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Clinical Case

PRECLINICAL TESTING OF THE DOUBLE IRON OXIDE-BASED HEALTH SUPPLEMENT

LAS PRUEBAS PRECLÍNICAS DEL SUPLEMENTO DE SALUD BASADO EN EL DOBLE ÓXIDO DE HIERRO

Tsykhanovska Iryna[1]; Shmatkov Daniyil[2]; Evlash Victoria[3]; Koshova Olena[4]; Bielikova Nadiia[5]; Bolgova Natalia[6].

[1] *Ukrainian Engineering Pedagogics Academy, Ukraine*

[2] *Ukrainian Engineering Pedagogics Academy, Ukraine*

[3] *Kharkiv State University of Nutrition and Trade, Ukraine*

[4] *National University of Pharmacy, Ukraine*

[5] *Research Center for Industrial Development Problems of the National Academy of Sciences, Ukraine*

[6] *Sumy National Agrarian University, Ukraine*

Correspondence to:
Tsykhanovska Iryna
Ukrainian Engineering Pedagogics Academy, Ukraine
d.shmatkov@uipa.edu.ua

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Didactic Association ANDALUCIA

editor@journalshr.com

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RESUMEN

El desarrollo y uso de nano-suplementos alimenticios y dietéticos en tecnologías de alimentos con una amplia gama de propiedades funcionales y tecnológicas es una dirección científica y práctica prometedora de la nutrición moderna y saludable.
El suplemento de salud basado en el doble óxido de hierro Fe_3O_4 tiene la forma de polvo ultrafino con un tamaño de partícula de (70–80) nm. Para el uso del suplemento de salud en el campo de la nutrición saludable, la nutrición durante la recuperación y la nutrición deportiva se realizó una prueba. Este artículo presenta los resultados de las pruebas de los datos de seguridad de un nuevo suplemento de salud en estudios preclínicos con animales de laboratorio e in vitro.

Los resultados de las ruebas citotóxicas indican que el suplemento de salud presenta un efecto citotóxico, solo en concentraciones (20 mg / ml; 10 mg / ml; 5 mg / ml). En concentraciones equivalentes a la ingesta humana diaria del producto, no se reveló ningún efecto tóxico en el cultivo de células de médula ósea roja de ratas, como lo demuestra la ausencia de Disminución estadísticamente significativa en el número de células viables de médula ósea. A concentraciones de 0.00002 mg / ml a los 90 minutos de incubación, el suplemento de salud produjo un efecto citoprotector, lo que incrementó estadísticamente la viabilidad de las células de la médula ósea de rata.

Por lo tanto, las pruebas realizadas demuestran que el suplemento de salud basado en el doble de óxido de hierro es suficientemente seguro, lo que implica la posibilidad de su uso generalizado en tecnologías de producción de alimentos saludables y permite recomendar el suplemento de salud para estudios adicionales con respecto a su Parámetros de seguridad.

Palabras clave: suplemento de salud, doble óxido de hierro, nutrición saludable, seguridad, citotoxicidad.

ABSTRACT

The development and use of food and dietary nano-supplements in food technologies with a wide range of functional and technological properties is a promising scientific and practical direction of modern healthy nutrition.
The double iron oxide-based health supplement Fe_3O_4 has the form of ultrathin powder with a particle size of (70–80) nm. For the use of this health supplement in the field of healthy nutrition, nutrition during recovery and sports nutrition a testing was conducted. This article presents the results of the testing of the safety data of a new health supplement in preclinical laboratory animal studies and in vitro.

The results of the cytotoxic testing indicate that the health supplement exhibits a cytotoxic effect only at concentrations (20 mg / ml; 10 mg / ml; 5 mg / ml). At concentrations equivalent to the daily human intake of the product, no toxic effect on the red bone marrow cell culture of rats was revealed, as evidenced by the absence of a statistically significant decrease in the number of viable bone marrow cells. In addition, at concentrations of 0.00002 mg / ml at 90 minutes of incubation, the health supplement produced a cytoprotective effect, statistically significantly increasing the viability of rat bone marrow cells.

Thus, the testing conducted proves that the double iron oxide-based health supplement is sufficiently safe, which entails the prospect for its widespread use in healthy food production technologies and allows recommending the health supplement for further studies with regard to its safety parameters.

Keywords: the double iron oxide-based health supplement, healthy nutrition, safety, cytotoxicity.

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1. INTRODUCTION

Proper nutrition is the most important factor that ensures human health, ability to work and resist external adverse effects, and determines the quality of life and life expectancy. Violation of the principles of rational, balanced nutrition inevitably leads to the spread of nutritional (non-infectious) diseases and, ultimately, to unjustified social losses. Scientific and technological advances, development of nano- and biotechnologies, innovative technological solutions allow creating a new generation of food products of high quality, efficiency and safety (Friel, 2017; Moore & Weeks, 2011).

The food ration of a modern person is characterized by the presence of so-called "empty calories" due to the consumption of canned food, the use of tough technological and culinary methods of its production and storage, leading to the loss of vital nutrients, primarily vitamins (Friel et al., 2017; Moore & Weeks, 2011). In addition, food safety issues arise due to anthropogenic contamination of raw materials and uncontrolled use of synthetic and identical to natural food additives (Elmadfa & Meyer, 2014). Implementation of modern innovative technological solutions in the food industry is complicated without the use of microingredients (food additives, flavors, auxiliary technological means, and functional ingredients). Through their use, deeper processing and careful use of agricultural raw materials, improvement of technological processes, reduction of production costs and product prices are ensured, higher organoleptic standards are achieved, competitiveness and availability of healthy food products for all groups of population are increased (Magni et al., 2017; Mikkelsen et al., 2014). Studies on the use of nanotechnologies are well known in the fields of sports and healthy nutrition (Dunford, 2010; Kuhlmeier et al., 2012; Neelam, 2017).

Health nano-supplements from natural minerals should be viewed as an important supplement to the integrated use of both natural health and dietary supplements (Tsykhanovska et al., 2018). Moreover, at the moment, modern pharmacology is not able to fully eliminate the deficiency of essential nutrients, which itself is the cause of nutritional diseases. In this regard, it is important to highlight that nano-supplements from natural mineral raw materials are natural substances and their complexes that have been familiar to the human body over millions of years of its existence and which were embedded in metabolic processes and encoded by the genetic apparatus in the ancestral memory (Homonnay et al. , 2016; Jalali et al., 2016; Tsykhanovska et al., 2018). As for modern micronutrientology, it faces the challenge of moving to a new level of knowledge about the mechanisms of food components and their active ingredients in the human body (Elmadfa & Meyer, 2014; Magni et al., 2017). Furthermore, the deficit of food that meets the physiological needs of a person is inevitable (El-Hack et al., 2017; Magni, 2017 et al.).

It must be added that the development of new products should be based on their complete safety, the absence of possible risks to human health and quality assurance, including nutritional value, which satisfies the need for the necessary macro and micronutrients and minor food components.

The key aspects in addressing this issue are the scientifically based search and selection of promising sources of raw materials with high sanitary, hygienic and biomedical indicators. The use of modern nano- and biotechnologies is one of the promising trends, which allows not only to significantly affect the organoleptic and physical-chemical indicators of raw materials and finished products, increasing their nutritional value, but also to give them specific functional properties (El-Hack et al., 2017; Homonnay et al., 2016; Jalali et al., 2016).

The term "nanoparticle" does not apply to individual molecules, but is usually used to refer to inorganic materials. These particles differ in their physical-chemical and functional-technological properties, as well as in their ability to adsorb various components on their surface and affect environmental conditions in different ways. Various types of nanoparticles are used in such industries as nutrition, pharmacy, medicine, drug delivery, therapeutic products, vaccine compositions, diagnostics, chemical industry, sport, biotechnology and biomedicine to protect human and animal health. The use of nanoparticles is a promising solution for producing food products for functional purposes, stimulating metabolic processes and organism power recourses, improving activity, metabolism and growth as well as the quality of finished products.

Nanotechnology has become an important element of pharmacy, and nanomaterials have found many applications in drug delivery systems to increase the therapeutic efficacy of drugs. Most modern “nanosystems” of drug delivery are conventional pharmaceutical forms, such as nano-micelles, nano-emulsions and nano-suspensions. In addition, nanotechnology will play an important role in future research areas of animal nutrition. Nano-supplements can be fitted into capsules or micelles of protein or other natural food, or food component (Andi et al., 2011). The use of nanoparticles of nutrients can improve the bioavailability of transported nutrients through the intestinal epithelial barriers and their sensitivity to the destruction of the gastrointestinal tract by digestive enzymes (Bunglavan et al., 2014; Sharma et al., 2007). The proposed nanoformulated substances can also improve the functionality of feed / food molecules in favor of the quality of the final product (Canham, 2007; Rui et al., 2016; Shi et al. 2011).

Consequently, iron oxide nanoparticles (Fe_2O_3 and Fe_3O_4) are effectively used to feed plants to improve metabolic processes: growth, photosynthesis, the ability to absorb hydrogen peroxide and superoxide anion, helping to reduce the rate of lipid peroxidation in membranes, increase the amount of ferritin, maintain Fe homeostasis and the balance of redox and immune systems (Homonnay et al., 2016; Jalali et al., 2016).

The use of health supplements in nano-form is known in feeds for birds and animals, particularly zeolite-hydrocolloid silver (Ognik et al., 2016); nano-capsules: commercial products, nano-Se, nano-Si (Shi et al., 2011). Their use helps to improve feed intake, body mass gain and feed efficiency. The researchers have attributed the positive effect of their action to the effect of nanoparticles on intestinal pathogenic bacteria and the relief of the intestine, and consequently, to better absorption of nutrients (Ognik et al., 2016).

The integration of health supplements in capsules or micelles of the food components, particularly proteins, polysaccharides or fats, is effective (Dziechciarek et al., 1998; El-Hack et al., 2017). Currently, the smallest micelles (nanocapsules) are used as carriers for essential oils, flavors, antioxidants, coenzyme Q10, vitamins, minerals to improve the bioavailability and digestibility of nutrients (El-Hack et al., 2017). It is known that the use of nano-supplements in food contributes to better digestion and assimilation of nutrients, due to the increased sensitivity to food digestion by digestive enzymes of the gastrointestinal tract and bioavailability through intestinal epithelial barriers (Bunglavan et al., 2014). At the same time, the improvement in the digestibility of nutrients is also associated with an increase in the lifetime of trypsin and peroxidase enzymes (from several hours to weeks), due to their “sticking” to nanoparticles (Sharma et al., 2007).

Scientists have successfully developed amylum-based nanoparticles (Dziechciarek et al., 1998), which behave like colloids in aqueous solutions and can be introduced into food during the mixing and emulsification stages, in order to give food systems the necessary functional and technological properties (Tsykhanovska et al., 2018). Thus, health supplements in nano-form improve the functionality of food in favor of the quality of the finished product at the molecular level.

This work is the introduction of nanotechnology in food production. Will nanotechnologies in the field of healthy food products be appreciated by consumers; will they become desirable and be in demand? The global value of food nanoproduct sales is growing, and, apparently, this trend will continue in the future. Since 2005, “Nano-food” conferences on the integration of nanotechnologies in the food industry have been held annually, and continuous improvement of the quality, health utility, product safety, prevention of certain diseases, creation of therapeutic and prophylactic and functional products are among the main objectives of their agendas (Aleshkov, 2016; Thangavel & Thiruvengadam, 2014).

Presently, the world market already has several hundred food products manufactured using nanotechnology. Basically, such products are common in the US, Asian and European markets. However, nano-supplements often have a toxic effect on the human body. The scientific community`s views are rather mixed when assessing the possibility of using nanotechnology in food production. The

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effects on the body of nanotechnology products require additional analysis. In this regard, in 2000, 57 countries all over the world signed the Cartagena Protocol, where the so-called precautionary principle was established. The official documents indicate that nanoparticles have an active, developed surface; the complex of physical and chemical properties and biological action (sometimes toxic) often radically differs from the properties of this substance in the form of continuous phases or macroscopic dispersions. All these determine both the positive effect of nano-supplements on the functional and technological characteristics and indicators of the quality of food products, and can cause specific exposures and risks when used in food products (Aigbogun et al., 2017; El-Hack et al., 2017).

At the state level, official certification of food nanoproducts was first introduced in Taiwan, where in 2005 the “Nano-Mark” certificate was developed. Products under this brand must meet at least two requirements: the size of nanoparticles must be within 1 to 100 nm; a nanoproduct must have fundamentally new consumer properties or improved characteristics namely because of its nano-dimensions. Nevertheless, the manipulations with the amount of nutrients at nanolevel open up possibilities for diversification and improving the functionality, physiology and quality of food products; and for the introduction of environmentally friendly and economical nanotechnologies (El-Hack et al., 2017).

For the purpose of creating specialized products that make an important contribution to the nutrition and health of modern humans, Ukrainian scientists have developed a double iron oxide-based health supplement with advanced technology that allows achieving the necessary functional and technological properties and reducing the complexity of the manufacturing process. Previous experiments proved that the double iron oxide-based health supplement in food systems exhibits a complex effect: antioxidant, bacteriostatic, emulsifying, moisture-binding, water-retaining, fat-retaining, stabilizing and structuring (Tsykhanovska et al., 2018). The polyfunctionality of the double iron oxide-based health supplement determines the main direction of its use – an improvement of the quality of food products and an extension of the shelf life when creating conditions for healthy and functional nutrition.

It should also be noted that, along with the main practical use in food technology, the double iron oxide-based health supplement, when regularly consumed, as an antioxidant and a source of easily digestible iron (II) will help to improve the overall metabolic processes in the human body, including iron deficiency disorders, and have a positive effect on the human organism, acting as an energy food supplement that has a positive effect on the gastrointestinal tract (GIT). Based on the above, this nano-object can be used to address the problem of preventing and treating metabolic disorders, namely iron deficiency disorders, by introducing the double iron oxide-based health supplement into various food products.

Accordingly, the use of the double iron oxide-based health supplement in various food technologies and in functional food establishes a functional and technological potential. But it is necessary to take into account the safety data of the supplement.

The double iron oxide-based health supplement in terms of chemical composition is identical to magnetite, a natural mineral of biogenic origin. Therefore, we will further consider the accumulated scientific experience in assessing the toxicity and safety data of magnetite nanoparticles (Fe_3O_4), which have been the subject of many medical and pharmacist studies.

Intravenous administration of an aqueous suspension of magnetite nanoparticles with a dispersity of (5–60) nm, revealed the following:

- a single injection of the suspension (100 mg of magnetite per 1 kg of body mass) causes reversible metabolic disorders of the liver, kidneys and heart of rats;

- repeated administration of the suspension (from 300 mg to 2 g of magnetite per 1 kg of body mass) causes agitation that continues throughout the duration of the testing (40 days) and is dose-dependent.

Furthermore, repeated administration of the magnetite suspension causes changes in the energy and plastic metabolism of hepatocytes, cardiomyocytes and nephrocytes of rats, while its single use does not affect the metabolic status of the

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studied cells. In addition, the studies revealed the prooxidant properties of magnetite nanoparticles and the ability to cause activation of the antioxidant system of the blood plasma of rats (Milto et al., 2011). The effect of magnetite nanoparticles of dispersion (60–80) nm on the human body was also revealed experimentally in vivo (Ilyukha et al., 2010). Cytomorphological studies were performed using the percentage of electronegativity of the nuclei of the population of epithelial cells of the individual. It was revealed that the time of the full effect of the medication on the body is (6 ± 0.5) hours, while the time of its active effect is (2.5 ± 0.5) hours. The increased activity of the stomach, duodenum and pancreas was recorded. All these characterize the food nano-additive as a rapidly digestible substance that positively affects the organs and organ systems of the human body, and certifies the nano-additive as nutritional. Synthetic magnetite can alter the activity of the surface of the stomach and pancreas cells (Gil et al., 2010). Among the factors that determine the toxicity of nanoparticles, besides dosing and the method of their administration, the most significant are: size, surface area, shape, composition, modifying coatings of nanoparticles. The findings indicate that changing the surface of nanoparticles is the main tool for minimizing toxic effects. A high ratio of the surface size to the volume of nanoparticles can potentially lead to adverse biochemical reactions. It was also revealed that at concentrations of (20–100) mg / ml for large magnetite nanoparticles (more than 40 nm), they exhibit low toxicity, LD_{50} = 10 g / kg, low level of mutagenic hazard, and inability to cause negative body reactions during intravenous, intraarterial, intramuscular and other routes of administration (Krishnan, 2010; Saiyed et al. 2003). Taking into account and summarizing the findings on the toxicity of ferrite nanoparticles (namely magnetite), it is necessary to note the need to test the toxicity of Fe_3O_4 nanoparticles of dispersion (70–80) nm (namely the double iron oxide-based health supplement) when used in food systems. Further testing was aimed at establishing indicators of the index of acute and chronic toxicity of nanoparticles of the double iron oxide-based health supplement during nonparenteral administration. The findings are the basis for food safety assurance.

It is worth noting here that the analysis of information sources clearly shows a lack of data on the cytotoxic effect of the double iron oxide-based health supplement.

It is crucial to include in the daily ration products with improved consumer properties and increased nutritional value, products with tailor-made properties (namely functional food products enriched with essential nutrients and micronutrients), as well as dietary food supplements along with traditional natural food products.

The double iron oxide-based health supplement as an antioxidant and a source of easily digestible iron (II) when consumed regularly will help to improve the overall metabolic processes in the human body, including iron deficiency disorders. The physical-chemical properties of the natural mineral Fe_3O_4 in the form of nanoparticles have been studied in considerable detail (Křišťan et al., 2014), but for its use on the basis of double iron oxide in the field of healthy nutrition and nutrition during recovery, it is necessary to test its effect on living organisms.

Since one of the important stages in the establishment and implementation of healthy, functional and restorative nutrition is the testing of the safety of new health supplements, the purpose of our research is the preclinical testing of some of the toxicity parameters of the double iron oxide-based health supplement.

2. METHODS

In the process of determination of the toxicological characteristics of substances (including health supplements), the first step is determining the acute toxicity, i.e. obtaining information about potential dangers this substance poses to human health under conditions of short-term exposure to high doses. Setting the parameters of acute toxicity in animals allows identifying the signs and symptoms of harmful effects on humans, as well as obtaining important information about the cumulative properties of the substance under study and its possible side effects on the human organs and systems.

The testing of the acute toxicity of the double iron oxide-based health supplement was conducted in laboratory animals. In the experiment, white non-linear male and female mice of about 25 ± 2 g body mass were used. In order to set the parameters of acute toxicity, the double iron oxide-based health

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supplement was administered intragastrically at a single maximum dose of the toxicity class of 5000 mg / kg. Observation of the animals was carried out daily within 14 days. The degree of toxicity of the double iron oxide-based health supplement was estimated by the change in the general condition and the influence on the dynamics of the body mass of the animals. Animal survival, their consumption of food and water were recorded. At the end of the observation period, on the 15th day of the experiment, the animals were euthanized by an overdosage of inhalation anesthetic. The autopsy and macroscopic examination of internal organs were conducted. The effect of the double iron oxide-based health supplement on the internal organs was estimated by the change in the relative mass of the liver, kidneys, lungs, heart, spleen, thymus, and genitals. For this purpose, the organs were removed, weighed on an electronic scale, and their relative mass was calculated by the following formula:

$$RM, \% = (\text{Organ mass (g)} / \text{Animal mass (g)}) * 100\%,$$

where: RM is the relative mass of the organ

In order to study the safety of the double iron oxide-based health supplement directly on a cellular level, its basic cytotoxicity was estimated through in vitro testing on a bone marrow cell culture model of male rats of about 180–200 g body mass with the use of trypan blue. Bone marrow cells were extracted from the thighs of animals in the cold. The final suspension contained 2.0–2.1 × 106 cells / ml. The suspension with the additive based on double iron oxide on the basis of sodium chloride was added to the tray for immunological reactions using a dispenser, upon which an equal proportion of a bone marrow cell suspension was added to each cell. The following concentrations of the test substance were studied: 20 mg / ml; 10 mg / ml; 5 mg / ml; 2 mg / ml; 0.02 mg / ml; 0.00002 mg / ml. Sodium chloride solution was used as a means of control. The evaluation of the cytotoxic effect of the health supplement was carried out after 15, 30, 60 and 90 minutes of incubation. To determine the viability of the cells, a coloring method with the use of 0.1% trypan blue solution was applied. Trypan blue is an acidic aniline coloring material that is not able to enter the cell through intact cell membranes, but selectively stains the dead cells. The quantification of viable cells was performed in a hemocytometer, the results were expressed as a percentage of all viable cells of their total number. The average values obtained in triplicate and their standard errors were calculated.

The laboratory animals used in the experiments were grown in the vivarium of the Central Research Laboratory of National University of Pharmacy, (Ukraine), which is equipped in accordance with modern sanitary and hygienic standards. The animals were kept in standard plastic cages of 6–8 heads in each cage in a separate room with controlled microclimate parameters at an air temperature of 20–24 ° C, a relative air humidity of 45–65%, and a light mode “12 hours day / night”. The room was aired daily and the air was sterilized with a UV lamp. Animals had free access to water. For drinking settled distilled tap water from the troughs was used. The animals were given pelleted complete feed for mice and rats ad libitum.

All the studies were conducted in compliance with the requirements (Directive 2010/63 / EU, 2010).

Statistical data processing was performed using the software package Statistica 10. The check for normality of distribution of quantitative data was carried out using the Shapiro-Wilk test. In case of absence of correspondence between the obtained data and the normal distribution, the total intergroup differences were assessed using the Kruskal-Wallis test. Pairwise intergroup comparisons of the indicators were performed using the Mann-Whitney U-test. If the obtained data corresponded to the normal distribution, ANOVA analysis of variance was used, and intergroup comparisons of the indicators were performed using the Newman-Keuls test. The critical level of significance was assumed equal to or less than 0.05. Quantitative indicators in the tables are given as the mean and its standard error or the minimum and maximum values of the sample. The sample size of n=24 animals was chosen.

3. RESULTS

A single intragastric administration of a high dose of the double iron oxide-based health supplement did not lead to the death of animals. Observation of the general physiological state showed the absence of

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signs of intoxication in experimental animals following the use of this product. During the entire observation period, the mice were mobile, had a satisfactory appetite, looked neat, responded normally to light and sound stimuli.

The assessment of the possible toxic effect of the double iron oxide-based health supplement on the animal organism was carried out on the basis of the integral indicator of the physiological state of animals – body mass. The dynamics pattern of this indicator during the observation period is given in table 1; Figs. 1-2 show the histograms of the distribution of the mass values of male (Fig. 1) and female (Fig. 2) mice that were administered the double iron oxide-based health supplement during the experiment.

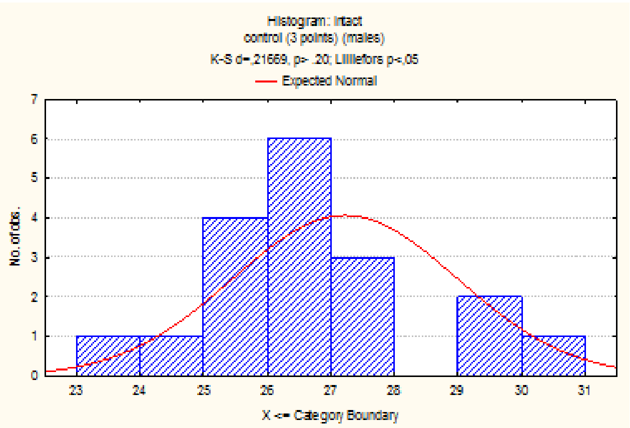

FIGURE 1. The histogram of the distribution of the mass values of male mice that were administered the double iron oxide-based health supplement.

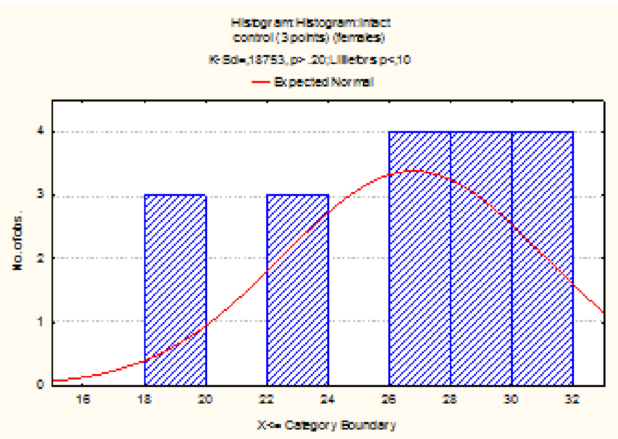

FIGURE 2. The histogram of the distribution of the mass values of female mice that were administered the double iron oxide-based health supplement.

Statistical analysis of the findings showed the absence of statistically significant differences in the mass of animals between the experimental and control groups, as evidenced by the level of significance given in Tables 1-2.

TABLE 1. The influence of the double iron oxide-based health supplement on the dynamics of the mass values after intragastric administration at a dose of 5000 mg / kg

	Animal groups					
	Males			Females		
Observation time	*ANOVA*	Intact control	Double iron oxide-based health supplement	*ANOVA*	Intact control	Double iron oxide-based health supplement
Reference mass	*p=0,4995*	25,33±1,22	26,17±0,70	*p=0,6697*	26,83±0,88	26,16±1,58
3 day	*p=0,3037*	25,33±1,23	26,67±0,80	*p=0,5993*	27,16±0,80	26,33±1,65
7 day	*p=0,3339*	25,84±1,41	27,16±0,71	*p=0,7128*	27,58±0,87	26,92±1,91
14 day	*p=0,3909*	26,67±1,39	27,83±0,71	*p=0,7510*	27,66±0,85	27,08±1,96

According to the obtained data, the mass of both male and female mice that were administered the supplement at a dose of 5000 mg / kg, varied within the values of the intact control group and did not significantly differ from the values of the control animals (Figs. 3-4) which indicates the absence of a toxic effect of the double iron oxide (code name “Magnetofood”) based health supplement on the organism of animals.

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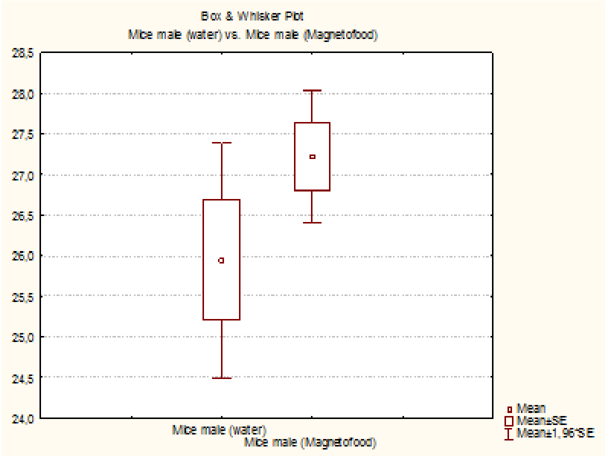

FIGURE 3. The box plot for average masses of male mice from the intact control group and mice that were administered the double iron oxide-based health supplement at a single dose of 5000 mg / kg**.**

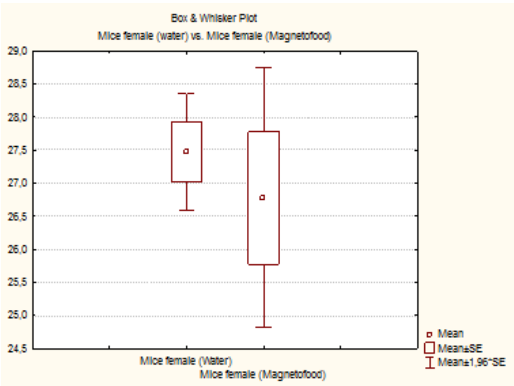

FIGURE 4. The box plot for average masses of female mice from the intact control group and mice that were administered the double iron oxide-based health supplement at a single dose of 5000 mg / kg.

The indicators of the relative mass of the internal organs of mice are given in tables 2 and 3.

TABLE 2. The effect of the double iron oxide-based health supplement on the relative mass of the internal organs of male mice (%) M (Min÷Max), n=6 (n is the number of animals in each group).

Indicators	Animal groups		*Kruskal-Wallis ANOVA by Ranks*
	Intact control	Double iron oxide-based health supplement	
Liver	4,41 (3,44÷5,73)	4,24 (4,11÷4,36)	*p=0,6547*
Kidneys	1,20 (0,90÷1,71)	1,22 (1,03÷1,53)	*p=0,4822*
Heart	0,44 (0,35÷0,62)	0,43 (0,36÷0,52)	*p=0,8480*
Lungs	0,74 (0,65÷0,84)	0,71 (0,56÷0,83)	*p=0,5653*
Spleen	1,02 (0,65÷2,30)	0,93 (0,54÷1,82)	*p=0,7484*
Thymus	0,21 (0,12÷0,41)	0,19 (0,10÷0,32)	*p=0,8480*
Testis	0,61 (0,49÷0,75)	0,69 (0,58÷0,81)	*p=0,0639*

TABLE 3. The effect of the double iron oxide-based health supplement on the relative mass of the internal organs of female mice (%) M (Min÷Max), n=6 (n is the number of animals in each group).

Indicators	Animal groups		Kruskal-Wallis ANOVA by Ranks
	Intact control	Double iron oxide-based health supplement	
Liver	4,79 (3,75÷6,75)	4,35 (2,78÷5,52)	p=0,5653
Kidneys	1,03 (0,85÷1,24)	1,01 (0,82÷1,24)	p=0,4062
Heart	0,46 (0,40÷0,56)	0,40 (0,30÷0,46)	p=0,0845
Lungs	0,81 (0,66÷0,91)	0,82 (0,57÷1,17)	p=0,8480
Spleen	0,72 (0,42÷1,24)	0,77 (0,51÷1,02)	p=0,6547
Thymus	0,17 (0,09÷0,20)	0,17 (0,12÷0,24)	p=0,5653

According to the obtained data, intragastric administration of the double iron oxide-based health supplement at a dose of 5000 mg / kg did not have a toxic effect on the internal organs of mice, as evidenced by the absence of statistically significant differences between the relative masses of organs of experimental and control animals of both sexes according to the results of the Kruskal-Wallis ANOVA by Ranks analysis.

The next stage of the research was the study of the cytotoxic properties of the double iron oxide-based health supplement. Basic cytotoxicity is the presence of pathological changes in the structure of cells and its functions following the use of the substances under study. One of the manifestations of basic cytotoxicity is the failure of the cell membrane integrity (Romanova & Dodonova, 2016).

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As shown in Fig. 5, with an increase in the concentration of the double iron oxide-based health supplement, its cytotoxic properties increased. At concentrations of 0.00002 mg / ml; 0.02 mg / ml; 2.5 mg / ml the double iron oxide-based health supplement did not cause a significant decrease in the number of viable bone marrow cells during the entire observation period, which indicates the absence of a cytotoxic effect of the researched product in these concentrations. In addition, at 90 minutes' exposure to the health supplement at a concentration of 0.00002 mg / ml, a statistically significant increase in the number of living cells was observed compared to control ($p = 0.0222$). The findings suggest the presence of a cytoprotective effect of the double iron oxide-based health supplement during long-term incubation with rat bone marrow cells at concentrations of 0.00002 mg / ml.

During the incubation of the double iron oxide-based health supplement at a concentration of 5 mg / ml, its cytotoxic effect was the lowest and did not depend on the exposure time. At concentrations of 10–20 mg / ml, a statistically significant decrease in the number of viable cells was observed. Moreover, with an increase in the contact time of the cells with the researched supplement, its cytotoxic effect increased, as evidenced by the decrease in the number of living cells ($p \leq 0.05$) in all the exposures under testing.

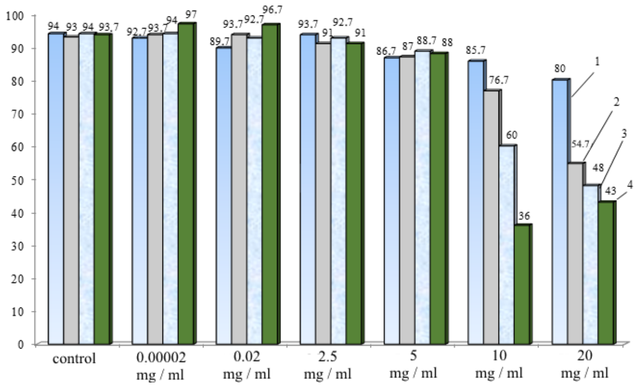

FIGURE 5. The results of the basic cytotoxic testing of the double iron oxide-based health supplement through in vitro testing on a bone marrow cell culture with the use of trypan blue. Note. The number of viable red bone marrow cells in control and after the exposure to the double iron oxide-based health supplement with different duration of the incubation period: 1 – 15 minutes; 2 – 30 minutes; 3 – 60 minutes; 4 – 90 minutes. The differences are statistically significant in relation to control (Newman-Keuls test, $p \leq 0.05$).

In view of this, the double iron oxide-based health supplement has a cytotoxic effect at rather high concentrations. With a reduction in the concentration of the researched additive, its toxic effect on bone marrow cells decreases. The toxicity of the researched product depends both on the concentration and the time of incubation. At the lowest research concentration, 0.00002 mg / ml with the highest exposure, a cytoprotective effect of the double iron oxide-based health supplement was revealed.

4. DISCUSSSION

The results of the testing in mice showed the absence of a toxic effect of the double iron oxide-based health supplement on the organism of both male and female animals after a single intragastric administration at a dose, which greatly exceeds the recommended daily human intake. With the use of the double iron oxide-based health supplement at a dose of 5000 mg / kg, no mortality of animals was found. The double iron oxide-based health supplement did not affect the body mass of males and females and the relative mass of the internal organs. Macroscopic examination revealed the absence of pathological changes in the internal organs of mice. In accordance with the generally accepted classification of substances by toxicity, the double iron oxide-based health supplement belongs to the class of low-toxic substances ($LD_{50} > 5000$ mg / kg).

The study of the cytotoxicity of the double iron oxide-based health supplement revealed that the researched product exhibits a cytotoxic effect depending on the dose and the time of contact with the culture of rat bone marrow cells. The double iron oxide-based health supplement exhibits a cytotoxic effect only at high concentrations (20 mg / ml; 10 mg / ml; 5 mg / ml). At concentrations equivalent to the daily human intake of the product (2.5 mg / ml; 0.02 mg / ml; 0.00002 mg / ml), the double iron oxide-based health supplement does not produce a toxic effect on the culture of red bone marrow cells of rats, as evidenced by the absence of a statistically significant reduction in the number of viable bone marrow cells. In addition, during long-term exposure

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to the lowest concentrations studied, the double iron oxide-based health supplement produced a cytoprotective effect, statistically significantly increasing the viability of bone marrow cells. It can be assumed that the cytoprotective effect of the double iron oxide-based health supplement is due to its antioxidant properties. This conclusion can be supported by the results of the studies of the antioxidant properties of magnetite. The ability of magnetite nanoparticles to increase the power of the antioxidant system of rat blood plasma is known (Milto, 2011) as confirmed by our testing.

The results of the testing of the acute toxicity of the double iron oxide-based health supplement are consistent with the data obtained by the other scientists who studied magnetite nanomedicines (Fe_3O_4) of identical chemical composition (Krishnan, 2010; Saiyed et al., 2003) and revealed the relative safety of their use. Scientists previously found that a single intravenous injection of an aqueous suspension of magnetite nanoparticles with a dispersion of (5–60) nm at a dose of 100 mg / kg of body mass causes reversible metabolic disorders of the liver, kidneys, and heart of rats. Repeated intravenous administration of a suspension of nanoscale magnetite particles is accompanied by activation of glycolysis in hepatocytes; in rat nephrocytes, the aerobic oxidation of substrates is activated. In both cell types, repeated intravenous administration of magnetite causes inhibition of NADPH-dependent processes of deoxidizing synthesis. The changes detected in intracellular metabolism with repeated administration of magnetite suspension persist throughout the experiment and are dose-dependent.

Studies on the effect of magnetite nanoparticles of dispersion (60–80) nm on the human body through in vivo testing are known. (Ilyukha et al., 2010). Cytomorphological studies revealed that the time of the full effect of the medication on the body is (6 ± 0.5) hours, and the time of its active action is (2.5 ± 0.5) hours. At the same time, activation of the GIT was observed; this characterizes the health nano-supplement as a rapidly digestible substance that positively affects the organs of the GIT system.

Among the factors that determine the toxicity of nanoparticles, apart from dosing and the method of their administration, their dispersion and morphology are the most significant. It is confirmed that changing the surface of nanoparticles is the main tool for toxic effect correction (Gil et al., 2010; Krishnan, 2010; Saiyed et al., 2003). Thus, at concentrations of (20–100) mg / ml for large nanoparticles (more than 40 nm), their low toxicity was revealed (LD_{50} = 10 g / kg).

Therefore, the studies conducted reveal the fact that the double iron oxide-based health supplement is sufficiently safe, and confirm the need for a more detailed study of the other toxicological parameters.

The disadvantages of the testing include a relatively small sample of laboratory animals. However, with regard to European convention for the protection of vertebrate animals used for experimental and other scientific purposes and good convergence with the results of the statistical analysis, the findings obtained are highly reliable.

The expediency and effectiveness of the use of antioxidant, bacteriostatic, emulsifying, moisture and fat retaining, stabilizing and structuring health supplements based on double iron oxide in various areas of healthy and sports nutrition require further research.

5. CONCLUSIONS

1. Comprehensive preclinical testing of the double iron oxide-based health supplement revealed the following:

• The intragastric administration of the double iron oxide-based health supplement at a dose of 5000 mg / kg did not affect the body mass dynamics of the animals and did not produce a toxic effect on the internal organs of the mice. The health supplement when intragastrically administered to both male and female mice is of low toxicity (LD_{50} > 5000 mg / kg);

• The cytotoxicity of the double iron oxide-based health supplement depends on the dose and duration of contact with the culture of red bone marrow cells. The safest concentrations (2.5 mg / ml; 0.02 mg / ml; 0.00002 mg / ml) have been revealed, at which the double iron oxide-based health supplement does not produce a cytotoxic effect. In addition, at a concentration of 0.00002 mg / ml with a long exposure to the rat bone marrow cells, the double

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iron oxide-based health supplement exhibits a cytoprotective effect due to the antioxidant properties of the nanoproduct.

2. The analytical overview of innovations in the sector of biologically active substances and health supplements revealed their use in nano-forms in healthy food production (Aigbogun et al., 2017; Aleshkov, 2016; Tsykhanovska et al., 2018;). So, for instance, baked goods with the addition of silver nanobiokomposite and minced meat products with the use of nano-activated fluids are known (Aigbogun et al., 2017; Aleshkov, 2016). However, the specific properties of nanoparticles both determine their positive effect on the functional and technological characteristics and quality indicators of food products and can cause specific risks when used in food products (Aigbogun et al., 2017; El-Hack et al., 2017). Therefore, when assessing the usefulness of including food nano-supplements into the process technology of food production, one of the key points is the testing of their safety indicators.

3. The double iron oxide-based health supplement in food systems exhibits a complex effect: antioxidant, bacteriostatic, emulsifying, water and fat retaining, stabilizing and structuring, and being an additional source of easily digestible iron produces a positive effect on the body. In addition, by acting as an energy-enhancing health supplement it improves the activeness of the stomach, duodenum and pancreas.

4. Thus, the use of the double iron oxide-based health supplement in functional food products builds a functional and technological potential for expanding the range of high-quality healthy food products.

5. Preclinical testing of the double iron oxide-based health supplement reveal sufficient safety levels of the nanoproduct, hence make it promising to use the health supplement in functional food products. However, controversial information about the safety of these nanoproducts determines the objectives of our further research, namely a more in-depth study of the toxicological parameters of the double iron oxide-based health supplement, including the study of its possible toxic properties during its long-term use.

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