Toxicity of Nanocomplexes Containing Gadolinium Orthovanadate Nanoparticles and Cholesterol

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Abstract

Previous studies have shown the ability of nanocomplexes (NCs), which consist of nanoparticles (NPs) of orthovanadates of rare earth metals ($GdYVO_4:Eu^{3+)}$ and cholesterol, to inhibit the growth of Ehrlich's ascites carcinoma (EAC). However, the biosafety of these NCs remains unclear. Our objective was to investigate acute and subchronic toxicity of NCs. NCs were administered to BALB/c mice in NPs concentration of 5.9; 29.5; 59.1 and 118.2 mg/kg. Acute toxicity was induced by single administration of NCs; subchronic – by repeated daily administration of NCs for 14 days. On day 15 and on day 31 for acute and subchronic toxicity, respectively, the percentage of animal survival, body weight, condition of visceral organs, and activities of γ-glutamyl transferase (GGT) and glucose-6-phosphate dehydrogenase (G-6-PDH) were determined. It was found that administration of NCs in concentration of 5.9 mg/kg and 29.5 mg/kg of NPs did not influence on survival of animals or have a negative impact on their performance status, morphological and quantitative characteristics of visceral organs and activities of the GGT and G-6-PDH in liver. For acute toxicity, the semi-lethal dose (LD_{50}) of nanocomplexes was determined (118.2 mg/kg of NPs). As to subchronic toxicity, it was found that repeated (for 14 days) administration of NCs containing 59.1 mg/kg of NPs decrease survival of animals to 50%. The coefficient of accumulation (C_{acum} = 7) indicates the low accumulative ability of NCs upon long-term use. Thus, from the LD_{50} and accumulation coefficient, NCs can be referred to low-toxic substances and used in conditionally therapeutic doses in oncological practice to develop nanostructured formulations of drugs.

Keywords Acute toxicity · Subchronic toxicity · Nanocomplexes · Orthovanadate

Introduction

For recent decades, rare earth metals have been of great interest to researchers in the field of biomedicine due to their unique physicochemical properties [1, 2, 3]. Nanoparticles (NPs) based on these metals are widely used as fluorescent probes for multimodal imaging, biodetecting, delivery of substances, biolabeling, etc. [1, 2].

Due to its atomic mass (Mr = 64), gadolinium (Gd) is a good electron emitter, which significantly enhances the radiation-induced destruction of cells pre-sensitized by NPs [4]. In addition, rare earth metal-based NPs have excellent photophysical properties, in particular high photostability over long periods of time, narrow radiation bands and high quasi-luminescent performance, which opens the prospects for their use as luminescent labels and probes in magnetic resonance imaging and X-ray computer tomography. In particular, nanomaterials based on gadolinium orthovanadate NPs ($GdVO_4:Eu^{3+}$) doped with lanthanide ions are very promising and have unique optical properties due to intense red emission [5]. The luminescence of $nGdYVO_4:Eu^{3+}$ nanomaterials is efficiently excited in the visible light spectrum; they have a significant Stokes shift (more than 200 nm) and no flicker effect, which eliminates the noise effect of autofluorescence of biological objects [6] and opens the prospects for their use as fluorescent labels and probes.

Vanadium is the 22nd most abundant element on earth (0.013% w/w), and it is widely distributed in all organisms.Vanadium compounds reach cellular compartmentalization after the recognition process of the specific carrier ligand by a particular cell surface receptor occurs (e.g., transferrin, albumin, IgG) and subsequent endocytosis [7]. Vanadium and its compounds in the cell can interact with different proteins and, thereby, define the biological activity as an inhibitor or activator and influence signaling pathways [8, 9]. Due to these properties the

range of potential medical applications of vanadium is growing, with the main applications driven by its antidiabetic (insulin-mimetic) properties, anti-parasitism, antiviral, antimicrobial and antitumor activities [10, 11]. The antidiabetic action of vanadium compounds is probably related directly to control of glucose and protein metabolism in this tissue [12, 13]. Moreover vanadium complexes have played a vital role in the development of modern chemotherapy because they can modify the pharmacological properties of known drugs when interacting with each other [14, 15]. For example, treatment with metformin was ineffective in reducing hyperglycemia and oxidative stress in a rat model of alloxan-induced diabetes. MetfDeca (Metformin-decavanadate) treatments ameliorate glucose and insulin levels, and reduce the levels of oxidized glutathione, reactive oxygen species, malondialdehyde, and 4-hydroxyalkenal; the superoxide and catalase activities, as well as glutathione levels were regulated [8]. The bis(ethylmaltolato)oxidovanadium(iv) (BEOV), was found to be the best compound to treat diabetes and it have demonstrated improved efficiency in comparison to a common inorganic vanadium salt such as vanadyl sulfate [11]. However, while the randomized controlled trials carried out with vanadium compounds have been promising, these studies did not utilize an optimally rigorous design satisfying FDA regulations in 2018 [16]. Among the arguments against the use of vanadium as an antidiabetic agent, there is an enhanced formation of reactive oxygen species, adverse effects on the immune system and the risk of mutagenesis [17].

Vanadium compounds are of interest to researchers as potential antitumor agents. Several animal and in vitro studies have clearly demonstrated the potential of simple and complex vanadium compounds in reducing or preventing neoplasia, and thus tumors, including cancer and its metastasis in various target issues [18, 19, 20, 21, 22, 23]. In this context, and as a first important example of this activity, Kopf-Maier P et al in 1986 reported about the effectiveness of vanadocene in the treatment of Ehrlich's ascites carcinoma (EAC) [24]. In EAC-bearing CF1 mice, which were injected with a vanadium complex [(C3 H5) VCl2] in doses of 100-600 mg/kg, the tumor size reduced due to damage to DNA and RNA of tumor cells. A recent research showed that vanadocene dichloride could selectively inhibit the proliferation of HeLa cells over the normal cells, due to the inactivation of serine-threonine protein kinase Aurora B [25]. A significant decrease in the number of tumors and their size was also observed in female Sprague-Dawley rats with 7,12-dimethylbenz(alpha)anthracene-induced breast tumors treated with ammonium methavanadate in drinking water [26]. In male and female Sprague-Dawley rats bearing hepatoma induced by diethylnitrosoamine with phenobarbital as a promoter, per os administration of sodium methavanadate led to a decrease in the number of tumor foci and inhibition of their growth at the early stages [27]. The complex bis(4,7-dimethyl-1,10-phenantroline)sulfatooxidovanadium(IV), commonly known as Metvan, impaired cell viability of human osteosarcoma (MG-63) and human colorectal adenocarcinoma (HT-29) cell lines in a low concentration range (0.25–5.0 μM) [18]. The decavanadate compound with inorganic cations, (NH4)4Li2V10O28.10H2O demonstrated dose-dependent antiproliferative activity on U87, MDA-MB-231 and IGR39 melanoma lines with IC_{50} values of 2 μg/mL, 18 μg/mL and 12 μg /mL, respectively [28]. Its effect on melanoma cells is higher than the chemotherapeutic temozolomide. Earlier studies have demonstrated that the decavanadate salt Na4{(HOCH2CH2)3NH}2[V10O28]•6H2O potently inhibited activities against human laryngeal carcinoma epithelial cell line (Hep-2), human breast cancer cell line (MDA-MB-231) and hepatocellular liver carcinoma cell line (HepG2), which was better than the positive control drug 5-fluorouracil (5-FU) [29]. Nevertheless, the inhibition of tumour proliferation by decavanadate compounds seems to impact certain enzymes such as alkaline phosphatase, ecto-nucleotidases or P-type ATPases [30]. Oxovanadium (IV) complex with the flavonoid chrysin is a promising compound, the anti-cancer activity of which was confirmed *in vitro* and *in vivo* [31]. In another investigation bis(ethylmaltolato)oxovanadium(IV), had improved efficacy compared to the vanadyl sulfate and was selected for Phase 1 and 2 clinical trials [32]. In research of Pisano M et al it was shown that that two vanadium (IV,V) species exhibit antiproliferative activity on melanoma A375 cell line by arresting cell cycle and triggering apoptosis through intracellular ROS production, ERK and Rb dephosphorylation and p21Cip1 overexpression [33]. Crans DC et al concluded in a review that Vanadate and VO(acac)2 can be regarded as a novel class of anticancer drugs acting through the activation of the MAPK/ERK pathway [34]. Thus based on extensive research, it was pointed out that vanadium had emerged as a promising element that inhibits carcinogenesis through pleiotropic modes of action. Vanadium could suppress the growth and spread of existing tumors by inhibited tumor cell proliferation, inducing apoptosis and limiting the invasion and metastatic potential of neoplastic cells [35]. From apoptotic mechanisms to miRNA, stem cell differentiation, autophagy and anoikis mechanistic processes, the nature of vanadoforms and their (sub)cellular biomolecular interactions emerges as an outstanding feature in the quest for optimized, selective and specific interactions leading to vanadium-induced death of cancer cells [36].

The antiproliferative potential of vanadium compounds can be increased by using them in nanoform. In particular, the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine synthesized nanocomplexes (NCs) consisting of gadolinium-yttrium orthovanadate ($GdYVO_4:Eu^{3+}$) and cholesterol [37]. They have been shown to be able to target tumor cells and inhibit their function [3, 38].

The uncertainty about the potential adverse effects of vanadium compounds on different cell structures upon systemic administration is a problem holding back their use in biomedical research. Moreover, taking into account the increased reactivity of vanadium NPs compared to its compounds in traditional form, due to increased number of surface atoms with decrease in sizes, the issue of the nanotoxicity to organs and tissues that are capable of accumulating vanadium remains unclear.

Assuming the issue of biological safety of nanomaterials to be contradictory and versatile, therefore it requires a comprehensive scientifically grounded approach. Thus the studies of potential risks of using

nanomaterials can be expedient when applying key characteristics of biological systems of various level organization (physiological, biochemical, hematological etc.) sensitive to toxic effect.

The NC toxicity can be studied through single or repeated administration to healthy animals. Single administration involves the use of certain harmless (including conditionally therapeutic), toxic or lethal doses to determine the morphological, biochemical, pathological and histological changes in experimental animals and the causes of their death. Repeated administration of test compounds is aimed at detecting all physiological and anatomical abnormalities and at elucidating how these anomalies are associated with dosing. In this case, animals are administered toxic, not therapeutic doses [39, 40].

Thus, our objective was to investigate the potential risks of using nanocomplexes consisting of gadolinium-yttrium orthovanadate nanoparticles and cholesterol during single and repeated administrations to experimental animals.

Materials and Methods

Experimental animals

The study was performed on 8-month-old BALB/c mice, which were kept in a standard vivarium of the Institute for Problems of Cryobiology and Cryomedicine (IPCC) of the National Academy of Sciences of Ukraine (Kharkiv). All procedures on animals have been approved by the IPCC Bioethics Committee and carried out in accordance with the General Principles of Animal Experiments approved by the First National Congress on Bioethics (Kyiv, 2004) and with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

Synthesis of nanocomplexes

Nanocomplexes (NCs) were synthesized at the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine (Kharkiv) according to the method [37]. Nanocomplexes are an aqueous dispersion of cholesterol (0.55 g/L) («Acros Organics», Belgium) using NPs of gadolinium-yttrium orthovanadate ($GdYVO_4:Eu^{3+}$) (1.3 g/L) as an stabilizer.

Aqueous solutions of NPs based on gadolinium-yttrium orthovanadates $Gd_{0.7}Y_{0.2}Eu_{0.1}$ VO_4 were obtained by colloidal synthesis [41]. The applied technique of colloidal synthesis allows for production of aggregation-stable hydrosols of NPs with controlled geometric parameters of the solid phase. Aqueous colloidal solutions of gadolinium-yttrium orthovanadate NPs were purified from impurities by dialysis using membranes "Cellu Sep H1" 3.5 KDa.

Concentrations of components included in the NCs were selected in accordance with the developer's recommendations [37]. The NPs size distribution in the NCs was determined by dynamic light scattering at a laser wavelength of 659 nm on a ZetaPALS/BI-MAS device (Brookhaven Instruments Corp., USA) at a scattering angle of 90º. Measurements were performed in special polystyrene measuring cells (BI-SCP, USA). Prior to measuring, the solutions were incubated at 25°C. Negatively charged NPs stabilize NCs through their localization on the periphery of cholesterol particles due to Van der Waals and hydrophobic interactions. The size of the synthesized NCs did not exceed 100 nm. The nanocomplex scheme is presented in Fig.1.

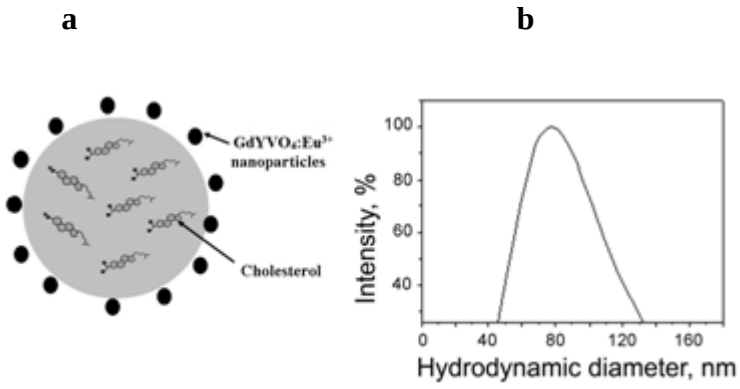

Fig. 1 Hybrid nanocomplex: (**a**) Schematic representation, (**b**) measurement of the hydrodynamic diameter

The general toxic effect of the drug was studied in acute and subchronic toxicity tests induced by single or repeated intraperitoneal administration of various concentrations of NCs to experimental animals in accordance with the international and national principles of Good Laboratory Practice (GLP). The study design is presented in Fig.2.

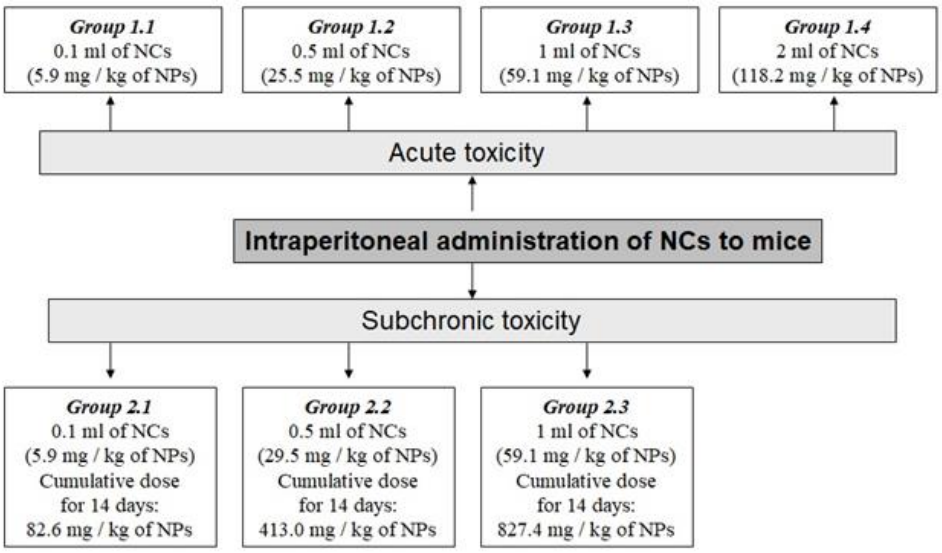

Fig. 2 Schematic design of the experiment. NCs (nanocomplexes), NPs (nanoparticles).

The choice of the NCs concentrations was justified by results of previous studies [38], which allow us to consider 0.1 mL of NCs with an NPs concentration of 5.9 mg/kg as a conditionally therapeutic dose in the treatment of experimental oncopathology – EAC. In addition, the results of a pilot series of experiments were taken into account, the purpose of which was to establish the dose-limiting toxicity of NCs (results are not presented). Basing on the data obtained and using the SPSS software package (IBM, USA), we calculated a semi-lethal dose (LD_{50}) causing the death of 50% of animals by VB Prozorovsky's method of probit analysis [42]. We found that this dose with 95% confidence interval is within the range of 100-130 mg/kg of NPs, so for further studies, the concentration of 118.2 mg/kg of NPs was selected.

Acute toxicity was induced by single intraperitoneal administration of various volumes of NCs solutions and thus the following experimental groups were formed (n = 10):

Group 1.1 - 0.1 mL of NCs per mouse with an NPs concentration of 5.9 mg/kg;

Group 1.2 - 0.5 mL of NCs per mouse with an NPs concentration of 29.5 mg/kg;

Group 1.3 - 1 mL of NCs per mouse with an NPs concentration of 59.1 mg/kg;

Group 1.4 - 2 mL of NCs per mouse with an NPs concentration of 118.2 mg/kg.

Animals of the control groups (n = 10) were once intraperitoneally injected (administrated) with 5% glucose (PAT Infuziia, Ukraine) (0.1, 0.5, 1, and 2 mL per mouse, respectively).

The experimental groups were formed by randomization using the body weight as the essential criterion (the inter- and intragroup deviations in the initial weight did not exceed ± 10%). The total duration of the study was 14 days.

On day 1 after the NCs administration, mice were constantly monitored. During the experiment, the following parameters of the general toxic effect of NCs were determined: the percentage of animal survival, performance status and behavior, excitability, food and water intake, motor activity of animals, impaired posture and coordination of movements, and body weight dynamics.

On day 15, the animals were withdrawn from the experiment by decapitation under ether anesthesia. Visceral organs were removed and grossly examined, including description of the appearance, size and consistency of the organs; relative organ weights (organ-to-body weight ratios) were calculated by dividing each animal's organ weight by their body weight; hematological and biochemical tests were performed; and pathologies of visceral organs (liver, kidneys, and spleen) were detected by histological examination.

Subchronic toxicity was induced by repeated daily intraperitoneal administration of NCs for 14 days to animals of experimental groups in doses calculated in accordance with the recommendations for potentially toxic substances [39]: conditionally therapeutic (minimum), maximum (which is at least 10-fold as high as the therapeutic dose), and intermediate (between minimum and maximum). Therefore, to study subchronic toxicity, the following experimental groups were formed (n = 10):

Group 2.1 - 0.1 mL of NCs per mouse with an NPs concentration of 5.9 mg/kg; the total cumulative dose of NPs for 14 days - 82.6 mg/kg;

Group 2.2 - 0.5 mL of NCs per mouse with an NPs concentration of 29.5 mg/kg; the total cumulative dose of NPs for 14 days - 413 mg/kg;

Group 2.3 - 1 mL of NCs per mouse with an NPs concentration of 59.1 mg/kg; the total cumulative dose of NPs for 14 days is 827.4 mg/kg.

Animals of control groups (n = 10) were also intraperitoneally administered corresponding volumes of 5% glucose (PAT Infuziia, Ukraine) for 14 days (0.1 mL, 0.5 mL and 1 mL per mouse). BALB/c mice, which were not injected, were taken as intact controls.

The total duration of the study was 30 days. During the experiment, the body weight dynamics and performance status were assessed; the percentage of animal survival was determined.

Proceeding from the results, the NCs coefficient of accumulation (C_{acum}) was determined as the ratio of the total dose of the substance that causes death of 50% of experimental animals after repeated administration to the dose that causes the same effect after single injection [39].

$C_{acum} = LD_{50}sub / LD_{50}acute$,

where C_{acum} is the coefficient of accumulation; LD_{50} sub – dose resulting in the death of 50% of animals upon subchronic toxicity; LD_{50} acute - dose resulting in the death of 50% of animals upon acute toxicity.

On day 31, the animals were withdrawn from the experiment by decapitation; visceral organs were removed and grossly examined; relative weights of organs were determined; hematological parameters were evaluated; appropriate biochemical tests were performed, and pathologies of visceral organs (liver, kidneys, and spleen) were detected.

Hematological parameters: red blood cell count, white blood cell count, hemoglobin concentration were determined by conventional methods [43].

Histological assay was performed on liver and kidneys. Tissue fragments were fixed in 10% neutral formalin solution and dehydrated with ethanol and ethanol-ether mixture (1:1); 7-9 μm thick paraffin sections were made. The sections were stained with hematoxylin-eosin. The histological specimens were examined with a Primo Star light microscope (Carl Zeiss, Germany) and photographed with an Axiocam 105 color camera (Carl Zeiss, Germany).

Biochemical parameters. To obtain a hepatocyte suspension, the organ was chopped up with scissors, mechanically homogenized in a Potter glass homogenizer in colorless Hanks solution at 0-2°C and then filtered through a 100 μm nylon filter (Falkon, USA). For biochemical tests, supernatants of the liver homogenates were obtained by centrifugation (5 min, 400 g). The total protein in the liver homogenate supernatants was determined using Total Protein Kit (Rendox, UK). Optical density was read on a CHEM 7 biochemical analyzer (ERBA Diagnostics Mannheim, Czech Republic) at 540 nm. The protein amount was calculated as per the manufacturer's instructions.

The γ-glutamyl transferase (GGT) and glucose-6-phosphate dehydrogenase (G-6-FDG) activities in the liver homogenate supernatants were spectrometrically determined using γ-GT (GGT) and G-6-PDH Kits (Rendox, UK) on the CHEM 7 biochemical analyzer (ERBA Diagnostics Mannheim, Czech Republic) at 365 nm. The enzyme activities were calculated as per the manufacturer's instructions and normalized to 1 mg of protein.

Statistical Analyses. Data were statistically processed in Microsoft Excel 2010 and Statistica 10.0 (StatSoft, Inc., US). The results are presented as the median with the lower and upper quartiles (Me [LQ; UQ]) according to the International System of Units recommended for clinical and laboratory practice. The data were analyzed by the Mann – Whitney U test, and *p* -values less than 0.05 were considered significant.

Results

Acute toxicity is adverse reactions that occur immediately after single administration of a potentially toxic substance. The main parameter characterizing the toxicity of substances is the dose that causes the death of 50% of animals (LD_{50}). This parameter allows referring the compound under investigation to a particular class of toxicity and further calculating its therapeutic range [5]. Upon induction of acute toxicity by administration of NCs to mice, no fatalities were registered in experimental groups 1.1, 1.2, and 1.3 throughout the follow-up period (Table 1). The survival rate of animals in the control groups receiving 5% glucose was 100%. Daily examination revealed no intoxication symptoms in any of the above groups throughout the follow-up period. There were no differences in the animals' performance status, behavior, food and water consumption between experimental groups 1.1, 1.2, and 1.3 and controls.

However, on follow-up day 1 onwards, animals of group 1.4 (2 mL of NCs [118.2 mg / kg of NPs]) developed intoxication signs, which increased during follow-up day 1 and reached their maximum manifestation on day 3. Ataxia, adynamia, tremor of the whole body, tousled hair; decreased eating and drinking behavior were observed. On day 3 after administration of this dose of NCs, the survival of the animals was 50%. No more than 20% of animals survived 14 days after administration (Table 1).

Thus, taking into account the ratio of the number of dead animals to the total number of animals in the group, we determined that the potential semi-lethal dose of NCs (LD_{50}), which causes death of 50% of experimental animals after intraperitoneal administration, is 118.2 mg/kg of NPs. This allows us to conclude that the NCs belong to danger class IV (low-toxic substances) according to the Good Laboratory Practice guidelines.

Table 1 Animal survival upon acute toxicity induced by administration of NCs with various concentrations of NPs

Group	Test substance	Dose	Survival, %				
			Day				
			0	3	7	10	14
Group 1.1	NCs	0.1 mL (5.9 mg / kg of NPs)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
	Glucose (control)	0.1 mL	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
Group 1.2	NCs	0.5 mL (29.5 mg / kg of NPs)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
	Glucose (control)	0.5 mL	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
Group 1.3	NCs	1 mL (59.1 mg / kg of NPs)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
	Glucose (control)	1 mL	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
Group 1.4	NCs	2 mL (118.2 mg / kg of NPs)	100 (10/10)	50 (5/10)	30 (3/10)	20 (2/10)	20 (2/10)
	Glucose (control)	2 mL	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)
Intact control	-	-	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)

Changes in body weight of laboratory animals in toxicity studies of investigational compounds are an important indicator that reflects their general condition. Weight loss during an experiment may indicate a systemic toxic effect of an investigational drug on the body. Table 2 shows the body weight of mice over time after single intraperitoneal administration of the test compound. It was found that on day 14 after 5% glucose injection, there were no statistically significant differences in the weights of animals compared to intact animals. Similar data were obtained after NCs administration in groups 1.1 and 1.2 (Table 2), where the body weight gain was 1.7 and 1.4 g, respectively, while in intact animals it was 2.0 g.

Group 1.3 and 1.4 became the exception: the body weight of mice statistically significantly decreased compared both with intact animals and with animals of the corresponding control group (Table 2).

Table 2 Changes in the body weight of mice upon acute toxicity induced by administration of NCs with various concentrations of NPs

Group	Test substance	Dose	Body weight		Gain
			Day		
			0	14	
Group 1.1	NCs	0.1 mL (5.9 mg / kg of NPs)	21.4 [20.4; 21.5]	23.1 [22.3; 23.6]	1.7
	Glucose (control)	0.1 mL	20.2 [18.5; 21.3]	22.4 [21.5; 23.1]	2.2
Group 1.2	NCs	0.5 mL (29.5 mg / kg of NPs)	21.4 [20.4; 22.7]	22.8 [21.9; 23.7]	1.4
	Glucose (control)	0.5 mL	18.9 [18.3; 20.5]	20.5 [20.4; 22.5]	1.6
Group 1.3	NCs	1 mL (59.1 mg / kg of NPs)	19.6 [19.2; 20.4]	19.7*# [19.3; 20.5]	0.1*#
	Glucose (control)	1 mL	20.06 [19.7; 21.3]	21.96 [22.8; 23.4]	1.9
Group 1.4	NCs	2 mL (118.2 mg / kg of NPs)	20.7 [20.3; 21.2]	18.5# [17.6; 19.6]	-2.2*#
	Glucose (control)	2 mL	20.1 [19.1; 20.6]	21.9 [19.5; 22.4]	1.8
Intact control	-	-	18,8 [18.2; 20.5]	20,8 [20.7; 22.5]	2

Notes: * - difference is significant compared to the intact control, ($p < 0.05$); # - difference is significant compared to the corresponding control (glucose), ($p < 0.05$).

The visceral organ-to-body weight ratios generally reflect the functional state of organs. There were no statistically significant differences in the kidney- and liver-to-body weight ratios between animals of all experimental groups (1.1, 1.2, 1.3, and 1.4), intact animals and animals receiving corresponding doses of 5% glucose (Table 3). As to the spleen-to-body weight ratio, it did not statistically significantly differ between groups 1.1 and 1.2 only and the corresponding control groups. In animals of groups 1.3 and 1.4, the spleen-to-body weight ratio rose in comparison accordance both with the intact control and with groups receiving 5% glucose.

Table 3 Relative weights of the visceral organs of mice upon acute toxicity induced by administration of NCs with various concentrations of NPs

Organ-to-body weight ratios	**Intact control**	**Test substance**	**Group**			
			1.1.	**1.2.**	**1.3.**	**1.4.**
Spleen-to-body weight ratio, %	6.87 [6.3; 8.0]	NCs	7.6 [6.8; 8.2]	8.5 [7.7; 9.0]	19.0*# [17.7; 20.0]	20.9*# [19.8; 22.2]
		Glucose (control)	8.4 [7.8; 9.4]	8.9 [7.5;10.3]	11.4* [10.7; 11.8]	10.5* [9.8; 11.2]
Kidney-to-body weight ratio, %	6.16 [5.8; 6.3]	NCs	6.5 [6.3; 6.9]	6.3 [6.3; 6.4]	6.3 [6.2; 6.5]	6.8 [6.3; 7.1]
		Glucose (control)	6.1 [5.7; 6.2]	6.5 [6.2; 6.6]	6.3 [6.15; 6.8]	5.4 [5.1; 6.3]
Liver-to-body weight ratio, %	67.0 [60.9; 70.1]	NCs	59.3 [57.2; 67.9]	64.6 [58.9; 67.5]	60.1 [57.4; 64.3]	68.7 [61.0; 70.1]
		Glucose (control)	56.3 [51.9; 61.0]	65.0 [61.6; 66.7]	63.7 [54.9; 65.3]	59.0 [56.6; 62.6]

Notes: * - difference is significant compared to the intact control, ($p < 0.05$); # - difference is significant compared to the corresponding control (glucose), ($p < 0.05$).

Hematological analysis of peripheral blood of animals is one of the most common and important laboratory tests, which reflects processes occurring in the body in response to different factors, including nanomaterial administration. When determining the red blood cell count and hemoglobin in the peripheral blood of animals of all experimental groups (1.1, 1.2, 1.3, and 1.4) on day 14 day of the study, we revealed no statistically significant differences between the experimental groups compared with the corresponding controls and intact animals (Table 4). As to the white blood cell count in the peripheral blood, there were no statistically significant changes in experimental animals (groups 1.1, 1.2. and 1.3), either, except for group 1.4, where a statistically significant increase in this indicator was noted in comparison both with the corresponding control and with intact animals. This may indicate the manifestation of protective capacities of the body in response to administration of a potentially toxic substance in the highest selected concentration.

Table 4 Hematological parameters: red blood cell count, hemoglobin and white blood cell count of mice upon acute toxicity induced by administration of NCs with various concentrations of NPs

Hematological parameters	**Intact control**	**Test substance**	**Group**			
			2.1.	**2.2.**	**2.3.**	**2.4.**
Red blood cell count x 10^9 cells / mL	7.6 [6.5; 8.3]	NCs	7.1 [6.5; 7.7]	6.9 [5.9; 7.7]	7.0 [6.0; 7.8]	6.1 [5.7; 7.1]
		Glucose (control)	8.0 [7.3; 8.8]	7.0 [6.2; 8.2]	7.4 [6.9; 8.7]	6.9 [5.9; 7.5]
Hemoglobin, g / L	142.5 [134.3; 151.8]	NCs	144.5 [135.0; 148.5]	138.5 [126.0; 146.8]	140.5 [134.0; 144.8]	135.5 [125.8; 146.0]
		Glucose (control)	143.0 [137.3; 156.3]	141.5 [135.5; 148.0]	147.0 [138.3; 152.3]	141.0 [134.0; 146.8]
White blood cell count x 10^6 cells / mL	3.6 [3.5; 3.7]	NCs	3.3 [3.1; 3.5]	3.3 [3.1; 3.5]	3.4 [3.0; 3.5]	4.1* [3.8; 4.6]
		Glucose (control)	3.2 [2.9; 3.5]	3.1 [2.8; 3.5]	3.4 [3.1; 3.5]	3.4 [3.0; 3.4]

Note. * - difference is statistically significant compared to the intact control ($p < 0.05$).

At the end of the experiment (day 15), gross examination of the visceral organs of animals of experimental groups 1.1, 1.2, and 1.3 revealed no changes in their location in the abdominal cavity. Moderate fat deposition was noted in the subcutaneous tissue. The peritoneum was transparent, smooth, without hemorrhage. No foreign contents

were found in the abdominal cavity. All liver lobes were well distinguished; the liver capsule was not tense; the organ surface was smooth, without nodules; the parenchyma section was uniformly red-brown. The kidneys had an easily removed capsule; their section was dark-red and dense, with preserved pattern of layers. The spleen was elastic, reddish-brown; the section showed fine-grained tissue. However, in one of the survivors of group 1.4 (20%), a significant solid neoplasm was found in the spleen (Fig. 3a). Microscopic examination of the organ revealed several alterations in its architecture: aplasia of lymphoid follicles, increase in the relative area of red pulp compared to white pulp, infiltration by lymphoblasts and lymphocytes (small, medium and large). There were foci of plasma cell genesis between the pulp cord sinuses (Fig. 3b). Decreased proliferation and differentiation of lymphoid cells associated with atrophy of lymphoid follicles were observed. There were no such changes in any of the control or experimental groups (except for group 1.4), where the spleen structure was similar to that in the intact control (Fig. 3c). Microscopy of the spleen of the control in group 1.4, which received 5% glucose, showed that its structure did not differ from that in the intact control. The gross appearance of the spleen was normal. Nodes were well differentiated; the ratio of stroma to parenchyma was normal. The capsule was not thickened. Follicles were of medium or small size, with light reactive centers.

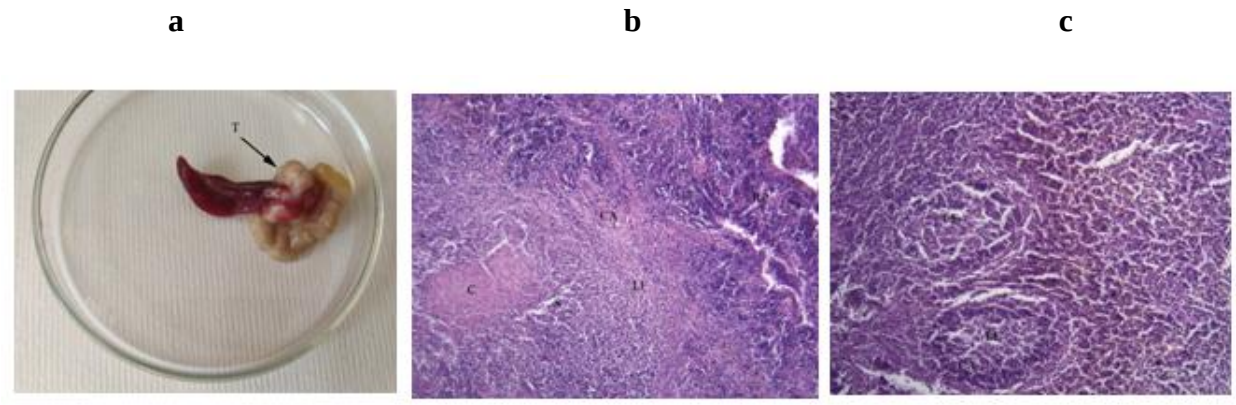

Fig. 3 Microphotographs of the spleen and its histological sections from mice of group 1.4 with a solid neoplasm on day 15 upon acute toxicity: **a** - gross appearance of the tumor-bearing spleen (group 1.4), **b** - microphotograph of a histological section of the spleen (group 1.4), **c** – microphotograph of a histological section of the spleen of the intact control, day 15 of the study (hematoxylin and eosin staining, 100×).
Notes: RP - red pulp; CA - central artery; LF - lymphoid follicle; C - cyst; S-sinus.

Microscopy of the kidneys of animals upon acute toxicity showed that the use of NCs at a concentration of 59.1 mg / kg of NPs resulted in development of focal edema in the interstitium and expansion of the lumen of both distal and of proximal tubules in the cortex and medulla in animals of group 1.3 (Fig. 4a). Partial dystrophy of renal tubuli was detected, with focal hyaline-drop dystrophy in single epitheliocytes. The glomeruli preserved their morphology. Administration of NCs with the highest concentration of NPs (118.2 mg / kg) to animals of group 1.4 led to the development of profound dystrophy and necrosis of tubuli (Fig. 4b). Diffuse infiltration of the interstitial tissue of the kidneys by neutrophilic leukocytes and hyalinosis of glomeruli were detected. Changes of proximal tubules were heterogeneous; the lumen was visualized in some of them and was expanded in others. Karyolysis was observed in epithelial cells of tubuli.

In animals of all control groups, which received 5% glucose, the renal cortex and medulla structures were well preserved, not differing from the intact control (Fig. 4c). Numerous glomeruli were evenly spaced and of normal size. The lumens of spiral and straight tubuli were bright. There was no tubulopathy. Similar histological structure of the kidneys was intrinsic to animals of experimental groups 1.1 and 1.2 (data are not presented). The cortical glomeruli were preserved, of normal size. The epithelium of spiral tubuli was cylindrical, with distinct boundaries. There was no interstitial inflammation.

a **b** **c**

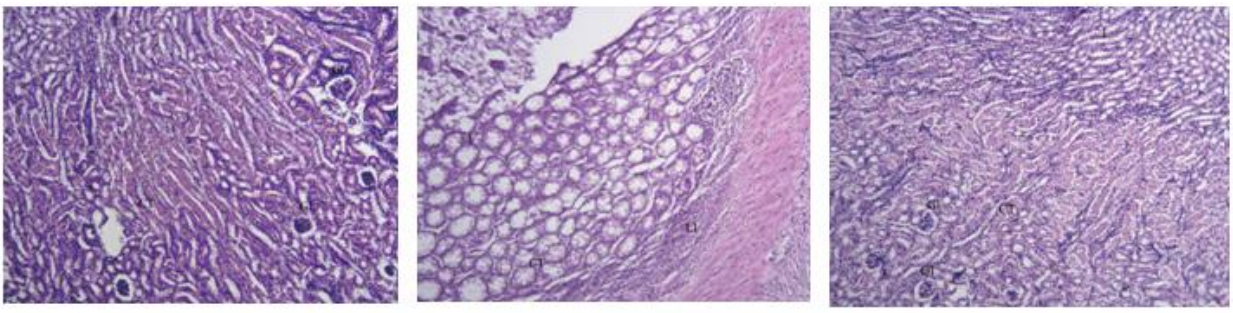

Fig.4 Microphotographs of histological sections of the kidneys from mice upon acute toxicity: **a** - group 1.3 administrated with NCs (concentration of NPs was 59.1 mg / kg); **b** - group 1.4 administrated with NCs (concentration of NPs was 118.2 mg / kg); **c** - intact control, day 15 of the study (hematoxylin and eosin staining, 100×). Notes: CT - collecting tubuli; Gl - glomerulus; I - interstitium; LI - leukocyte infiltration.

A similar dependence of the severity of pathomorphological findings on the NPs concentration in the NCs solution was noted for the liver. Thus, in animals of group 1.3, we observed dilation of the portal vein, accumulation of lymphoid cells around it, fatty degeneration of individual cells (Fig. 5a). There were lymphoid nodules; each of them consisted of several types of lymphoid tissue cells: lymphocytes, lymphoblasts, macrophages and plasma cells. This is consistent with other researchers' data on the hepatotoxic effects of vanadium compounds [44, 45]. In particular, it was reported [46] that intraperitoneal administration of vanadyl sulfate at a dose of 5 or 10 mg / kg body weight increased most hepatotoxic markers, decreased the activities of antioxidant enzymes and caused severe damage to the liver tissue.

Administration of the maximum concentration of NPs (118.2 mg / kg) in the NCs solution led to the greatest changes in the liver structure of animals of group 1.4. There were foci with signs of necrosis and large areas with granular, small-drop and small-vacuolar hepatocyte dystrophy. Hepatic ducts were dilated; leukocyte infiltration within the lobules among hepatocytes was noted (Fig. 5b). The numbers of hepatocytes with multiple nucleoli, polyploid and hyperchromic nuclei increased. These events were associated with regeneration of the plate structure of the central lobules of the liver and residual dystrophic phenomena with characteristic changes in hepatocytes. Thus, in animals of group 1.4, the maximum concentration of NPs (118.2 mg / kg) in the solution locally enhanced necrotic/dystrophic processes in the liver on the one hand and led to an increase in the area of sites of active hepatocyte proliferation with large, often polyploid nuclei, indicating tissue regeneration, on the other.

It should be noted that administration of NCs at a dose of 5.9 and 29.5 mg / kg (groups 1.1 and 1.2) and glucose (control groups) caused no pathological changes in the liver structure, which was generally similar to that in intact animals (Fig. 5c). The liver had a typical structure; polygonal lobules were clearly visible; liver plates were not damaged. Hepatocytes were polygonal shape, with basophilic cytoplasm and nuclei moderately filled with chromatin. Triads formed by the interlobar artery, vein, and bile duct were well visible in the interlobular connective tissue.

A comprehensive approach to analysis of the action of NCs as a potentially toxic substance requires determination of biochemical parameters. The activity of gamma-glutamyltransferase (GGT) is of great clinical importance, because its increased level indicates either destruction of liver cell membranes or induction of microsomal oxidation under the influence of toxic agents. Assay of the GGT activity after induction of acute toxicity demonstrated a statistically significant (almost 2-fold) increase in the activity after administration of NCs in concentrations of 59.1 mg / kg and 118.2 mg / kg compared with the corresponding controls, which were administration with glucose (Fig. 5d), indicating activation of metabolic processes at the cellular level, in particular development of oxidative stress.

On day 15 after NCs administration was evaluated by glucose-6- phosphate dehydrogenase (G-6-PDH) activity of the murine liver. It was found that, in all control groups of mice administrated with corresponding concentrations of glucose, the G-6-PDH activity did not differ from that in intact animals. Administration of low doses of NCs (5.9 and 29.5 mg / kg of NPs) did not lead to statistically significant changes in the G-6-PDH activity, either, compared with the corresponding controls (Fig. 5e). Increasing the dose of NPs to 59.1 mg / kg in the NCs solution (group 1.3) led to the maximum increase in the enzyme activity on day 15 after induction of acute toxicity, indicating activation of the energy system to prevent toxic effects of NCs. At the same time, in group 1.4 (118.2 mg / kg of NPs), there was a statistically significant decrease in the activity of enzymatic constituent, judging from the G-6-PDH level. The decrease in the activity of this enzyme associated with the peak values of the GGT activity in this group confirms dysregulation of the enzymatic constituent energetic systems of liver after administration of the maximum concentration of NCs. G-6-PDH is known to be the first pentose phosphate glycolysis enzyme to provide

energy to cells. Its main function is to reduce NADP to NADPH, necessary to reduce of oxidized glutathione form. Reduced glutathione is required for the ROS-binding.

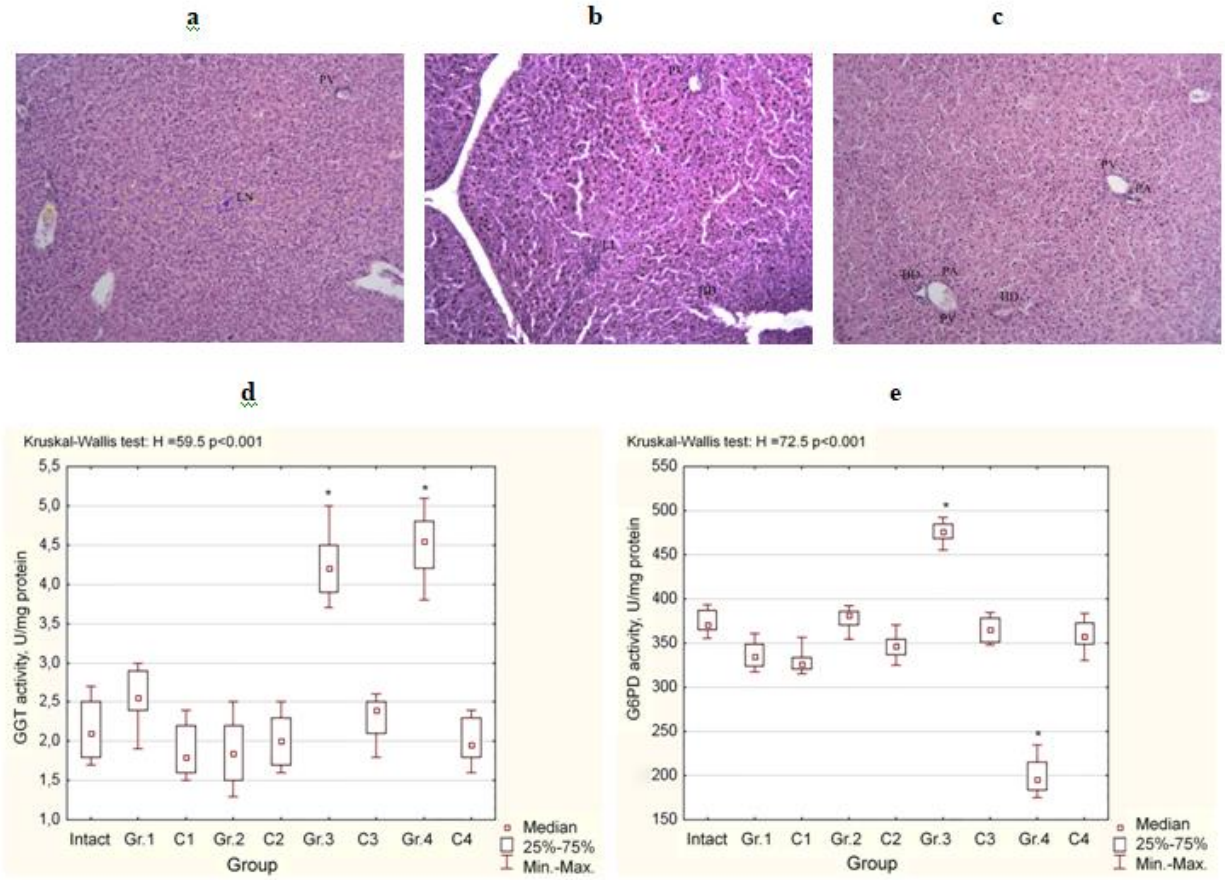

Fig.5 Liver parameters of animals upon acute toxicity: microphotographs of histological sections of the livers tissue from mice: **a** - group 1.3 (concentration of NPs was 59.1 mg / kg), **b** - group 1.4 (concentration of NPs was 118.2 mg / kg), **c** - intact control ((hematoxylin and eosin staining, 100×); biochemical parameters: **d** - activity of gamma-glutamyltransferase; **e** - activity of glucose-6-phosphate dehydrogenase.
Notes: LN - lymphoid nodules; PV - portal vein; LI - leukocyte infiltration; HD - hepatic duct; HA - hepatic artery; BD - bile duct.

GGT is a microsomal enzyme that can be isolated from hepatocytes and gall bladder epithelium, as well as increases in GGT in liver, gall bladder and pancreatic diseases. The extracellular metabolism of glutathione, which is the main antioxidant molecule in cells, is GGT-controlled. The concentration of glutathione in cell also depends on the activity of the membrane enzyme GGT, which catalyzes the breakdown of glutathione in the γ-glutamyl cycle. The detachment of GGT from the membrane and an increase in its activity in cytoplasmic fraction of the liver tissue is a direct consequence of oxidative stress [47]

Thus, having modeled acute toxicity, we revealed that administration of NCs containing NPs in doses of 5.9 mg and 29.5 mg / kg of body weight did not cause mortality of animals, did not have a negative impact on their performance status, morphological and quantitative characteristics of visceral organs or biochemical parameters.

Studies of subchronic toxicity are aimed at assessments of the effects of long-term exposure to a potential drug on the body and conducted for several doses: the maximum dose is supposed to cause intoxication or death of some animals, and the minimum dose – to have a therapeutic effect. Upon subchronic toxicity induced by repeated administration, the performance status of mice was satisfactory and no death was observed during the 31-day follow-up period in experimental groups 2.1 and 2.2. All experimental animals of these groups were almost identical in terms of hair condition, food and water consumption, and behavior. At the same time, on day 5 after the experiment onset in group 2.3, 10% of mice died and, as early as on day 20, 50% of mice were dead, without further increase in the mortality until the end of the follow-up period (Table 5).

Table 5 Indices of mice upon subchronic toxicity induced by administration of NCs with various concentrations of NPs

Indices	Intact control	Test substance	Group		
			Group 2.1.	Group 2.2.	Group 2.3.
Survival, %	100	NCs	100 (10/10)	100 (10/10)	50 (5/10)
		Glucose (control)	100 (10/10)	100 (10/10)	100 (10/10)
Body weight gain, g	3.8	NCs	1.17*#	1.0*#	0.9*#
		Glucose (control)	2.63	3.03	3.07
Spleen-to-body weight ratio, %	7.9 [7.4; 8.7]	NCs	7.8 [7.5; 8.6]	9.34 [8.7; 10.2]	8.1 [7.7; 8.3]
		Glucose (control)	7.6 [7.3; 8.1]	7.4 [6.4; 8.3]	8.0 [6.9; 8.8]
Kidney-to-body weight ratio, %	5.5 [5.1; 5.6]	NCs	5.8 [5.6; 5.8]	5.8 [5.3; 6.4]	3.9*# [3.8; 4.0]
		Glucose (control)	5.6 [5.2; 6.2]	5.8 [5.6; 6.1]	5.19 [4.9; 6.0]
Liver-to-body weight ratio, %	51.7 [50.6; 53.3]	NCs	54.3 [49.2; 55.8]	54.2 [52.9; 56.4]	57.6*# [56.5; 60.1]
		Glucose (control)	52.97 [50.9; 56.6]	49.9 [46.8; 52.9]	48.5 [47.4; 51.3]

Notes: * - difference is significant compared to the intact control, (p <0.05); # - difference is significant compared to the corresponding control (glucose), (p <0.05).

Upon subchronic toxicity, in all experimental groups after 30 days, the body weight gain was positive, but almost 3-fold as little as that of intact animals and control animal, which received 5% glucose (Table 5). It should be noted that administration of the highest dose of NCs led to the greatest slowing of the weight gain in animals of group 2.3.

The relative weights of visceral organs generally reflect their functional state. Analysis of the data showed that in all experimental groups (2.1, 2.2, and 2.3) the spleen-to-body weight ratio remained within the normal limits and did not show statistically significant differences from the control value after administration of corresponding glucose concentrations (Table 5). However, in group 2.3, the kidney-to-body weight ratio statistically significantly decreased (by 30%) and there was an upward trend in the liver-to-body weight ratio (by 16%) in experimental animals compared with the corresponding control values.

Table 6 Hematological parameters: red blood cell count, hemoglobin and white blood cell count of mice upon subchronic toxicity induced by administration of NCs with various concentrations of NPs

Hematological parameter	Intact control	Test substance	Group		
			2.1.	2.2.	2.3.
Red blood cell count x 10^9 cells/mL	7.5 [6.9; 7.8]	NCs	8.1 [7.8; 8.5]	6.2 [5.8; 7.0]	4.5*# [4.1; 4.9]
		Glucose (control)	8.5 [7.8; 8 .7]	7.9 [7.5; 8.3]	7,3 [6.8; 7.5]
Haemoglobin, g/L	140.5 [135.5; 148.3]	NCs	148.5 [144.5; 153.3]	132.5 [129.0; 137.0]	112.0*# [108.8; 115.3]
		Glucose (control)	155.0 [141.0; 157.8]	144 [137.5; 150.5]	145.0 [140.8; 153.8]
White blood cell count x 10^6 cells/mL	3.6 [3.5; 3.7]	NCs	3.2 [2.8; 3.5]	3.5 [2.8; 3.7]	4.7*# [4.1; 5.0]
		Glucose (control)	3.6 [3.1; 3.7]	2.7 [2.4; 3.5]	3.4 [3.1; 3.7]

Notes: * - difference is significant compared to the intact control, (p <0.05); # - difference is significant compared to the corresponding control (glucose), (p <0.05).

As to the hematological parameters, on day 31 of modeling subchronic toxicity after administration of NCs in groups 2.1 and 2.2, we observed no significant deviations both from the intact control values and from the values in the corresponding 5% glucose groups (Table 6). In group 2.3, there was a 1.7-fold decrease in the red blood count and 1.3-fold decrease in the hemoglobin level compared with the intact control, indicating the development of anemia in animals of this group. Mice of group 2.3 had 1.3-fold higher the white blood count compared to group of intact animals. The red blood count and the hemoglobin level of group 2.3 were reduced to 1.3 and 1.7 fold respectively, compared with the intact control, indicating the development of anemia in animals of this group.

Gross examination at the end of the experiment (31 days) revealed no changes in the location of the organs in the abdominal cavity in mice of all experimental groups. The livers of all animals, except for group 2.3, were uniformly red-brown; their capsules were not tense, the organ surface was smooth, without nodules. In animals of group 2.3, the liver size increased; the blood supply of the liver was reduced; the color changed to pale-pink; and the increased granulosity was detected. The kidneys of animals of all experimental groups (2.1, 2.2, and 2.3) were bean-shaped, full-blooded, and dark-red, with the preserved structure in the section; the boundary between the cortex and medulla was distinct.

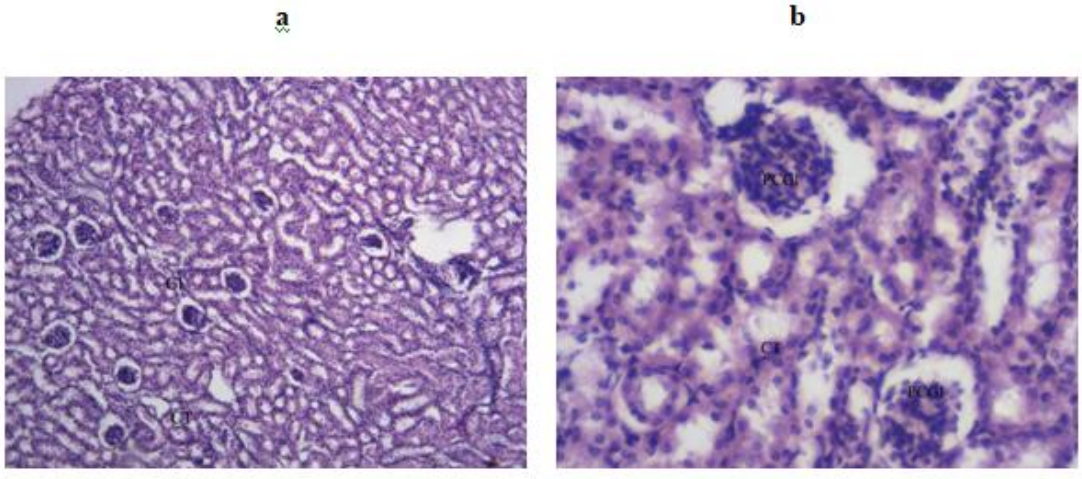

Fig. 6 Microphotographs of histological sections of the kidneys from mice upon subchronic toxicity group 2.3 administrated with NCs (cumulative dose was 827.4 mg/kg of NPs): **a** - the overall structure of the organ, (hematoxylin and eosin staining, 100×); **b** - epithelium of spiral tubuli, (hematoxylin and eosin staining, 400×). Notes: CT - collecting tubuli; Gl - glomerulus; I - interstitium; LI - leukocyte infiltration.

However, histological assay revealed the dependence of structural changes in the kidneys on the NCs dose. The most conspicuous alterations in the organ histology upon subchronic toxicity were recorded in animals of group 2.3, which received a cumulative dose of 827.4 mg / kg of NPs. In this group, dystrophy of spiral tubuli, profound hyperemia and damaged glomeruli (Fig. 6a) were noted. In the cortex and medulla of the kidney, focal edema in the interstitium and dilation of the lumens both of distal and of proximal tubuli were observed. There was a partial dystrophy of the renal tubuli, with hyaline-drop dystrophy of individual epitheliocytes; apoptotic cells were found (Fig. 6b).

Histological analysis of the liver microstructure confirmed the dependence of morphological pathologies of the organ on the NPs dose. Thus, no deviations from the norm were seen in the livers of animal groups 2.1 and 2.2. However, in the livers of animal group 2.3, which received NPs at a dose of 59.1 mg/kg for 14 days (cumulative dose = 827.4 mg/kg), hypertrophic findings caused by compensatory-adaptive processes prevailed in the organ structure: increase in the number of binuclear hepatocytes, expansion of vessel lumens of the portal tracts (Fig. 7a). There were increases in dystrophic and inflammatory events: an increase in the number of cells of the lymphohistiocytic lineage, hydropic dystrophy of hepatocytes in subcapsular and periportal areas, and an increase in sites with blood stasis and hemorrhage signs. There was a macrophage reaction, hyaline-drop dystrophy of individual hepatocytes; apoptotic cells were found (Fig. 7b).

Parameters that characterize the functional state of liver enzymes are the main markers of toxicity of compounds. Upon subchronic toxicity, daily administration of NCs caused a dose-dependent increase in the GGT activity (Fig. 7c). There was a 2- and more than 10-fold increase in the GGT activity in groups 2.2 and 2.3, respectively. It should be noted that the bloodstream does not contain GGT; its activity is inherent mainly to cells of the liver and kidneys, which are destructed because of toxic effects, releasing their contents into blood.

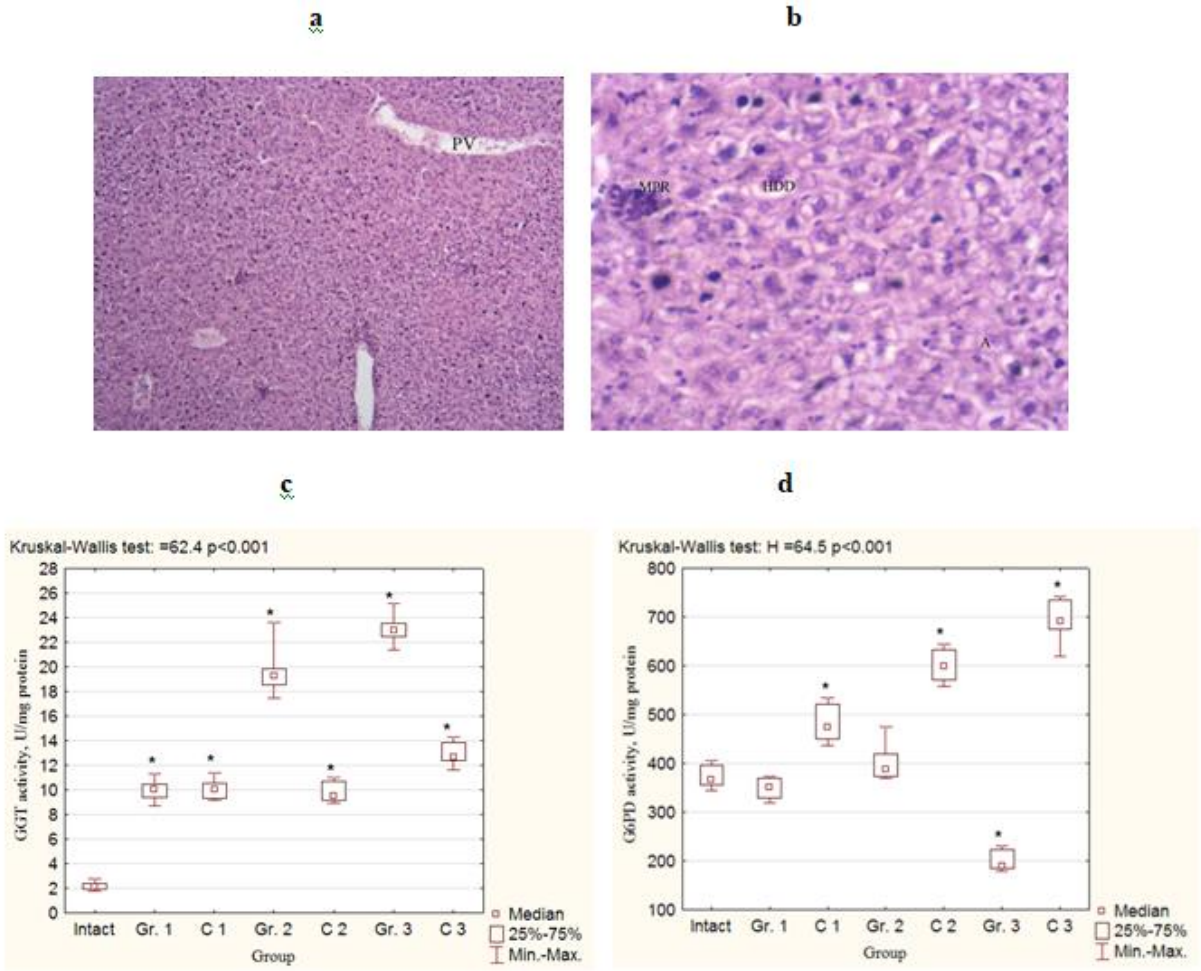

Fig. 7 Liver parameters of animals upon subchronic toxicity: microphotographs of histological sections of the livers tissue from mice group 2.3 (cumulative dose was 827.4 mg/kg of NPs): **a** - the overall structure of the organ (hematoxylin and eosin staining, 100×); **b** - dystrophic changes of hepatocytes (hematoxylin and eosin staining, 400×); hematoxylin-eosin staining; biochemical parameters: **c** - activity of gamma-glutamyltransferase; **d** - activity of glucose-6-phosphate dehydrogenase.
Notes: PV - portal vein; HDD - hyaline-drop dystrophy; MPR - macrophage reaction; A - apoptosis

Repeated administration of NCs for 14 days did not lead to significant changes in the G-6-PDH activity in animals of groups 2.1 and 2.2, but in animals of group 2.3 it resulted in a 2-fold decrease in this indicator (Fig. 7d). Thus, like upon single administration of the maximum dose of NCs (Fig. 5d,e), repeated administration of NCs in the highest doses dysregulated of the activity enzymes of pentose phosphate glycolysis in the liver (Fig. 7c,d). This is evidenced by insufficient activity of G-6-PDH in animals of group 2.3 in combination with the maximum values of the GGT activity in the liver homogenate supernatants.

The ability of potentially toxic compounds to get accumulated in the body upon repeated administration over a long period of time is an important indicator of subchronic toxicity. This process can be characterized by C_{acum} – a quantitative toxicometric indicator, which is defined as the ratio of the total dose of the substance that causes the death of 50% of experimental animals after repeated administration to the dose that causes the same effect after single injection. The value of the coefficient of accumulation is more than 5 indicates a relatively weak accumulation of the substance, of 3 to 5 – a moderate accumulation, of 1 to 3 – a strong accumulation, and of less than 1 – superaccumulation [39]. Over 14 days, the total cumulative dose was 827.4 mg/kg of NPs and it decrease survival of animals to 50%. Thus, C_{acum} is equal to 827.4 (mg/kg)/118.2 (mg/kg) = 7. According to the above classification of chemicals by accumulation degree, NCs can be referred to drugs with a weak ability to accumulation when being used for a long period.

Similar data were obtained by Belkina IO et al in a study of toxicity, accumulation dynamics and excretion of rare earth orthovanadate-based NPs orally administered to rats [46]. The researchers showed that oral administration of $GdVO_4$ NPs to sexually mature male rats for 30 days did not lead to death of animals or to changes in the relative weights of their visceral organs, except for an increase in the relative weights of the spleen and anterior tibialis. Even longer (70-day) oral administration of $GdVO_4$ NPs did not affect the relative weights of most organs, except for an increase in the relative weight of the ventral prostate. This was associated with a decrease in ALT activity in the testes. No other negative effects on the reproductive system were detected [46].

This allowed the researchers to classify the orally administered rare earth orthovanadate NPs as virtually non-toxic compounds (toxicity class V) [48]. Moreover, it was found that certain doses of $GdVO_4$ NPs could positively affect the reproductive function of rats with age-related and neonatally-determined pathology of sexual function [48, 49]. The positive effect of gadolinium orthovanadate NPs on the reproductive function of male rats with a modeled reproductive pathology was manifested as normalization of spermatogenesis, serum testosterone, triglyceride, total cholesterol, and arginine levels, alanine aminotransferase activity in the liver, fertility and fecundity and as reduction of embryonic mortality, contributing to normalization of the estimated reproductive potential [50].

Thus, it can be affirmed that orthovanadate gadolinium yttrium NPs and NCs based on such NPs in certain doses and conditions of administration are promising as innovative drugs for the treatment of pathologies of various etiologies.

Conclusions Our results indicate that, under conditions of acute and subchronic toxicity, NCs administered at the effective conditionally therapeutic dose for the treatment of oncopathologies (5.9 mg/kg) and at the 5-fold dose compared to the conditionally therapeutic one (29.5 mg/kg) had no adverse effects on the performance status, morphological or quantitative characteristics of the visceral organs, or on biochemical parameters of healthy mice. The 20-fold dose (118.2 mg/kg) compared to the conditionally therapeutic one (5.9 mg/kg) in the acute toxicity experiment decrease survival of animals to 50% and significantly changed the histology of the visceral organs in survivors, which was associated with dysregulation of the enzymatic constituent energetic systems of liver. In the subchronic toxicity experiment, 50% death and significant changes in the microstructure of the liver and kidneys of experimental animals were observed when NPs were administered at a dose of 59.1 mg/kg for 14 days (the total cumulative dose amounted to 827.4 mg/kg). This indicates that long-term use of such doses of NCs exerts toxic effects on the body. Nevertheless, the results of this study suggest that the conditionally therapeutic dose of NCs had no negative impact on the vast majority of the indicators under investigation, justifying the prospects for the development of nanostructured forms of drugs.

Author Contributions: AG: conceptualization, experimental design and edited the manuscript. NB: experimental data acquisition, drafted the manuscript. YuG: carried out the experiments and writing – original draft preparation. MB: did statistical processing, recorded and/or analyzed the data. TD: reviewed literature, interpreted the results. LO: histological examinations. NV: carried out the experiments, review and editing. VK synthesized NPs and NCs. All the authors have read and agreed to the final version of the manuscript.

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Data Availability

Raw data were generated at Department of Cryopathophysiology and Immunology, Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine. Derived data supporting the fndings of this study are available from the corresponding author: Mykola Bondarovych on request.

Declarations

Ethics Approval The study was approved by the Ethics Committee of Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine (minutes #2 dated March 11, 2020).

Consent for Publication All authors have approved the submission.

Conflict of Interest: Anatoliy Goltsev, Natalia Babenko, Yuliia Gaevska, Mykola Bondarovych, Tetyana Dubrava, Lyudmila Ostankova, Nataliia Volkova, and Vladimir Klochkov have no conflicts of interest, financial or otherwise.

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