. However, despite such promising applications, manganese oxide nanoparticles can present certain hazards to the health and safety of people and cause serious socio-economic and ethical issues [4]. Exposure can take place at the workplace during the production process, or in the non-production environment involving the product consumers, via inhalation or consumption of drinking water.

Manganese oxide nanoparticles due to their small size and high penetrating capacities can penetrate though the blood-brain barrier and even small concentrations regardless the route of entry can cause morphofunctional disruptions of various parts of the central nervous system [17]. Assessment of the potential risks of impact of manganese oxide nanoparticles is focused, mainly, on inhalation as the most probable route of entry into the body. According to several authors [16], we can assume that in certain conditions, manganese oxide nanoparticles can have unfavorable and toxic effects on health via inhalation intake. They include an active catalytic generation of reactive oxygen species (ROS) registered in alveolar epithelial cells of humans after 24 hours of exposure [9], and an increase in the levels of extracellular and intracellular oxidized forms of glutathione by 30 and 80%, respectively [16]. Manganese oxide nanoparticles (IV) sized less than 30 μm, administered via inhalation intake, can penetrate into PC-12 neuron-like cells of the cerebrum via the olfactory nerve [8, 13], and accumulate in the cerebrum cells – astrocytes [8, 13]. This process is accompanied by a slight inhibition of mitochondrial activity and a dose-dependent decrease in the concentration of dopamine and its metabolites: dihydroxyphenylacetic acid and homovanillic acid. This process is accompanied by a multiple increase in the ROS [11] and presents in experimental animals in the form of neurodegenerative disruptions after 2-3 weeks of exposure [8, 13], activation of proteolytic cleavage mediated by caspase-3 and protein kinase Sδ (enzymes involved in the processes of apoptosis, necrosis and inflammatory processes), as well as phosphorylation of the activation loop [5, 10, 12, 19]. According to other authors, intranasal introduction of manganese dioxide nanoparticles (IV) to rats at the dose of 2.63 mg/kg of the body mass for 6 weeks results in neurotoxicity to increase the relative refractory period of the caudal nerve [17]. Intratracheal administration of the analyzed compound at a similar dose for 6 weeks leads to a considerable decrease in the body mass, lengthening of the absolute refractory period of the tail nerve, and reduction in the mobility of animals [17]. Neurotoxicity of manganese dioxide nanoparticles (IV) after intratracheal administration at a dose of 2.63 mg/kg and 5.26 mg/kg is also manifested in the increase the latent period of the cortical potential (total response of large populations of neurons in the cortex to the coming simultaneous flow of impulses arising under the influence of afferent stimulus) in the visual, auditory and somatosensory areas. This effect can be explained by the disruption in the function of neuron membranes resulting from peroxidation of membrane lipids followed by disruptions in calcium homeostasis [6]. It was observed that after a 24-hour exposure of alveolar epithelial cells in culture to manganese oxide nanoparticles, the level of extracellular and intracellular forms of reduced glutathione increased by 30% and 80%, respectively. The concentration of oxidized glutathione grows after a 24-hour exposure to manganese oxide nanoparticles which can be connected with the activation of the synthesis of γ- glutamyl-cysteine synthetase and increased activity of the transport system of glutamate and cysteine amino acids, which are substrates for the synthesis of glutathione, in its reduced form [9].

Today there is a growing interest to the use of manganese oxide nanoparticles as a sorbent and catalyst in the integrated treatment of liquid radioactive waste which is hazardous to public health. Here, there is a possibility for entry of manganese oxide nanoparticles with waste water into the surface waterbodies which serve as a source of the local drinking water. For this reason, the study of toxic effects associated with exposure to the oral intake of manganese oxide nanoparticles with drinking water is an important step in the assessment of their safety.

Earlier studies showed that after one-time administration of nanodispersed manganese oxide in an aqueous suspension via a probe, particle size = 15-29 μm, the median lethal dose (LD_{50}) for mice BALB/C equals 2340±602.6 mg/kg of the body mass, for Wistar rats - 2577 ± 669.6 mg/kg [3].

The purpose of this study is to assess the effects of subchronic exposure to nanodispersed manganese oxide in a water suspension on the cen-

tral nervous system, and the activity of oxidation and antioxidation processes in rats by the oral intake with drinking water.

Materials and methods. In the experimental studies, we used manganese oxide (MnO) in aqueous suspension, particle size = 15-29 μm, manganese oxide concentration = 41,37±2.5 mg/ml. The particles had a filamentous shape and a nonspherical surface area = 150,23 m^2/g. The synthesis of the aqueous suspension of nanosized MnO carried out in the multiphase dispersions laboratory at the Institute of Technical Chemistry, Ural Branch of the Russian Academy of Sciences, utilizing standard LCD templates which relate to nanotechnologies and help produce materials with unique textural and structural characteristics (high specific surface area, uniform pore size distribution and their ordering) [20]. To prevent the growth of particles, we used nanoreactors – micelles of the surface-active agent (SAA), - cetyl trimethyl ammonium bromide (CTAB, $C_{16}H_{33}(CH_3)_3NBr$) obtained from Sigma-Aldrich. CTAB is not part of the final product since it is removed by piercing or extraction at the final stage. The synthesis was carried out in the following stages: CTAB was dissolved in alcohol at room temperature under vigorous stirring for 30 minutes (CTAB/EtOH=1:10). A previously prepared aqueous solution of 0.4m $MnSO4 \cdot 5H_2O$ was added to the alcoholic solution of CTAB and stirred for 24 hours to form a microemulsion system. Then, with constant stirring, dropwise 0.05 M aqueous solution of $KMnO_4$ was slowly added. The resulting reaction mixture was stirred for 24 hours to complete the reaction. A dark-brown residum was washed from soluble reaction products with distilled water. CTAB was removed by extraction with ethanol (The degree of extraction was not less than 98%). Residual CTAB concentration in the aqueous suspension of nanodispersed MnO was determined after extraction by HPLC. The residual content of CTAB in the suspension was below the determination threshold (0.00001 mg/ml). Redistilled water was sued as the suspension matrix.

The distribution of MnO particles in aqueous suspension by size and particle shape was controlled by the methods of dynamic laser scattering (DLS) and scanning electron microscopy (SEM). Specific area of the MnO (S_{BET}) particles surface was calculated using the Brunauer-Emmet-Taylor method [2] after degassing of the test material under vacuum at 350 ° C for 3 hours. The concentration of MnO in aqueous suspension was determined with the help of ICP-MS method. Immediately before the study and weekly during the 90 days of the experiment, we performed dispersing of the aqueous suspension of nanosized MnO to evenly distribute the particles ultrasonically at room temperature during continuous pulsation at 65% power for two minutes.

The results of the assessment of physicchemical parameters of manganese oxide corresponded with the data earlier published by producers.

The study focused on the analysis of the ratio of major transmitters of the central nervous system: "exciting" neurotransmitter - glutamate affecting NMDA-receptors and He-NMDA-receptors, as well as the "braking" neurotransmitter - γ-aminobutyric acid (GABA) affecting primarily GAMKd- GABA B receptors and neurons (reagent kits Immundiagnostik, Germany). The activity of lipid peroxidation (LPO) at the level of cell membranes was analyzed by the content of the initial and final products of LPO - lipid hydroperoxides (Biomedica, Slovak Republic) and malondialdehyde (MDA), respectively. The activity of antioxidant system was analyzed by the content of antioxidant enzyme - Cu / Zn-superoxide dismutase (Cu/ZnSOD) (eBioscience, Austria), total antioxidant status (TAS) (Immundiagnostik, Germany) in blood serum. Determination of the biochemical parameters was performed using the kits according to the protocol for the automatic microplate enzyme immunoassay analyzer Infinite-F50 (Tecan, Austria).

The experimental studies were carried out on male and female Wistar rats (4 weeks old), with a body mass of 120±10 g, obtained from Andreyevka nursery at the Scientific Center of Biomedical Technologies of the Russian Academy of Sciences. Before the start of the experiment, the animals were held in quarantine for 14 days, housed in groups of 2 per cage made of polypropylene, standard size. The animal room with cages was ventilated and kept at 23,0±2,0 ^oC (temperature), 60,0±5,0 % humidity. The animals were fed a semi-synthetic diet, the nutrition and biological value of which completely satisfied the physiological needs. Access to food and water was not limited. The studies and procedures were conducted in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals intended for use in any experiment or for other scientific purpose (Strasburg, 1986), and the Ethical Committee at the Federal Budgetary Scien-

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The doses of nanodispersed MnO in aqueous suspension (260, 50, 10 and 5 mg/kg of the body mass/day) which totals 1/10, 1/50, 1/250 and 1/500 LD50 were administered intragastrically via a probe with the account for the body weight, during the period of 90 days (once a day – in the morning, 7 days a week). The dose was calculated and changed in accordance with the change in the volume of the aqueous suspension of MnO (in ml) without dilution. The volume of the aqueous suspension did not exceed the maximum volume recommended by OECD for aqueous solutions of the analyzed substances (20 ml/kg/day) and totaled 1.5 ml. A total of 100 Wistar rats participated in the experiment; the animals were distributed into 5 groups (group № 1-4 – experimental, group № 5 - control, with 10 species of males and females in each group). The control group of animals received distilled water in a similar manner and same volume. To determine the levels of biochemical indicators in blood serum, blood was taken from the tail vein of all the experimental animals before the start of the experiment (baseline), and on the 30th, 60th, and 90th day of the experiment.

Elimination of animals from the experiment was conducted on the 91st day, using carbon dioxide euthanasia. To analyze neurotoxic effects of nanodispersed manganese oxide, samples of the cerebrum were taken from the experimental animals, with the help of a special tool aimed for morphological studies.

The samples (cerebrum) were fixed in 5% aqueous neutral buffered formalin solution at a ratio of 1:50. The fixed tissue was dehydrated in alcohols of increasing concentration, then soaked in chloroform and paraffin, then embedded in homogenized paraffin medium Histomix. The paraffin blocks were used to obtain 4mm thick histosections using Sannomiya microtome model JUNG SM 2000R (Leica, Germany). Histological sections were stained by the standard technique with Erlich's hematoxylin and eosin. Microscopic preparations were performed using the light-optical microscope Axiostar (Carl Zeiss, Germany). Photomicrographs made with the help of the light-optical microscopy device «MEIJI» (Techno, Japan) equipped with a camera «microscopy VISION» (VISION, Canada) at magnification 100 ×, × 200, × 400

The analysis was performed using Statistica 6.0. Mathematical processing of the results was conducted with the help of parametric methods of statistics. To assess the statistical significance of the obtained data, we used Student's criterion (t). The differences in the obtained results are statistically significant at $p \leq 0,05$ [1].

Results and discussion. Assessment of the parameters of lipid peroxidation in the experimental animals showed a significant increase in lipid and MDA peroxidation on the 30th day of the experiment in groups 1-3 as compared to the baseline level and indicators of the control group – by 1.4-1.9 and 1.6-2.0 times respectively ($p<0,001$). The elevated level of lipid and MDA peroxidation in blood serum remained on the 90th day, the difference with the baseline level and the control group totaled 1.3-1.9 times ($p<0,001$). The indicators in group 4 throughout the entire experiment were not significantly different as compared to the baseline level and the control group (see Table 1).

Analysis of the activity of the antioxidant system showed a significant decrease in the level of antioxidant enzyme ZnSOD in blood serum in rats from groups 1-3. At the same time, the biggest decrease throughout the experiment was registered in rats from group 1. The level of Cu/ZnSOD on the 30th day decreased as compared to the baseline and control levels, on average, by 4.6 times ($p<0.001$), on the 60th and 90th day – by 2.5 times ($p<0,001$). In rats from groups 2 and 3, the level of Cu/ZnSOD in blood serum decreased on the 30th day as compared to the baseline and control levels by 1.4-1.9 times ($p<0,002$) and remained at a significantly low level until the 90th day of the experiment ($p<0,002$). Similar changes in the level of TAS in blood serum compared to the baseline and control levels were registered in rats from groups 1-3 ($p<0,001$). At the same time, the maximum decrease in TAS – by 5.1 times – as compared to the control group and by 5.4 times as compared to the baseline was observed in rats from group 1 on the 30th and 90th day ($p<0,001$). In rats from groups 2 and 3, the decrease in TAS in blood serum on the 30th day totaled as compared to the control level 2,2-2,4 times, and as compared to the baseline level – 2,3-2,6 times ($p<0,001$). On the 90th day of the experiment, the decrease in TAS in blood serum

Table 1.

Assessment of the activity of oxidation and antioxidation processes in Wistar rats after peroral exposure to nanodispersed MnO in aqueous suspension

Group, №	Indicator (M±m)			
	TAS (μmol /l)	Cu/ZnSOD (ng/ml)	Lipid hydroperoxide (μmol /l)	MDA (μmol /ml)
30th day				
Baseline level	174.1±10.13	5.01±1.01	30.76±2.64	1.62±0.20
Control	164.26±8.29	5.20±1.01	32.07±3.04	1.60±0.12
1	31.95±3.35*^	1.10±0.04*^	62.21±5.50*^	3.12±0.01*^
2	75.36±6.94*^	2.67±0.36*^	48.88±6.07*^	2.74±0.29*^
3	68.97±4.73*^	3.67±0.25*^	46.71±5.67*^	2.61±0.34*^
4	153.21±5.13	5.12±0.13	33.12±3.03	1.72±0.51
60th day				
Control	173.95±4.64	5.28±0.51	32.08±0.19	1.59±0.16
1	35.51±4.69*^	2.23±0.25*^	35.34±4.39	3.15±0.22*^
2	68.14±12.76*^	3.38±0.32*^	68.23±12.61*^	2.50±0.34*^
3	68.87±7.35*^	4.15±0.32*^	91.35±8.24*^	2.34±0.45*^
4	164.8±6.20	5.20±1.01	32.07±3.04	1.69±0.15
90th day				
Control	163.5±7.3	5.12±0.13	33.12±3.03	1.73±0.19
1	31.95±3.35*^	2.05±0.39*^	62.2±9.58*^	3.24±0.29*^
2	68.97±6.21*^	4.78±0.27*^	44.86±3.25*^	2.44±0.20*^
3	99.42±4.73*^	3.61±0.17*^	46.71±0.22*^	2.25±0.52*^
4	157.5±10.9*	5.17±0.13	32.12±2.11	1.75±0.22

*p<0,05 as compared to the value of the indicator before the start of the experiment ^p<0,05 as compared to the value of the indicator in the control group

in rats from group 2 totaled as compared to the baseline level 2.5 and 2.4 times, in group 3 – 1.6 and 2.4 times respectively ((p<0.001). In rats from group 4, the level of TAS was not significantly different as compared to the baseline and control levels throughout the entire experiment.

Assessment of the level of transmitters of the central nervous system in the experimental animals on the 90th day showed that in groups 1-3, the level of "exciting" neurotransmitter - glutamate in blood serum was significantly higher as compared to the baseline and control levels. At the same time, the maximum increase (by 3.8 times) was registered in group 1 (p<0.001). In rats from groups 2 and 3, the level of glutamate increased by 2-2.5 times (p<0.001). In rats from groups 4, the level of glutamate was not significantly different as compared to the baseline and control levels. The level of “braking” amino acid – GABA in blood serum was significantly lower as compared to the baseline and control levels. In rats from groups 1, GABA level decreased by 2.3 times, в group 2 – by 2.7 times (p<0.001), in group 3 – by 2.5 times (p<0.001). In rats from groups 4, the level of this indicator in blood serum was not significantly different from the baseline and control levels (see Table 2).

Assessment of histological preparations of the cerebrum of experimental animals showed the presence of morphological changes in the structure of tissues in a dose-dependent aqueous suspension of nanosized MnO. The test substance during peroral introduction at the dose of 260 mg / kg of the body mass / day causes in cerebral cortexi cerebellum a sharp vascular congestion with erythrocyte diapedesis and formation of focal hemorrhages (Figure 1a, 1b), subarachnoid hemorrhage, brain swelling with expansion of perivascular and pericellular spaces (Figure 2a), foci of demyelination of nerve fibers determined by the uneven staining of white matter fibers, and the presence of pallors with indistinct boundaries (Figure 2b).

Table 2.

Assessment of the balance of CNS transmitters under peroral exposure to nanodispersed manganese oxide in aqueous suspension on the 90th day of the experiment

Group, №	Indicator (M±m)	
	glutamate (μmol/l)	GABA (μmol/l)
Baseline level	242.1±18.2	0.32±0.03
Control	243.4±33.2	0.31±0.04
1	917.5±117.6*^	0.14±0.02*^
2	607.5±56.7*^	0.12±0.04*^
3	474.8±82.3*^	0.13±0.02*^
4	254.1±12.2	0.30±0.02

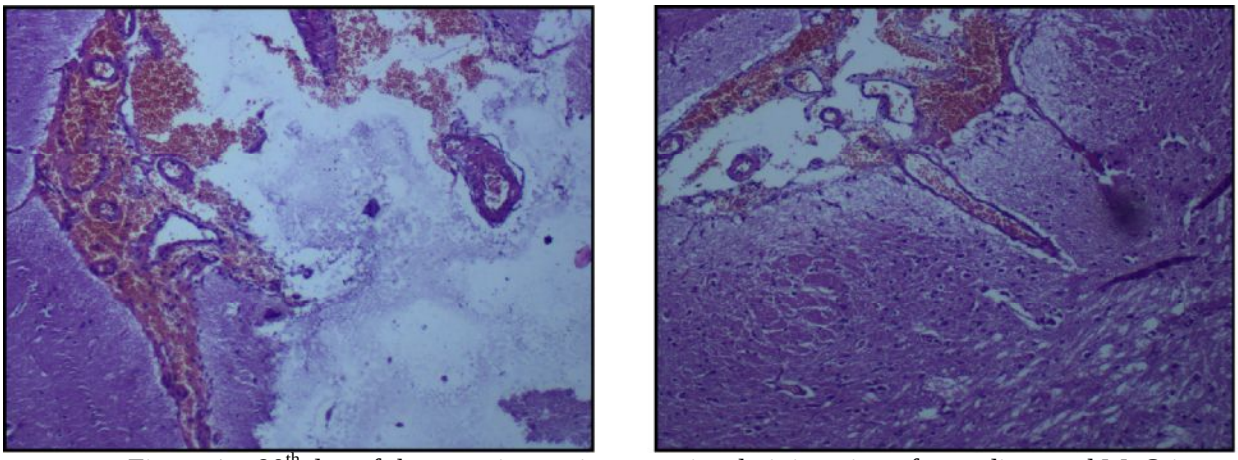

Figure 1 – 90th day of the experiment, intragastric administration of nanodispersed MnO in aqueous suspension at the dose of 260 mg/kg of the body mass/day, magn. x 200. H&E staining: *a* – cerebral cortex, *b* – cerebellum

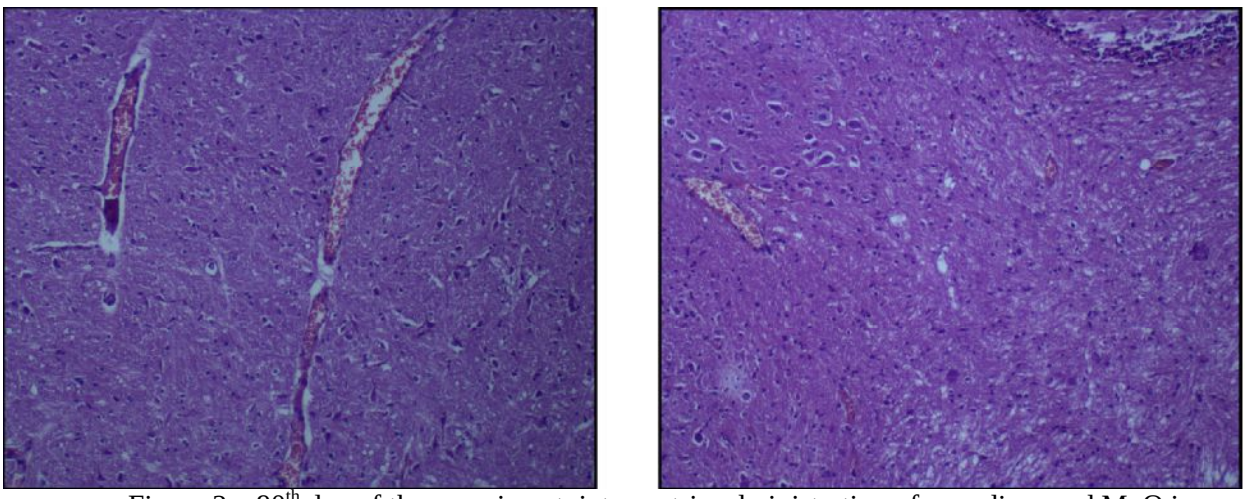

Figure 2 – 90th day of the experiment, intragastric administration of nanodispersed MnO in aqueous suspension at the dose of 260 mg/kg of the body mass/day, magn. x200. H&E staining: *a* – cerebral cortex of a rat, *b* – cerebellum of a rat

Peroral administration of nanodispersed MnO in aqueous suspension at the dose of 50 mg/kg results in focal expansion of perivascular and pericellular spaces in cerebral cortex, the differentiation of which is preserved (Figure 3a). The vessels of the substances of the analyzed tissue are thin-walled and have a moderate hyperemia, endothelium is flattened, there are small focal subarachnoid hemorrhage and small individual portions of pale colored deteriorated nerve fibers (Figure 3b) indicating, a focal degeneration of neurons.

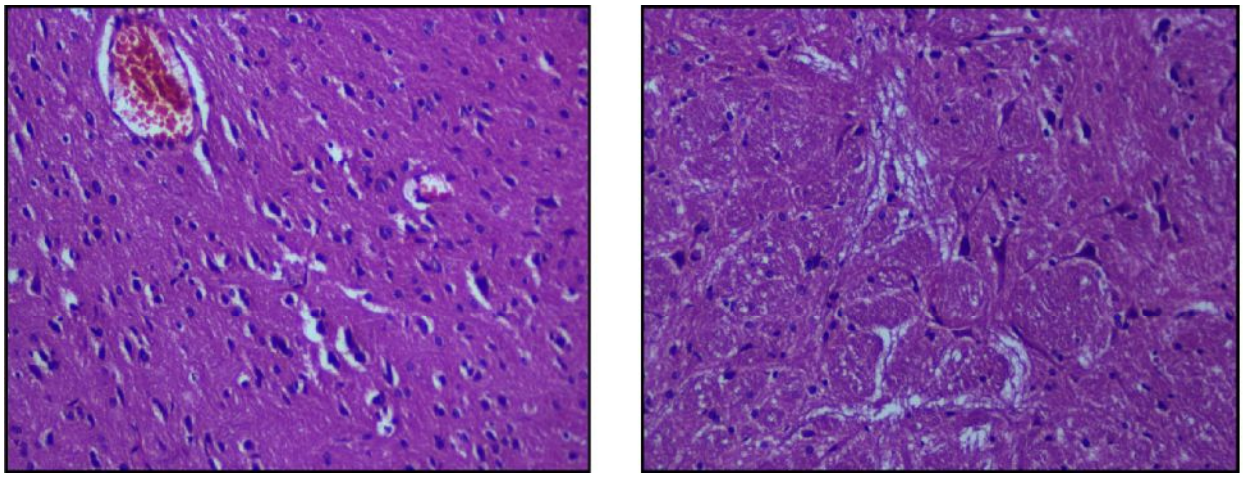

Figure 3 – Cerebral cortex of a rat on the 90th day of the experiment, intragastric administration of nanodispersed MnO in aqueous suspension at the dose of 50 mg/kg of the body mass/day, magn. x400. H&E staining: *a* – expansion of perivascular and pericellular spaces in cerebral cortex of a rat, *b* – deteriorated nerve fiber in cerebral cortex of a rat

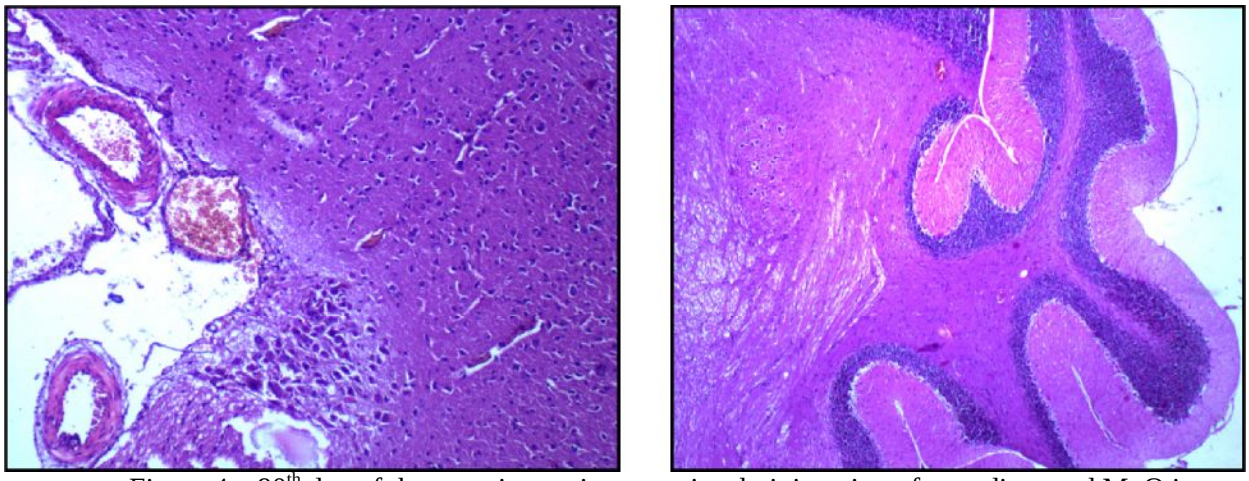

Figure 4 – 90th day of the experiment, intragastric administration of nanodispersed MnO in aqueous suspension. H&E staining: *a* – cerebral cortex of a rat, dose = 10 mg/kg of the body mass /day, magn. x200; *b* – cerebellum of a rat, dose 5 mg/kg , magn. x100

Peoral intake of nanodispersed MnO at the dose 10 mg/kg of the body mass/day causes an insignificant expansion of perivascular spaces (Figure 4a). At the exposure of 5 mg/kg, the morphological picture of the cerebrum and cerebellum of a rat corresponds with the control group (Figure 4b) and is characterized by the preservation of the pattern of the structure in all the sections.

The layers of the cerebral cortex are well differentiated. The outer granular layer is made up of a dense cluster of many small neurons; the pyramidal layer of the cerebral cortex is wide and comprises of polymorphic neurons. The internal granular layer of the cerebral cortex and cerebellum tissues is thin, discontinuous, formed by small pyramidal and stellate cells; the ganglion cell layer of the cerebral cortex tissue is large, with dark polymorphic

nuclei, diffused; in the polymorphic cell layer, there is a wide variety of neurons of various shapes and sizes. The ganglion cell layer of the cerebellum tissue is made up of one row of Purkinje cells with well-developed eosinophilic granular cytoplasm, round dark nuclei. The molecular layer of the cerebellum tissue is loose, with a small amount of small cells. The white matter in the cerebrum cortex and cerebellum is made up of bundles of uniformly stained nerve fibers and rounded glial cells. The vessels of the brain matter are thin-walled, of mild to moderate blood supply.

The observed effects (imbalance in the oxidation and antioxidation processes, CNS transmitters, morphological changes in the structures of the cerebrum cortex and cerebellum) suggest a negative impact on the experimental animals of nanodispersed MnO in aqueous suspension, administered intragastrically via a probe, for the period of 90 days, at the following doses: 260 mg/kg, 50 mg/kg, and 10 mg/kg of the body mass/day. Administration of nanodispersed MnO in aqueous suspension at a dose of 5 mg/kg did not cause significant changes in the analyzed biochemical indicators and morphological changes as compared to the baseline and control levels.

Generalization of the obtained materials helped us describe the toxic effects of nanodispersed MnO administered perorally. Activated lipid peroxidation resulting from a direct damaging effect on nanodispersed MnO on the bilipid layer of the cytoplasmic membrane can serve as a trigger [11, 16]. This effect is manifested on the system-level including an increase in the level of lipid hydroperoxides, MDA. This leads to inhibition of antioxidant processes [11]. Insufficiency of antioxidant processes is confirmed by reduced levels of Cu/ZnSOD and TAS in blood serum. The study showed that neuronal membranes and astrocytes serve as target cells for the aqueous suspension of nanodispersed MnO, administered perorally or inhaled [7, 10, 12]. This can be explained by the pronounced ability of nanodispersed MnO, as well as other metals, to penetrate from the gastrointestinal tract into the blood. From the bloodstream, MnO nanoparticles reach the brain tissue when entering through the capillary endothelial cells of the blood-brain barrier with subsequent accumulation in brain cells - astrocytes [10, 12]. Astrocytes damaged due to increased lipid peroxidation of cell membranes and the formation of reactive oxygen species may lose the ability to capture and neutralize excessive amounts of "exciting" amino acids - glutamate, which contributes to the excitotoxic effect [21]. This is manifested in increased concentration of glutamate and decreased GABA in blood serum.

Morphological changes in the cerebral tissues confirm the pathogenic effects of Mno nanoparticles determined during the assessment of biochemical indicators. Development of degenerative changes may be explained by a direct oxidation impact of nanoparticles on neurons, glial cells and vascular endothelium. The observed effects may be explained only by a direct contact of nanoparticles with the cerebral tissues which suggest a possible penetration through the hematoencephalic barrier.

Conclusion. The study showed that the aqueous suspension of MnO nanoparticles administered intragastrically to Wistar rats via a probe at the doses of 260 mg/kg , 50 mg/kg , and 10 mg/kg of the body mass/day during the period of 90 days causes the activation of lipid peroxidation (associated with increased levels of lipid hydroperoxides and MDA in blood serum), decreased activity of the antioxidant system (associated with reduced concentrations of TAS and Cu/ZnSOD in blood serum), damaged membranes of astrocytes and neurons, and distortion of the neurotransmitters ratio (associated with increased glutamate and decreased GABA in blood serum), vascular congestion, subarachnoid hemorrhage, brain swelling, expansion of the perivascular and pericellular spaces, foci of demyelination of nerve fibers, focal degenerative changes in the vascular endothelium.

The aqueous suspension of MnO nanoparticles administered at the dose of 5 mg/kg of the body mass/day does not cause the above effects.

Today there are not enough regulatory and methodological documents that would regulate the use of nanomaterials, in particular those entering a human body with drinking water. In order to assess the safety of such products, including nanodispersed MnO, consumed perorally with drinking water, it is necessary to continue the studies beyond the determined toxicity.

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