https://doi.org/10.47470/0869-7922-2024-32-2-87-98
Оригинальная статья

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# Assessment of the general toxic effect of subchronic exposure to copper oxide nanoparticles in an *in vivo* experiment on rats

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ABSTRACT

***Introduction.*** Reduced emissions of hazardous pollutants into the ambient and workplace air registered at modern industrial enterprises is an additional positive effect of updating production facilities. The problem of exposure to nanoparticles, however, remains relevant, but the focus of world science is shifting towards the toxicity of lower doses.

*The purpose of the study* is to assess the general toxic effect of subchronic exposure to copper oxide nanoparticles (CuO NPs) in an *in vivo* experiment on outbred male rats.

***Material and methods.*** Stable suspensions of copper oxide nanoparticles were administered to male rats in a single dose of 1 mg/kg body weight thrice a week for six weeks. Health status of the experimental animals was then evaluated by certain criteria to establish the general toxic effect of the subchronic exposure. Student's *t*-test was used to assess statistical significance of differences between the exposure and control groups.

***Results.*** We found a decrease in the intensity of intracellular energy processes, morphological and functional changes in the liver, kidneys and spleen, including those associated with the activation of cellular immunity in the exposed animals.

***Limitations.*** The study was limited to examining general toxicity in an experimental study of subchronic exposure of male rats to copper oxide nanoparticles using only one dose.

***Conclusion.*** The severity of the changes observed suggests that, judging by the assessed parameters of general toxicity, the dose approximates the threshold one for rats.

***Keywords:*** *toxicity; experiment; nanoparticles; rats*

***Compliance with ethical standards.*** The study was approved by the institutional Ethics Committee of the Yekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers (protocol No. 2 of April 20, 2021).

**For citation:** Ryabova Yu.V., Sakhautdinova R.R., Minigalieva I.A., Sutunkova M.P. Assessment of the general toxic effect of subchronic exposure to copper oxide nanoparticles in an *in vivo* experiment on rats. *Toksikologicheskiy vestnik / Toxicological Review.* 2024; 32(2): 87–98. https://doi.org/10.47470/0869-7922-2024-32-2-87-98 (In Russian)

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**Authors contribution:** *Ryabova Yu.V.* – data collection and analysis, draft manuscript preparation; *Sakhautdinova R.R.* – data collection and analysis, draft manuscript preparation; *Minigalieva I.A.* – study conception and design, draft manuscript preparation; *Sutunkova M.P.* – study conception and design, editing. *All co-authors* – approval of the final version of the article, responsibility for the integrity of all parts of the article.

**Acknowledgment.** The authors express their gratitude to the team of the Ural Multiple Access Center "Modern Nanotechnologies" of the Ural Federal University named after the first President of Russia B.N. Yeltsin and personally to its director Vladimir Ya. Shur, Prof., Dr. Sci. (Phys.-Math.), for the synthesis of nanoparticle suspensions.
**Conflict of interests.** The authors declare no conflict of interest.
**Funding.** The study had no sponsorship.

Received: December 25, 2023 / Accepted: March 10, 2024 / Published: April 27, 2024

https://doi.org/10.47470/0869-7922-2024-32-2-87-98
Original article

## Introduction

The targeted production of nanoparticles and/or their unintentional formation during industrial high-temperature processes leads to their negative effects on humans through inhalation, oral or dermal exposure. The toxic effect of CuO NPs has been widely studied. According to the results of the study by Anreddy et al. (2018), oral administration of a single dose of CuO NPs 5.50 mg/kg body weight per day for 14 days to Wistar rats caused a change in the activity of antioxidant enzymes such as glutathione, catalase, superoxide dismutase, as well as an increase in lipid peroxidation products. Liver toxicity has been shown. With oral daily intake of CuO NPs at a dose of 100 mg/kg body weight for 2 weeks, Abdelazeim et al. (2020) revealed a significant increase in the activity of liver enzymes and the level of inflammatory markers, an increase in the DNA fragmentation coefficient, changes in the antioxidant balance and the histomorphological picture of the liver. Oral exposure to CuO NPs for five consecutive days at doses of 32, 64 and 512 mg/kg body weight per day caused changes in hematological and biochemical parameters, histopathological abnormalities in the bone marrow, stomach and liver, mainly consisting of an inflammatory reaction, ulceration. Subchronic intraperitoneal exposure of rats to CuO NPs at a single dose of 10 mg/kg body weight caused changes in various functional and biochemical indicators of the body, including pathological changes in the microscopic structure of the liver, spleen, kidneys, brain and an increase in the DNA fragmentation coefficient. Ghonimi et al. (2022) with intraperitoneal administration of CuO NPs for 9 days in single doses of 5–25 mg/kg body weight, using morphometric and immunohistochemical methods, they revealed changes in the condition of the liver and kidneys, up to necrotic changes in tissue. At the same time, the modern trend towards the implementation of multifaceted measures aimed at reducing emissions of pollutants necessitates the consideration of harmful chemical factors in toxicological experiments in smaller doses.

The purpose of the study is to evaluate the subchronic general toxic effect of CuO NPs under in vivo experimental conditions on outbred male rats.

## Material and methods

Subchronic intoxication was modeled in outbred white male rats by repeated intraperitoneal injections 3 times a week for 6 weeks. The initial body weight of the animals was 235.4±6.7 g, the average age at the beginning of the experiment was 3–4 months. The range of fluctuations in body weight did not exceed ±10%. The keeping, feeding, care of animals and their removal from the experiment were carried out in accordance with generally accepted requirements. The work was approved by the Local Ethics Committee of the Federal Budgetary Institution Yekaterinburg Medical Research Center for Prophylaxis and Health Protection in Industrial Workers, protocol number No. 2 of 04/20/2021.

Experimental animals were administered a solution of a stable suspension of NPs in a total dose of 18 mg/kg body weight in a volume of 2 ml ("Experiment", 12 rats, a single dose of 1 mg/kg body weight) or deionized water in a similar volume ("Control", 12 rats). The choice of dose was determined both by the results of the literature search and by the authors' own previous studies. Suspensions of CuO NPs with a size of 21±4 nm were obtained at the Center for Collective Use "Modern Nanotechnologies" of Ural Federal University using laser ablation. The choice of dose was determined both by the results of the literature search and by the authors' own previous studies.

The choice of intraperitoneal administration model was due to the need for precise dosing of the substance per kilogram of animal body weight. The timing and frequency of administration were determined by the research team's own experience: with this model of administration used, it is possible to achieve subchronic intoxication over a 6-week period without causing excessive suffering to the animals.

At the end of the exposure, the animals were killed by complete decapitation, and the blood was used for hematological and biochemical analyses. Hematological parameters were determined using a Methic 18 hematological analyzer. Biochemical parameters were determined using a Cobas Integra 400 plus analyzer and corresponding diagnostic kits, or using manual techniques. The leukocyte formula was manually calculated in blood smears stained according to Romanovsky–Giemsa. The activity of succinate dehydrogenase (SDH) was assessed by the amount of formazan granules in blood lymphocytes. Tissue smears of cross sections of organs were stained according to Leishman, assessed for 300 cells of the kidneys, spleen and liver and in a Carl Zeiss Primo Star light binocular microscope at a magnification of ×100 and ×1000.

The statistical significance of intergroup differences was assessed using Student's *t*-test at a significance level of $p<0.05$ with Bonferroni correction; the sample was previously checked for normality using the Kolmogorov–Smirnov test.

https://doi.org/10.47470/0869-7922-2024-32-2-87-98
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## Results

At the end of the experimental period, no statistically significant changes were recorded in the morphometric parameters of some internal organs, including the final weight of the animals in the experimental group, an indirect indicator of the general toxic effect (Table 1). When visually examining the internal organs of the experimental animals, no pronounced changes were noticed compared to the control group.

The hematological parameters of the blood of rats did not change, with the exception of an increase in thrombocrit by almost 30% compared to the control value. At the same time, no studies have been conducted to clarify which platelet fraction accounted for this increase: young (immature), mature or old platelets. Also was noted, although not statistically significant, a clear decrease in the level of hemoglobin in the blood and the level of red blood cells (Table 2).

The indicators of the body's antioxidant status did not change, although the activity of catalase, an enzyme that protects the cell from oxidative damage, increased slightly. Undoubtedly, under the influence of CuO NPs, the activity of SDH in blood lymphocytes, one of the key links in the process of energy supply to the cell, decreased statistically significantly (Table 3).

When assessing the condition of internal organs using impression cytology in the group exposed to CuO NPs, an increase in the percentage of degenerative cells of the proximal, but not distal tubules of the rat kidneys was found (Fig. 1).

In the liver of rats, under the influence of CuO NPs, the percentage of degeneratively changed hepatocytes in fingerprint smears increased (Fig. 2, *a–d*) and the activity of alanine aminotransferase (ALAT) in the blood serum increased (Fig. 2, *b*).

In the spleen of rats, impression cytology revealed changes in the ratio of the proportion of cells of different types: the ratio of macrophages and

*Table 1*

**Changes in weigh and mass indicators of the control and exposed animals**

| Group | Initial weight of the animal, g | Final weight of the animal, g | Body weight gain, % | Liver weight, g per 100 g body weight | Kidney weight, g per 100 g body weight | Spleen weight, g per 100 g body weight | Brain mass, g per 100 g body weight |
|---|---|---|---|---|---|---|---|
| Control | 239,17 ± 4,12 | 292,92 ± 9,58 | 22,28 ± 2,73 | 2,42 ± 0,45 | 0,437 ± 0,08 | 0,185 ± 0,03 | 0,522 ± 0,09 |
| Experiment | 231,67 ± 4,14 | 270,00 ± 5,16 | 21,02 ± 1,40 | 2,76 ± 0,50 | 0,443 ± 0,08 | 0,167 ± 0,03 | 0,547 ± 0,09 |

*Table 2*

**Changes in blood parameters of control and exposed animals**

| Index | Group | |
|---|---|---|
| | Control | Experiment |
| Red blood cells, $10^{12}$/L | 8,46 ± 0,72 | 7,75 ± 0,62 |
| Hemoglobin, g/l | 172,18 ± 13,30 | 150,57 ± 4,72 |
| Hematocrit, % | 20,44 ± 1,61 | 18,54 ± 1,27 |
| Red blood cell distribution width, % | 16,79 ± 0,39 | 15,71 ± 0,50 |
| Platelets, $10^9$/L | 614,18 ± 36,52 | 659,78 ± 56,62 |
| Average platelet volume, fl | 5,97 ± 0,15 | 6,12 ± 0,15 |
| Thrombocrit, % | 0,18 ± 0,01 | **0,23 ± 0,01*** |
| Platelet distribution width, % | 13,85 ± 0,80 | 14,17 ± 0,48 |

*Table 3*

**Changes in indicators of antioxidant status and bioenergetic metabolism in control and exposed animals**

| Group | SH-groups, mmol/L | Reduced glutathione in blood hemolysate, μmol/L | Catalase in blood serum, μmol/L | Peroxidase in blood serum, μmol/L | SDH activity in blood lymphocytes, number of formazan granules in 50 lymphocytes |
|---|---|---|---|---|---|
| Control | 1,46 ± 0,17 | 9,33 ± 2,40 | 0,41 ± 0,09 | 22,31 ± 0,73 | 575,78 ± 6,10 |
| Experiment | 1,25 ± 0,19 | 12,22 ± 2,78 | 0,48 ± 0,11 | 22,40 ± 0,87 | 507,00 ± 8,12 * |

https://doi.org/10.47470/0869-7922-2024-32-2-87-98
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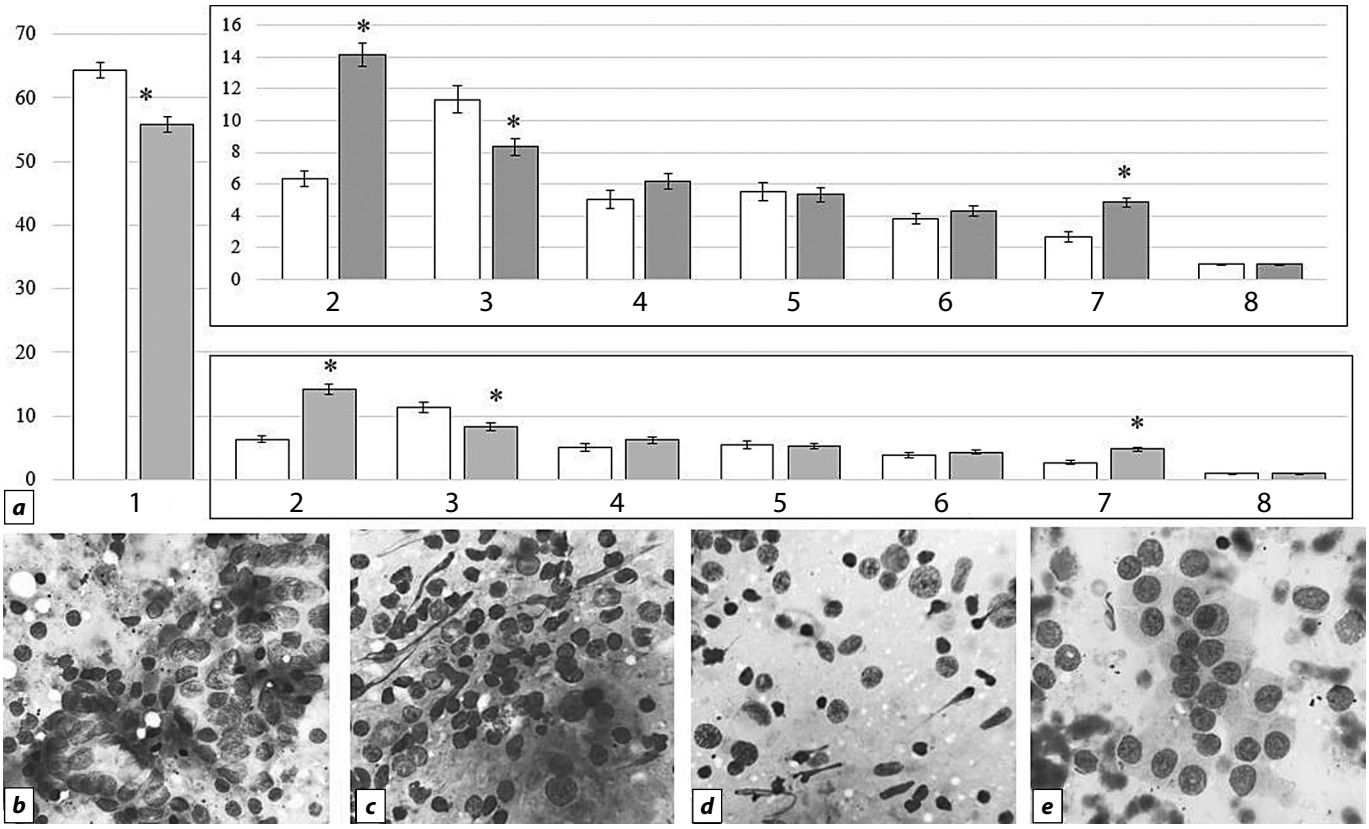


**Fig. 1.** Changes in the proportion of cells of different types in kidney imprint smears: *a* – graphical representation of changes in the proportion of cells of different types in kidney imprint smears, %; *b* – degenerated cells of proximal tubules, %; *c* – degenerated cells of distal tubules, %; *b, d* – groups of renal epithelium at 100,000× magnification; *c, e* – groups of renal epithelium with eosinophilia and degenerative dystrophic changes; *e* – image E shows "bare nuclei" at 100,000× magnification. * – Statistically different from the control group ($p<0.05$).

1 – proximal tubule cells in the renal imprint; 2 – degenerative cells of proximal tubules; 3 – cells of distal tubules; 4 – degenerative cells of distal tubules; 5 – neutrophils; 6 – monocytes; 7 – eosinophils; 8 – fibroblasts.

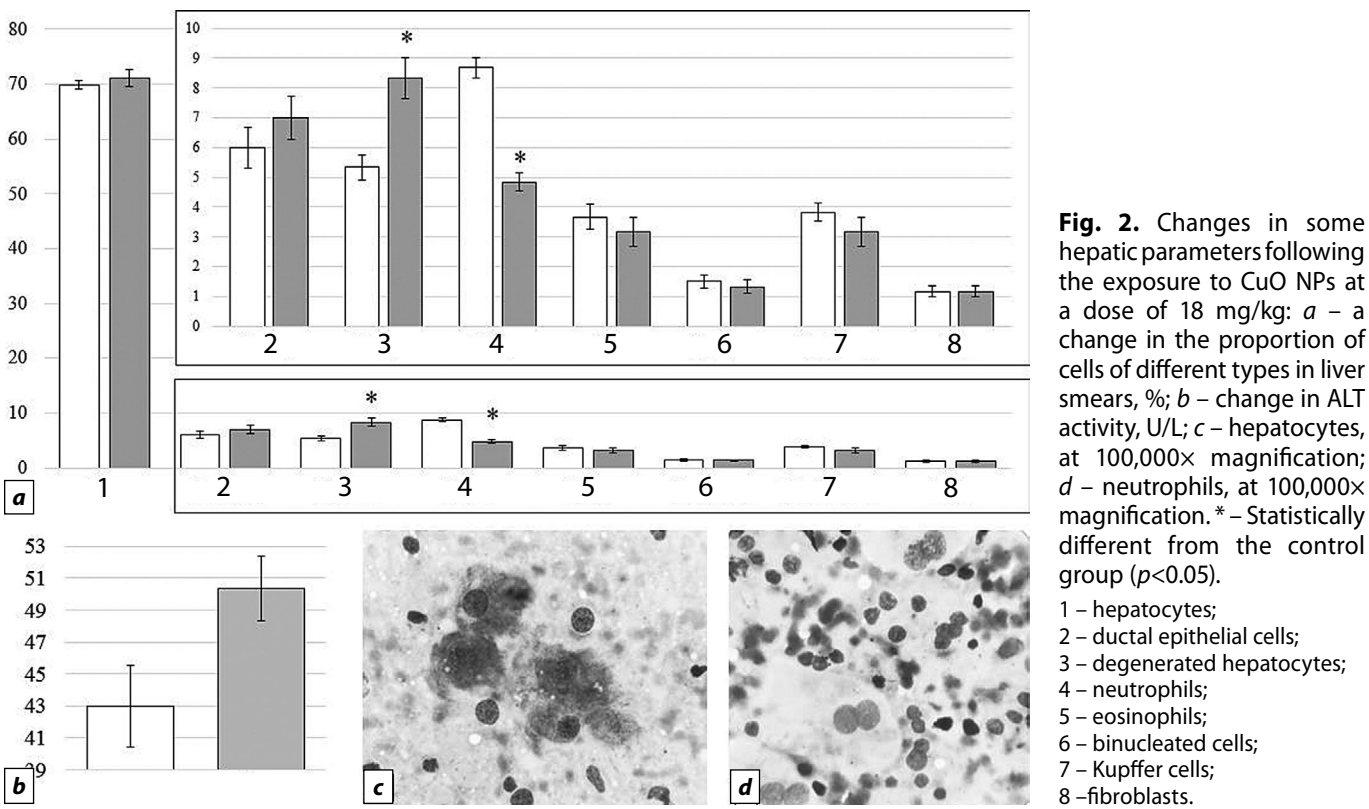


**Fig. 2.** Changes in some hepatic parameters following the exposure to CuO NPs at a dose of 18 mg/kg: *a* – a change in the proportion of cells of different types in liver smears, %; *b* – change in ALT activity, U/L; *c* – hepatocytes, at 100,000× magnification; *d* – neutrophils, at 100,000× magnification. * – Statistically different from the control group ($p<0.05$).

1 – hepatocytes;
2 – ductal epithelial cells;
3 – degenerated hepatocytes;
4 – neutrophils;
5 – eosinophils;
6 – binucleated cells;
7 – Kupffer cells;
8 –fibroblasts.

https://doi.org/10.47470/0869-7922-2024-32-2-87-98
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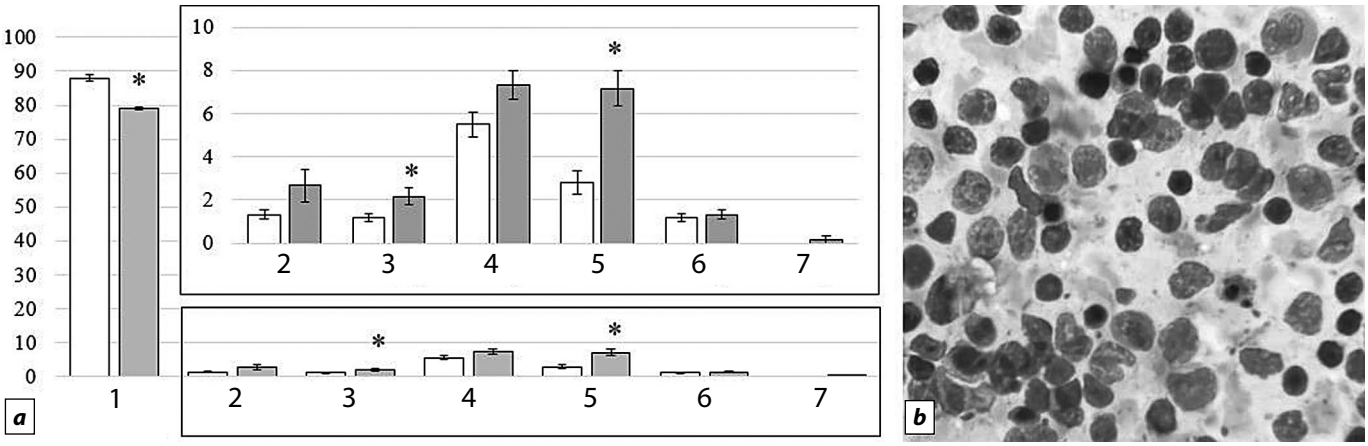


**Fig. 3.** Changes in the proportion of cells of different types in imprint smears of the spleen: *a* – changes in the proportion of cells of different types in imprint smears of the spleen; *b* – the area with eosinophilia at 100,000×magnification. * – Statistically different from the control group ($p<0.05$).

1 – mature lymphocytes and prolymphocytes in the spleen imprint; 2 – plasma cells; 3 – macrophages; 4 – neutrophils; 5 – eosinophils; 6 –lymphoblasts; 7 – reticular cells.

eosinophils increased, the number of lymphocytes and prolymphocytes decreased (Fig. 3).

## Discussion

In our study, with subchronic exposure, CuO NPs had a general toxic effect at a dose of 18 mg of body weight, which was expressed in changes in only some indicators of the rat's body state.

Thrombocytosis found in the blood of animals is likely a consequence of a low-grade inflammatory process, since platelets serve as an important link between the coagulation and immune systems, and the inflammation itself can be mediated by the action of CuO NPs. We judged its presence by the increase in the number of immunocompetent cells in smears of internal organs. In particular, the percentage of eosinophils, neutrophils and macrophages in the spleen (see Fig. 3) and eosinophils in the kidneys (see Fig. 1) increased. Our results are consistent with the data of Cho W.S. et al., indicating the ability of CuO NPs to cause inflammatory reactions involving eosinophils.

An increase in the percentage of degenerative cells of the proximal, but not distal tubules of rat kidneys was shown (see Fig. 1), which is consistent with well-known data on the dynamics of damage to parts of this organ under the influence of toxic agents and is associated with different functional loads on them. Note the possible role of eosinophils in damage to kidney tissue, whose proportion in the fingerprint smears was increased: they are able to induce oxidative stress, which provokes cell death.

In the liver of rats, under the influence of CuO NPs, the percentage of degeneratively changed hepatocytes in fingerprint smears increased and the activity of ALT in the blood serum increased (see Fig. 1), which can be considered as a manifestation of the hepatotoxic effect characteristic of CuO NPs. It was previously demonstrated by other authors, but using higher doses: Ghonimi et al. (2022) with intraperitoneal administration of copper nanooxide for 9 days in single doses of 5–25 mg/kg body weight, with oral daily consumption of a daily dose equal to 100 mg/kg body weight for 1 month and a single dose of 5.50 mg/kg body weight for 14 days.

The antioxidant status did not change, which is likely due to the dual nature of copper, which is an integral part of many biological processes. Oxidative damage induced by copper is closely related to exposure to excess copper ions, including associated with disruption of copper metabolism, which is probably not achieved sufficiently for a visible change in the studied parameters upon subchronic exposure to a dose of NPs (which inevitably partially dissolve when exposed to into the body) equal to 18 mg/kg body weight. At the same time, a decrease in the activity of SDH in blood lymphocytes, one of the key links in the process of energy supply to the cell, was recorded (see Table 3). It is probably associated with changes in adaptation processes against the background of developing intoxication and is a manifestation of the general toxic effect of copper. The mechanism is presumably due to the fact that mitochondria are targets for almost all types of damaging agents, including oxidative stress provoked by copper compounds. The diverse functionality of SDH, in addition to its role in the Krebs cycle and the respiratory chain, is associated with a wide range of disorders when it is inhibited. The latter necessitates further study of the mechanism of SDH inhibition under the influence of CuO NPs and possible long-term effects.

https://doi.org/10.47470/0869-7922-2024-32-2-87-98
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## Conclusion

The results obtained indicate that copper oxide nanoparticles in a total dose of 18 mg/kg body weight of rats with subchronic intraperitoneal exposure have a toxic effect, manifested by a change in the morphofunctional state of the liver, kidneys and spleen with activation of cellular immunity, as well as a decrease in the intensity of intracellular bioenergetic processes. The data obtained can be used to expand knowledge about the specific effects of copper oxide nanoparticles on the mammalian body.

## REFERENCES

1. Anreddy R.N.R. Copper oxide nanoparticles induces oxidative stress and liver toxicity in rats following oral exposure. *Toxicol. Rep.* 2018; 5: 903–4. https://doi.org/10.1016/j.toxrep.2018.08.022
2. Abdelazeim S.A., Shehata N.I., Aly H.F., Shams S.G.E. Amelioration of oxidative stress-mediated apoptosis in copper oxide nanoparticles-induced liver injury in rats by potent antioxidants. *Sci. Rep.* 2020; 10(1): 10812. https://doi.org/10.1038/s41598-020-67784-y
3. De Jong W.H., de Rijk E., Bonetto A., Wohlleben W., Stone V., Brunelli A. et al. Toxicity of copper oxide and basic copper carbonate nanoparticles after short-term oral exposure in rats. *Nanotoxicology.* 2019; 13(1): 50–72. https://doi.org/10.1080/17435390.2018.1530390
4. Privalova L.I., Katsnelson B.A., Loginova N.V., Gurvich V.B., Shur V.Y., Valamina I.E. et al. Subchronic toxicity of copper oxide nanoparticles and its attenuation with the help of a combination of bioprotectors. *Int. J. Mol. Sci.* 2014; 15(7): 12379–406. https://doi.org/10.3390/ijms150712379
5. Ghonimi W.A.M., Alferah M.A.Z., Dahran N., El-Shetry E.S. Hepatic and renal toxicity following the injection of copper oxide nanoparticles (CuO NPs) in mature male Westar rats: histochemical and caspase 3 immunohistochemical reactivities. *Environ. Sci. Pollut. Res. Int.* 2022; 29(54): 81923–37. https://doi.org/10.1007/s11356-022-21521-2
6. Minigalieva I.A., Katsnelson B.A., Panov V.G., Privalova L.I., Varaksin A.N., Gurvich V.B. et al. In vivo toxicity of copper oxide, lead oxide and zinc oxide nanoparticles acting in different combinations and its attenuation with a complex of innocuous bio-protectors. *Toxicology.* 2017; 380: 72–93. https://doi.org/10.1016/j.tox.2017.02.007
7. Huang H.S., Chang H.H. Platelets in inflammation and immune modulations: functions beyond hemostasis. *Arch. Immunol. Ther. Exp. (Warsz).* 2012; 60(6): 443–51. https://doi.org/10.1007/s00005-012-0193-y
8. Angelé-Martínez C., Nguyen K.V.T., Ameer F.S., Anker J.N., Brumaghim J.L. Reactive oxygen species generation by copper(II) oxide nanoparticles determined by DNA damage assays and EPR spectroscopy. *Nanotoxicology.* 2017; 11(2): 278–88. https://doi.org/10.1080/17435390.2017.1293750
9. Cho W.S., Duffin R., Poland C.A., Duschl A., Oostingh G.J., Macnee W. et al. Differential pro-inflammatory effects of metal oxide nanoparticles and their soluble ions in vitro and in vivo; zinc and copper nanoparticles, but not their ions, recruit eosinophils to the lungs. *Nanotoxicology.* 2012; 6(1): 22–35. https://doi.org/10.3109/17435390.2011.552810
10. Chevalier R.L. The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulotubular junction. *Am. J. Physiol. Renal Physiol.* 2016; 311(1): F145–61. https://doi.org/10.1152/ajprenal.00164.2016
11. Blanchard C., Rothenberg M.E. Biology of the eosinophil. *Adv. Immunol.* 2009; 101: 81–121. https://doi.org/10.1016/S0065-2776(08)01003-1
12. Gaetke L.M., Chow C.K. Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology.* 2003; 189(1–2): 147–63. https://doi.org/10.1016/s0300-483x(03)00159-8
13. Sutunkova M.P. Experimental studies of toxic effects' of metallic nanoparticles at iron and nonferrous industries and risk assessment for workers` health. *Gigiena i sanitariya.* 2017; 96(12): 1182-1187 (in Russian). https://doi.org/10.47470/0016-9900-2017-96-12-1182-1187
14. Guo C., Sun L., Chen X., Zhang D. Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regen. Res.* 2013; 8(21): 2003–14. https://doi.org/10.3969/j.issn.1673-5374.2013.21.009
15. He H., Zou Z., Wang B., Xu G., Chen C., Qin X. et al. Copper oxide nanoparticles induce oxidative DNA damage and cell death via copper ion-mediated P38 MAPK activation in vascular endothelial cells. *Int. J. Nanomedicine.* 2020; 15: 3291–3302. https://doi.org/10.2147/IJN.S241157
16. Rustin P., Munnich A., Rötig A. Succinate dehydrogenase and human diseases: new insights into a well-known enzyme. *Eur. J. Hum. Genet.* 2002; 10(5): 289–91. https://doi.org/10.1038/sj.ejhg.5200793

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