Study of the influence of anthropogenic factors on organism

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Abstract. The use of iron nanoparticles of various forms in industry continues to grow; therefore, there are risks of environmental damage and toxicity. The study was conducted on Wistar rats (n = 36). Nanoparticles of iron oxide and cobalt ferrite were injected once intraperitoneally at doses of 2 and 2.3 mg/kg (45 nm). On the 1st, 7th and 21st day of the experiment, the emotional and motor activities were assessed using the Open Field and Black and White Chamber tests. The results presented demonstrate that the liver and spleen are target organs that are able to accumulate nanoparticles; iron nanoparticles have a neurotropic effect and may have an anxiolytic effect. Despite the negative results obtained, the prospects of using nanoparticles cannot be denied. The use of a systematic and comprehensive approach to the assessment of risks and toxicity of nanomaterials will allow creating conditions to build a unified concept of nanosafety.

1. Introduction

The intensive development of nanotechnology has led to the introduction of their results in areas such as medicine, biology, agriculture, mechanical engineering, chemical industry and optics [1–5]. This intensive development of the nanoindustry leads to pollution of the environment by nanoparticles, which are then able to penetrate into the living organism [6–7]. However, despite this, the mechanisms of toxicity of nanoparticles and the potential risk to human health are not well understood.

In the field of biomedicine, various forms of iron nanoparticles are widely used [8–11]. Iron oxide nanoparticles Fe₃O₄ are used as an effective contrast agent for biological imaging, or as a carrier of biomolecules (drugs, nucleic acids) for their controlled delivery to certain organs and tissues. Much attention is paid to CoFe₂O₄ cobalt ferrite nanoparticles due to its wide therapeutic and agricultural applications [12]. They are used in magnetic resonance imaging due to the high coercivity, anisotropy and high magnetostriction; in drug delivery; cell labeling; destruction of cancer cells by magnetically induced thermal heating [13–14]. Along with this, many scientists report that metal nanoparticles can be the cause of some idiopathic diseases [15–16]. Studies show the presence of metal nanoparticles in pathologically altered tissues in inflammatory processes and tumors [17].

Today, the use of iron nanoparticles of various forms in biomedicine and industry continues to grow; therefore, there are risks of environmental damage and biological toxicity. In this regard, the determination of the causes of the pathological effects of iron nanoparticles and the assessment of their toxic effects on the body are the subject of a new direction in experimental research.
2. Purpose of research
The purpose of this work was to study the physiological and behavioral responses of the body when introducing various forms of iron nanoparticles.

3. Materials and methods of research
The study was conducted in an experimental biological clinic using 36 Wistar male rats weighing 250–280 g. The experiments were carried out in accordance with the Geneva Convention protocols and the principles of good laboratory practice (National Standard of the Russian Federation, GOST R 53434-2009), as well as The Guide for the Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996). Three groups were formed (n=36): a control group and two experimental groups. The animals of the experimental groups were injected intravenously once with iron oxide and cobalt ferrite nanoparticles at doses of 2 and 2.3 mg/kg, respectively. The control group of animals received an equivalent volume of 0.9 % of the isotonic sodium chloride solution intravenously.

The used nanoparticles were produced by the company «Advanced powder technology» (Tomsk). The Fe$_3$O$_4$ iron oxide nanoparticles obtained by chemical method corresponded to the following physical and chemical characteristics: Fe$_3$O$_4$ composition – 99 %, size – 45 nm, with a specific surface area of 20 m$^2$/g, Z-potential – 15±0.5 mV. CoFe$_2$O$_4$ cobalt ferrite nanoparticles were obtained by gas-phase method with a composition of 70 % iron and 30 % cobalt, 45 nm in size, with a specific surface area of 15 m$^2$/g, Z potential – 15±0.2 mV.

Assessment of the physiological state was carried out on days 1st, 7th, and 21st by integral indicators, including a study of the dynamics of body weight and organs, the volume of daily intake of food and water, changes in external signs and the degree of activity of laboratory animals. The emotional, motor activity and tentative research behavior of rats were studied in the Open Field and Black and White Chamber tests.

Blood sampling was performed on the 1st, 7th and 21st day of the experiment. A blood test was performed at the Central Scientific Center «BSC RAS», Orenburg. Determination of morphological parameters of blood was carried out on an automatic hemanalyzer URIT-2900 Vet Plus (China). The determination of blood biochemical parameters was performed using a CS-T240 biochemical analyzer (China) using DiaVetTest veterinary reagents (Russia).

The main data obtained in the studies were processed using the Excel and Statistica 6.0 programs. Assessment of the statistical significance of the effects was evaluated by the Mann-Whitney U-test. The data obtained are presented as the median (Me) and the 25th–75th quartile (Q25–Q75).

4. Results of research
All laboratory animals survived until the end of the experiment period and did not show a clear signal of pathology or abnormal behavior (refusal of food or water).

When evaluating psychophysiological indicators, it was found that the appearance of laboratory animals of the I and II experimental groups did not differ from the control throughout the experiment. According to the scale of changes in external signs of rats, the coat color was bright, the cover was free of contamination and hair loss. The degree of animal activity was evaluated on a scale of changes in rat activity. The animals of the experimental groups were mobile and had a good appetite.

Physiological changes occurring in the body of rats under the influence of injected nanoparticles were evaluated by changes in body weight and organs. On the 7th day after the introduction of nanoparticles in the first experimental group, a decrease in body weight was observed by 23 %, in the second group by 6 %.

When comparing the experimental groups with the control group, it was noted that the body weight was lower by 23 % (p<0.01) in group I; group II – by 4.7 % (p<0.01) on the seventh day. By the end of the study, this trend continued.

The mass of internal organs such as the heart, kidneys, lungs, and brain did not change during the entire experiment when different forms of iron nanoparticles were introduced. However, the mass of the liver and spleen increased. Figures 1 and 2 show the mass coefficients (MC) of these organs.
MK is used in toxicology to assess the status of internal organs, so you can detect the target organ of the toxicant. On the first day of the experiment, there was a slight increase in the mass coefficient of the liver in both experimental groups (p<0.05), which continued to increase during the experiment and by 21 days was almost twice as high in groups I and II (p <0.05). The spleen mass coefficient statistically significantly increased by 1 day in the second experimental group – 1.6 times higher than that in the control (p<0.05). By 7 days in both experimental groups, MK was also higher than control values (p<0.05). Thus, the liver and spleen are target organs that are able to accumulate nanoparticles.

The change in the behavioral activity of animals is clearly illustrated by the results of the Open Field and Black and White Chamber tests.

When taking into account changes in orientational-motor activity on the first day in animals of the I and II experimental groups relative to the control group, a decrease in the total HDA was noted: in the I group by 20.8 %, in the II by 14.5 %. There was a statistically significant decrease in total VDA in group I by 33 % (p <0.05). The level of emotional stress on the first day of the experiment, in particular total grooming, was higher in control animals, in experimental group II it was significantly lower by 75 % (p<0.01), the number of defecations in both experimental groups increased, while in group II there was a significant increase of 200 % (p <0.05). Also on the first day there was a change in the indicators characterizing the orientational research reaction – in group I there was a statistically
significant decrease in the rate of sniffing holes by 80 % (p<0.01). In the group that received the injection of CoFe₂O₄ iron ferrite nanoparticles, on the contrary, an increase was noted; the value of this indicator is 60 % (p<0.05).

The Open Field test is a strong stressing agent for rats, to which animals subsequently adapt and get used to, which was observed on the 7th day of the experiment in the control group – a decrease in almost all test parameters was noted. However, in the experimental groups, such a pattern was not observed, especially with the introduction of iron oxide Fe₃O₄ nanoparticles. So, the total HDA in group I significantly increased by 63.8 % (p<0.01) relative to the control group, the total number of racks increased by 114.2 % (p<0.01), total grooming and inspection of holes by 33 % (p<0.05). In the II experimental group, the values of the indicators were unreliable.

On the 21st day of the experiment, the following trend was noted: the level of total HDA in the experimental groups was lower by 2.6 % (p<0.01) and 7.7 %, respectively, relative to the control; indicators of grooming and defecation in both groups were lower than control values by 66.6 % and 100 % (p<0.01). Relatively 7 days in the experimental groups, on the 21st day of the experiment, activity as a whole decreased.

Thus, the introduction of iron oxide Fe₃O₄ nanoparticles and CoFe₂O₄ iron ferrite cobalt in doses of 2 and 2.3 mg/kg, respectively, affects the behavior of animals.

The results of the Black-and-White Chamber test were as follows: after the introduction of nanoparticles on the first day of the experiment in the experimental groups I and II, an increase in the number of peepings relative to the control group was observed by 40 % (p<0.05) and 100 % (p<0.01), respectively. By the 7th day, this indicator decreased, however, was higher than that in the control – in the I experimental group by 14.2 %; II – by 28.6 % (p<0.01). By the end of the experiment, the activity decreased, the values were close to the control (Figure 3).

The time spent in the white compartment was significantly longer in the experimental groups (Figure 4). The greatest biological effect was noted in the group with cobalt iron ferrite nanoparticles.

![Graph 1](image1.png)  
**Figure 3.** Dynamics of the number of peepings on the 1st, 7th and 21st days of the experiment

![Graph 2](image2.png)  
**Figure 4.** Duration of stay of animals in the white compartment on the 1st, 7th and 21st days of the experiment
Thus, the results of the Black and White Chamber test indicate an increase in research activity and a decrease in anxiety in animals of the experimental groups; similar results were also observed in the Open Field test.

Iron nanoparticles have a neurotropic effect and may have an anxiolytic effect. This was especially seen in the group injected with CoFe$_2$O$_4$ cobalt iron ferrite nanoparticles. It is known that metal-complex compounds of cobalt and iron have a sedative effect [18].

Summing up the results of behavioral testing, we can conclude that iron nanoparticles of various forms have a neurotropic effect, which is clearly visible throughout the experiment.

5. Conclusion
The main direction of biomedical use of iron nanoparticles of various forms is intravenous injection for magnetic resonance imaging or drug delivery. Providing data on toxicity, toxicokinetics and safe dose levels of these particles is very valuable for medicine.

The toxicity of iron nanoparticles, their oxides and compounds to humans, animals and the environment is an important issue since there are still disputes regarding the toxic effects and mechanisms of action of these nanoparticles. This indicates the need for research on the safety of nanoparticles of this metal.

With intravenous injection of iron oxide Fe$_3$O$_4$ nanoparticles and CoFe$_2$O$_4$ iron ferrite to laboratory animals at doses of 2.0 and 2.3 mg/kg, respectively, all laboratory animals survived until the end of the experiment and did not show an obvious signal of pathology or abnormal behavior (refusal of food or water). A decrease in body weight of laboratory animals was noted against the background of an increase in the mass of internal organs. It was found that the liver and spleen are target organs that are able to accumulate nanoparticles.

According to the results of Open Field and Black and White Chamber behavioral testing, iron nanoparticles have a neurotropic effect and may have an anxiolytic effect.

Thus, the study demonstrated negative effects with the introduction of iron oxide nanoparticles Fe$_3$O$_4$ and cobalt iron ferrite CoFe$_2$O$_4$. Despite the negative results obtained, there is no denying the prospects for using nanoparticles. It is important to understand that the real danger of using nanoparticles can be that the necessary assessments will not be carried out in time and precautions will be developed. Therefore, only the use of a systematic and integrated approach to assessing the risks and toxicity of nanomaterials will create the prerequisites for building a unified concept of nanosafety.

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