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THE STUDY OF ACUTE TOXICITY OF NANOSULFUR

Abstract. Acute oral toxicity of nanosulfur size of about 75 nm was studied in female's mice. LD₅₀ values were between 300 – 2000 mg/kg for females in mice. Toxic signs were manifested in the form of depression locomotor activity. The thoracic and abdominal cavities were meticulously examined. At necropsy and histology we revealed flatulence colon, dystrophic changes in the liver and kidneys. Hepatocytes are filled with small and medium-sized lipid droplets. These results indicate that nanosulfur is more toxic than powdered sulfur.

Keywords: nanosulfur, nanomaterial, acute toxicity, nanotoxicology.

Introduction

Broad activity against bacteria, fungi, and insect, parasitizing on the skin, is shown for sulfur nanoparticles. The degree of this efficiency depends on the polymorphism, the size and shape of sulfur. In addition lower toxicity of elemental sulfur to mammalian cells makes sulfur nanoparticles very promising for production on their basis of antimicrobial preparations [1-4]. There are also data about anti-tumor activity of elemental sulfur [5]. However, if the toxicity of deposited microcrystalline sulfur is well studied, its nanofoms requires deep research [6]. It is known that the structure and arrangement of atoms or molecules in the crystal have an effect on the biological activity of pharmaceutical substances [7]. Besides the polymorphism of crystals, the particle size also influences the properties of the material. It is shown that the particle size of sulfur, selenium, zinc, copper, titanium affect their bioavailability, activity and toxicity, and not in all cases this dependence is linear [8-17]. In connection with this the acute toxicity of nanosulfur with particle size about 75 nm conducted on laboratory mice was studied.

Materials and methods of research

Investigated substance - nanosulfur with the size of the coherent scattering blocks of 75 nm [18]. As the carrier distilled water was used.

Acute toxicity was investigated on white outbred female mice weighing 20-24 g in accordance with the guidelines of the Organization for Economic Cooperation and Development (OECD) No. 423 [19]. Class of toxicity was assessed by the classification and marking of chemical substances and mixtures (GHS) [19]. Nanosulfur solution was administered intragastrically once by pathfinder in a volume of 0.5 ml. The animals were kept in a vivarium RSBE "Scientific and practical center of sanitary-epidemiological examination and monitoring." Mice were in cages with litter from wood chips, preliminary seasoned under the influence of UV rays. Litter was changed 2 times a week. The ambient temperature was 21 ± 2°C, humidity 50 ± 10%, artificial light regime 12:12. For animals it was selected mixed diet comprising mainly food containing natural ingredients (tubers, grains). Feeding animals was 2 times a day at the same time. Access to water was ad libitum. Marking of animals was performed with marker on wool section on the legs, back and head. Euthanasia was performed in compliance with the rules of humane treatment of laboratory animals in a CO₂ chamber, containing 70% CO₂ at a flow rate of 30 l/min (ethics commission № 38 of 10.26.2015). At necropsy the lungs, heart, spleen, liver, kidneys, gastrointestinal tract were studied. Organs were fixed in 10% neutral formalin. Histological specimens

were prepared by the standard technique [20]. From the paraffin blocks it were sectioned with 5-7 microns thick at semi-automatic microtome ERM3000, sections were stained with hematoxylin-eosin. The preparations were examined by direct light microscope DM1000 (Leica, Germany).

The mathematical processing of the results was performed in Microsoft Excel-2010. After receiving the primary data regardless of the experiment it was calculated the arithmetic mean value and standard deviation. In order to identify significant differences between the experimental values it was used the Student's coefficient. Reliability values $P > 0,05$ was considered as not significant.

Results and discussion

After intragastric administration of nanosulfur in a dose of 2000 mg/kg, for the first three hours one mouse died, during the first day - the second one. After the administration up to 2 days the animals response to external stimuli has been greatly reduced. Mouse huddled in cells corners and almost did not move. Breathing was rapid. With abdomen opening of dead mice it was found bloating colon loops (Figure 1).



Figure 1 - Necropsy of mouse abdominal cavity, which received 2000 mg/kg of nanosulfur

Esophagus is permeable and unchanged. Duodenum and small intestine are without any pathological changes. The contents of the stomach is in pale yellow color with a slight shade of gray, with a faint odor of sulfur dioxide, indicating the suspending of nanosulfur. One animal has survived, and was left till the end of the experiment. On the 15th day the animal was euthanized and undertook necropsy (Figure 2).



Figure 2 - Mice necropsy on the 15th day of the experiment, received 2000 mg / kg of nanosulfur

At necropsy of the mouse from the group of 2000 mg/kg subjected to euthanasia, the following was found (Figure 2). Chest cavity was free from the liquid, pleura surface without changes; rose pink lungs,

aerial, full-blooded selected proportions. In the heart coronary vessels are clearly traced. Diaphragmatic cupula is not changed. The surface of the visceral organs, intestinal loops (small and large), mesenteric lymph nodes were unchanged. However, the colon is swollen. The spleen is dark - cherry color, it has been increased, with the longitudinal section it is not left on the scraping blade scalpel. Kidney - light - brown, kidney capsule is taken off hard. At section the border between the brain and the cortical layer is well-differentiated, pelvises are extended and a little bit swollen. The stomach contents was noted, the mucous fold structure has been preserved. When cross-section of the duodenum the pale yellow and odorless homogeneous chyme flowed. Colon loops are swollen. Reproductive system organs were without changes. When probing the uterine horns are passable. Oral cavity is free, mucous is unchanged.

As observed mortality of animals at a dose of 2000 mg/kg, according to the manual [19] nanosulfur dose was reduced to 300 mg/kg. Animal mortality at 300 mg/kg was missed throughout the observation period (14 days). In the first experiment hours, the following toxic symptoms were observed: animals huddled together, increasing of response to external stimuli (noise). All symptoms disappeared within 4 hours after administration of the test substance. Dynamics of changes in animal body weight during the experiment is presented in Table 1.

Table 1 - Change in body weight of mice after single intragastric administration of nanosulfur in a dose of 300 mg/kg, $M \pm m$

Experimental conditions	The average weight of the animals, g		
	1 st day	8 th day	15 th day
Control animals (solvent)	22,0±0,5	22,9±0,5	24,2±0,5
Nanosulfur	22,4 ± 0,8	22,4 ± 1,2	22,0 ± 1,9

Investigation of the body weight dynamics of mice, treated with nanosulfur solution, showed no significant reduction in this parameter. After 15 days all animals were taken from the experiment by euthanasia in the CO₂ chamber and macroscopy of internal organs of experimental animals was conducted (Figure 3).



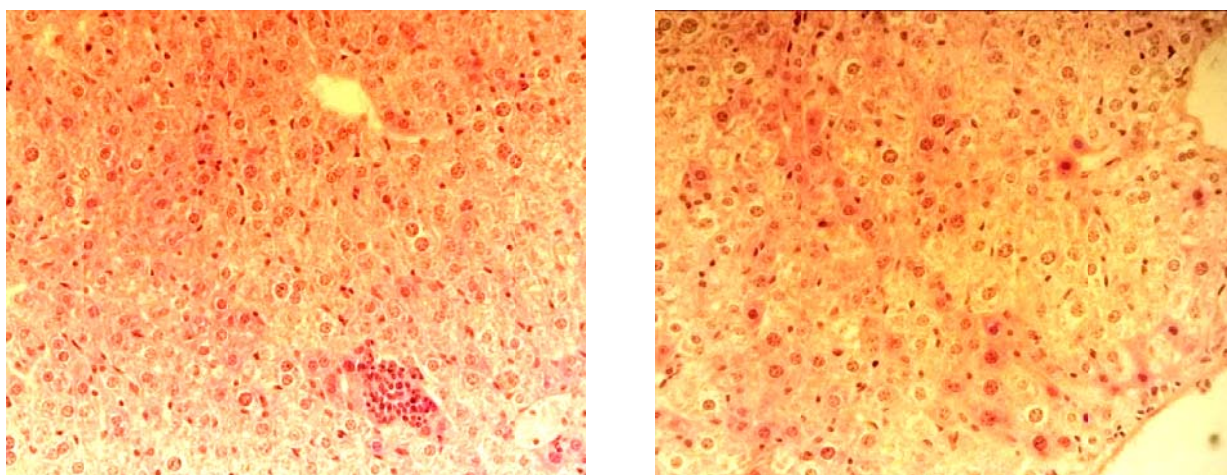
Figure 3 - Necropsy of mouse received 300 mg/kg of nanosulfur

Positions of organs were anatomically correct, the peritoneum was smooth and shiny, the thoracic and abdominal fluid was not found. The liver is a dark brown color, the edge looked round at the blade ventral side, at section there is a faint scraping, all of the blades surface are flat and smooth. Spleen is dark cherry color, enlarged, swollen capsule looked tense, shiny, when a cut a white pulp was traced. Faint scraping is on the cut edge. Kidneys are bean-shaped, smooth, shiny, elastic, capsule was easily removed. At the cutting off the border of cortex and medulla is clear, cortex prevailed. Pelvic organs were intact. Subcutaneous lymph nodes were not enlarged.

Histologic examination of the mice liver in the control group showed a typical morphological pattern, similar to that of the organs, without pathological changes. In the study of kidneys structural changes are

not detected. At histological sections of the spleen pathological and morphological changes in the structural components of the organ was not observed. The structures of the lung, heart and stomach were intact.

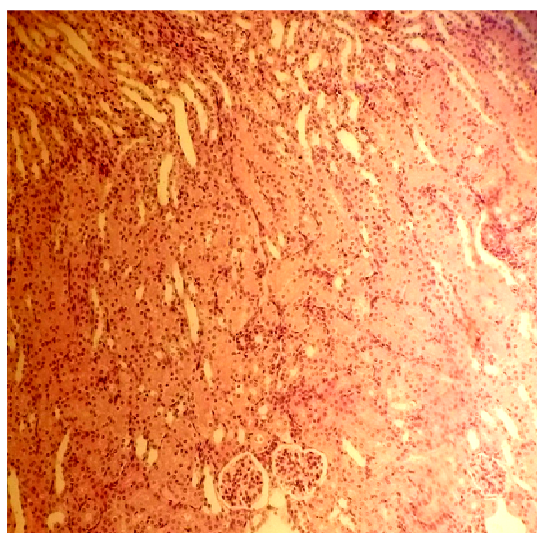
Histological examination of the mice tissues from the group of 2000 mg/kg showed liver hepatocytes with hyperchromatic nucleus and homogeneous eosinophilic cytoplasm. Hepatocytes are in small- and medium-droplet steatosis. Organ strom is focally infiltrated by lymphoid cellular elements. The activation of Kupffer cells was detected. Expanding the Disse's space is mainly in the periportal zone. Focal perivascular edema (Figure 4).



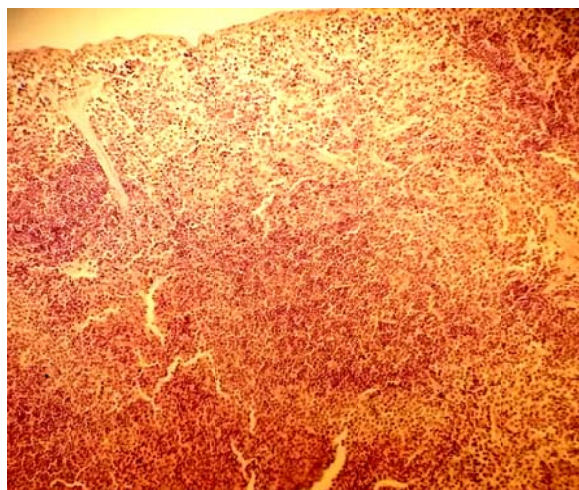
(Scaling-up: about 40, approx. 10; stain: hematoxylin-eosin)
Figure 4 - Histostructure of mouse liver from the group of 2000 mg/kg

The kidneys are marked dystrophic changes in the proximal tubules, in some tubules epithelial cells completely cover the gap. In the lumens of the distal tubules, Henle's loops it is notes homogeneously colored contents (Figure 5). The spleen was observed pronounced depletion of red and white pulp of lymphocytes delymphatisation of peripheral zones of the follicle and swelling of the stroma (Figure 6).

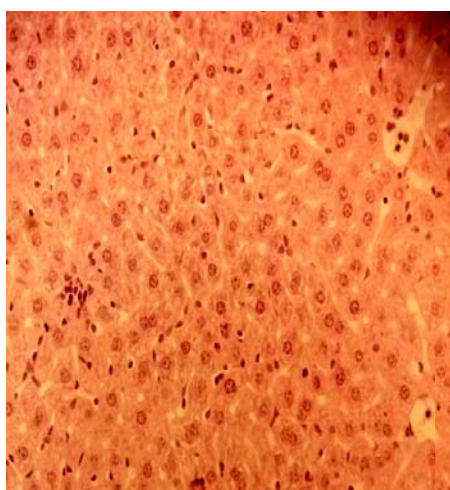
The liver of mice received 300 mg/kg of nanosulfur, had normal lobular structure (Figure 7). Hepatocytes are of rarely normal structure with two or more cores. Weak activation of Kupffer cells are revealed. Pathological changes in the kidneys were not found. Spleen study showed a slight decrease in white pulp, with the depletion of the red and white pulps with lymphocytes (Figure 8).



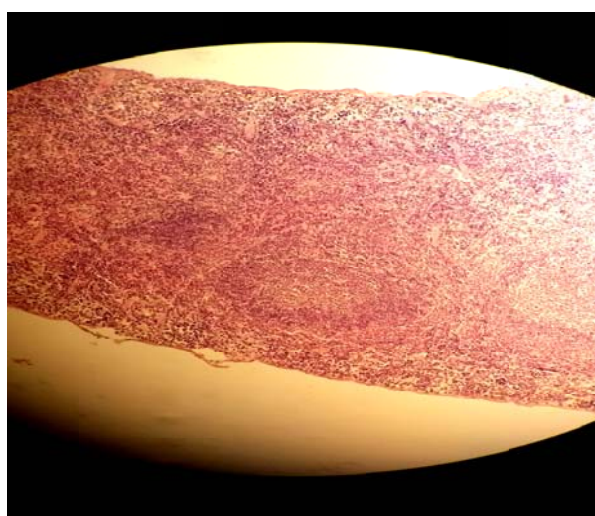
(Scaling-up: about 20, approx. 10; stain: hematoxylin-eosin)
Figure 5 - Histostructure of mouse liver from the group of 2000 mg/kg



(Scaling-up: about 20, approx. 10; stain: hematoxylin-eosin)
Figure 6 - Histostructure of mouse spleen from the group of 2000 mg/kg



(Scaling-up: about 40, approx. 10; stain: hematoxylin-eosin)
Figure 7 - Histostructure of mouse liver from the group of 300 mg/kg



(Scaling-up: about 20, approx. 10; stain: hematoxylin-eosin)
Figure 8 - Histostructure of mouse spleen from the group of 300 mg/kg

Thus, the study of acute toxicity of nanosulfur allowed to establish its extent and hazard class. The average lethal dose was >300<2000 mg/kg. According to the Globally Harmonized System of Classification and Labelling of Chemicals test substance - nanosulfur refers to 4 class of toxicity. Necropsy allowed to establish that the target organs of the toxic lesions are the liver and kidneys. For comparison, the mean lethal dose of elemental sulfur with a single oral administration is higher than 2000 mg/kg [21].

Conclusion

Currently the study of acute toxicity on mice have shown that nanosulfur refers to 4 class of toxicity with LD50 range from 300 to 2000 mg/kg. Histological examination revealed the target organs of toxic lesions, which are the liver and kidneys.

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НАНОКҮКІРТТІҢ ЖЕДЕЛ УЫТТЫЛЫҒЫН ЗЕРТТЕУ

Аннотация. Нанокүкірттің жедел уыттылығын мөлшері 75 нм болатын аналық тышқандарға жұтқызу арқылы зерттелді. Орта өлім-жітімге әкелетін доза 300 – 2000 мг/кг ауқымында болатыны көрінді. Қозғалыс

белсенділігінің төмендеуіне сәйкес улану симптомдары байқалды. Некропсия және гистологиялық зерттеу кезінде тоқ ішектің ісінуі мен бауыр және бүйректе дистрофикалық өзгерістері пайда болды. Гепатоциттер ұсақ және орта май тамшыларынан құралды. Нәтижесінде, нанокүкірттің ұнтақталған күкіртке қарағанда ұйтты екені анықталды.

Түйін сөздер: нанокүкірт, наноматериал, жедел ұйттылық, нанотоксикология.

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ИЗУЧЕНИЕ ОСТРОЙ ТОКСИЧНОСТИ НАНОСЕРА

Аннотация. Острую токсичность при пероральном введении наносера размером около 75 нм изучали на самках мышей. Было показано, что средняя смертельная доза находилась в диапазоне 300 – 2000 мг/кг. Наблюдались токсические симптомы в виде снижения активности животных. При некропсии и гистологическом исследовании обнаружили вздутие толстого кишечника, дистрофические изменения в печени и почках. Гепатоциты содержали мелкие и средние жировые капли. В результате было установлено, что наносера токсичнее, чем молотая сера.

Ключевые слова: наносера, наноматериал, острая токсичность, нанотоксикология.

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