Part 1. Veterinary medicine

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EFFECT OF ZINC CARBONATE NANOPARTICLES SUBCHRONIC INTAKE ON ANTIOXIDANT STATUS OF MALE RABBITS

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Summary. An urgent scientific task is to develop modern and safe zinc-based nanoparticles that can fulfill rabbits' need for this essential mineral. This research primarily focuses on enhancing the bioavailability of zinc and reducing its toxic properties. To evaluate the antioxidant effect of zinc carbonate nanoparticles, 24 sexually mature male Hiplus rabbits were used, which were randomly divided into two groups of four animals and three replicates each. The rabbits in the experimental group were orally administered zinc carbonate nanoparticles obtained by the co-precipitation method and stabilized with polyvinylpyrrolidone at a dose of 100 mg/kg body weight for 30 days. Animals in the control group received distilled water according to a similar procedure. At the end of the experiment, blood samples were taken from all animals for biochemical studies. The presence of redox activity of these nanoparticles in the organism of male rabbits was established: after administration in the experimental group of animals, a significant decrease in peroxidation markers was noted: the level of thiobarbiturate-active products by 24.5%, diene conjugates by 18.7% and lipid hydroperoxides by 16.2% ($p < 0.05$). A significant increase in the total antioxidant activity (by 40.2%), the content of enzymatic and non-enzymatic components of the antioxidant defense system was confirmed by the expressive antioxidant effect of zinc carbonate nanoparticles: superoxide dismutase activity by 68.9%, catalase by 18.3%, glutathione peroxidase by 27.6%, glutathione reductase by 34.6% and reduced glutathione content by 15.7% ($p < 0.05$). Thus, it can be argued that there is an antioxidant effect of zinc carbonate nanoparticles for male rabbits. The authors of the article consider the study of the effect of these nanoparticles on the state of sexual function in rabbits, as well as pharmacokinetic studies, to be prospects for further research

Keywords: bioavailability, toxicity, blood, biochemical parameters

Introduction. Among the large number of mineral supplements in rabbit diets, zinc (Zn) is one of the most important, especially for males, due to its multifaceted effects, including the ability to improve immune status, digestion, and absorption of nutrients, and the activity of many enzymes (Michalak et al., 2022; Abdel-Wareth et al., 2023b).

The importance of zinc as a cofactor for numerous metalloenzymes plays a leading role in the gastrointestinal tract, metabolism of proteins, fats, and carbohydrates (Malik et al., 2021; Abd El-Hack et al., 2024). The components of the diet used for rabbits (cereals, corn) contain a large amount of phytates, which significantly limit the absorption of zinc and other trace elements (Abdel-Wareth et al., 2020; Abdelnour et al., 2025).

According to Abdel-Wareth et al. (2023a), the average zinc content in rabbit diets is in the range of 30–110 mg/kg without additional addition of this trace element and in the amount of up to 250 mg in diets enriched with 100 mg of zinc oxide in macroform or 60 mg of nanoparticles (NPs).

Recently, there has been a growing interest in using dietary zinc oxide nanoparticles (ZnO-NPs) to enhance performance due to their antioxidant properties, bioavailability, and ability to modulate the rabbit immune system (Abdel-Wareth et al., 2022). ZnO-NPs have both local and systemic antibacterial effects, which have been successfully used in treating wounds in rabbits and other bacterial and surgical diseases in animals and poultry (Awad et al., 2022; Abbas et al., 2023; Obaid et al., 2024). Additionally, an experimental study on rabbits confirmed that ZnO-NPs can accelerate bone growth and mineralization (Zalama et al., 2022; Shokri et al., 2024; Nelogi et al., 2025). Some studies have shown that zinc-based NPs can serve multiple functions — not only as a source of a helpful trace element but also as a therapeutic agent (Hanini et al., 2016). Zinc-based NPs can also be used to develop nanocontainers of theranostic value, as the binding of zinc ions to metallothionein-1X proteins — overexpressed in kidney tumor cells — can increase therapeutic potential, as demonstrated in a rabbit model (Zeng et al., 2025).

However, researchers are mostly interested in the new properties acquired by zinc in the nanoform (Alqahtani, 2025). Thus, on the one hand, there is a decrease in its toxicity compared to inorganic sources, while its bioavailability increases (from 50–60% to 95–99% percent with different routes of administration) (Salimi et al., 2019; Hassan et al., 2024). It is worth noting that nanocrystalline zinc oxide is the most common nanocompound. A large number of studies have focused on ZnO-NPs, including their use as a feed additive for various species of mammals and poultry (Swain et al., 2016; Fatima et al., 2024). Although the studied dosages of ZnO-NPs *in vivo* models vary significantly (1–1,000 mg/kg b. w.), there are many limitations associated with the introduction of ZnO-NPs into animal husbandry practice (Youn and Choi, 2022; Rahman et al., 2022; Herrera-Rodríguez et al., 2023).

In vivo studies in rats, mice, and rabbits demonstrate the ability of zinc-based NPs to cause toxic effects, such as cyto-, embryo-, nephro-, neuro-, and hepatotoxicity (Bashir et al., 2022; Abouzeinab et al., 2023; Parashar et al., 2024). For example, the LC_{50} of ZnO-NPs is less than 25 μg/ml for rabbit corneal cells in an *in vitro* experiment (Lee and Park, 2019). These toxic effects are mainly caused by high doses (500–1,000 mg/kg b. w.) of ZnO-NPs, with different durations of administration (Fujihara and Nishimoto, 2024; Kahil et al., 2024). Among the main pathogenetic mechanism of their toxicity is oxidative stress, and there are also phenomena of damage to cell membranes, cell mitochondria, DNA, etc. mediated by it (Pei et al., 2023; Serhiienko et al., 2025). Therefore, in recent years, researchers have been developing new methods of synthesis aimed at reducing the toxicity of zinc-based NPs, in particular, using green chemistry methods, organic ligands, etc. (Zhang et al., 2022; Bozer, Dede and Güven, 2024; Elshaer et al., 2025). For example, recent studies of the authors of the article have shown the absence of a general toxic effect of zinc carbonate nanoparticles ($ZnHCO_3$-NPs) synthesised by the co-precipitation method (in doses of 25–200 mg/kg b. w.) on male rats, in particular, hepatotoxic, nephrotoxic, and haematotoxic effects (Naumenko et al., 2023; Koshevoy et al., 2025).

At the same time, there is no data on their redox activity, and therefore it is relevant to study their effect on the antioxidant defense system, which was the **aim** of this study, using model animals — male rabbits, in a subchronic experiment with $ZnHCO_3$-NPs administration of 100 mg/kg b. w. for 30 days.

Materials and methods. The study included 24 adult male rabbits of the Hiplus breed aged 30 weeks and weighing 4.2 ± 0.1 kg. Rabbits for the study were obtained from a private farm located in Kharkiv Region (Ukraine). The animals were kept in the vivarium of the Department of Veterinary Surgery and Reproductology of the State Biotechnological University. Before the experiment, the animals were kept in a preparatory period without the use of pharmacological adaptogens. The concentrated feed and fresh tap water were available *ad libitum* (Tverdokhlib et al., 2024). The rabbits were housed in a well-ventilated room at 25 ± 1°C and with a relative humidity of 55 ± 5% with a regular 12 hours light/12 hours dark cycle (Koshevoy et al., 2022). All animals were randomly divided into two groups: an experimental group and a control group. Each group had 12 animals, with four animals in each group and three replicates. Male rabbits of the experimental group received once a day $ZnHCO_3$-NPs at a dose of 100 mg/kg body weight for 30 days. The control group received the same volume of distilled water.

Synthesis of zinc carbonate nanoparticles and their characterization. Initially, 50 ml of Na_3Cit (Sigma Aldrich, USA) 0.075 M water solution was mixed with 50 ml of $Zn(Ac)_2$ (Sigma Aldrich, USA) 0.1 M water solution. Then, 50 ml of Na_2CO_3 (Sigma Aldrich, USA), 0.15 M water solution was added to the mixture under vigorous stirring. The obtained mixture was heated to 85°C using a water bath for 45 min at constant stirring. The obtained colloidal solution was dialyzed against water for 120 min in a cellulose dialysis sack (pore d = 2.5 nm, MWCO 12,000 kDa). Water was changed every 30 min. The final pH value of the solution was 7.5. Thereafter, 150 ml of 0.6% polyvinylpyrrolidone, used as a stabilizing agent, was added to the solution to obtain 300 ml of zinc carbonate nanoparticles ($ZnHCO_3$-NPs). The final concentration of solid phase ($ZnHCO_3$-NPs) was 2 g/l with 0.3 w% of polyvinylpyrrolidone. Morphology of the as-synthesized $ZnHCO_3$-NPs was analyzed by scanning electron microscopy (SEM, JSM-6390LV, JEOL Company, USA) (Fig. 1a). A TEM image was acquired by a TEM-125K electron microscope (Selmi, Ukraine) using a 100 kV electron beam (Fig. 1b).

Sampling and biochemical methods. Blood samples were taken from the lateral saphenous veins on the 30^{th} day of the study. Pharmacological preparations for sedation and anesthesia of animals were not employed at the time of blood sampling. In the next step, 2 ml of blood samples were taken at the same time throughout the study in tubes with separating gel. The content of OS markers in blood serum was estimated using spectrophotometric methods to determine the concentrations of thiobarbituric acid-reactive compounds (TBA-RC), diene conjugates (DC), and lipid hydroperoxides (LHP). The TBA-RC method is based on the binding of malondialdehyde with thiobarbituric acid to form a stable trimethine complex (wavelength of λ = 532 nm); DC determination is based on the value of the molar extinction coefficient for conjugated dienes of polyunsaturated higher fatty acids (at λ = 233 nm). The concentration of LHP was determined via protein precipitation with trichloroacetic acid and lipid extraction with ethanol. Upon the addition of ammonium thiocyanate to the ethanol lipid extracts, a colorimetric reaction occurred (at λ = 480 nm).

The total antioxidant activity (T-AOC) of plasma was assessed using FRAP analysis (the principle is to determine the antioxidant power of ferrum). A solution of ferric sulfate was used to create a standard curve, and the results were expressed in mmol of Fe^{2+} formed per liter of plasma. Plasma T-AOC was determined spectrophotometrically. Superoxide dismutase activity (SOD) was calculated by the degree of reaction inhibition by the enzyme to reduce nitroblue tetrazolium in the presence of nicotinamide adenine dinucleotide and phenazine methosulfate (at λ = 540 nm). Catalase activity was determined based on the ability of hydrogen peroxide to form a stable complex with ammonium molybdate, color intensity at λ = 410 nm.

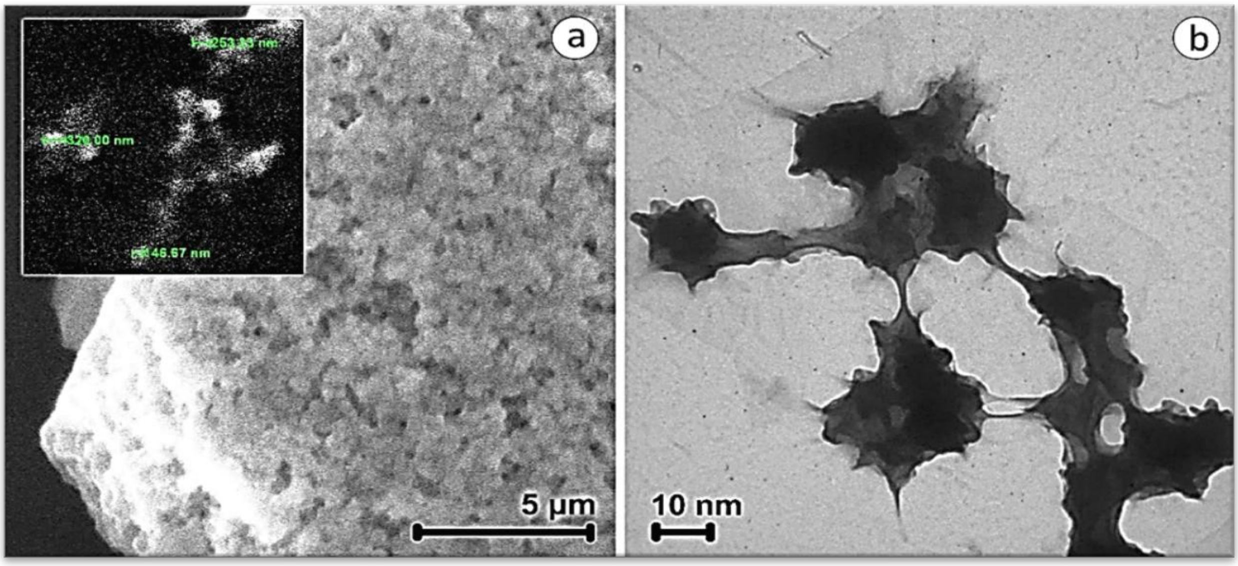

Figure 1. Scanning (a) and transmission (b) electron microscopy images of the synthesized zinc carbonate nanoparticles.

Glutathione peroxidase activity (GSH-Px) was measured based on the oxidation rate of the reduced glutathione in the presence of tBHP in the color reaction with 5,5-dithiobis-2-nitrobenzoic acid (at $\lambda = 412$ nm). Glutathione reductase activity (GSH-Rd) was calculated by reducing the content of nicotinamide adenine dinucleotide phosphate at 37°C for 1 min (at $\lambda = 340$ nm), and finally, reduced glutathione (GSH) was assessed by the Butler method using Ellman's reagent (at $\lambda = 412$ nm). Enzymes and non-enzyme antioxidants were spectrophotometrically determined following Vlizlo (2012).

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the State Biotechnology University.

Mathematical and statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS), version 22. An ANOVA test was performed to compare data from the control and experimental groups, with normality determined using the Shapiro-Wilk test. Significant differences between groups were confirmed by the Tukey test. Statistical significance was considered to be a P-value less than 0.05.

Results and discussion. The antioxidant effect of subchronic administration of zinc carbonate nanoparticles was assessed by changes in biochemical parameters of male rabbits' blood. First of all, it was important to find out whether the administered dose of $ZnHCO_3$-NPs affected the level of peroxidation products. For this purpose, the content of thiobarbiturate-active products (TBA-RC), diene conjugates (DC), and lipid hydroperoxides (LHP) was evaluated, the results of which are shown in Fig. 2.

In the animals of the control group, the content of TBA-RC was 0.94 ± 0.04 µmol/l, while in the experimental rabbits, it was lower by 24.5% (0.71 ± 0.03 µmol/l, $p < 0.05$). At the same time, there was a decrease in the content of DC by 18.7% (1.09 ± 0.05 µmol/l, $p < 0.05$). Additionally, compared with the control data, the content of LHP decreased to 0.62 ± 0.03 U/l, which was lower by 16.2% ($p < 0.05$). Further, the indicators of the antioxidant defence system were evaluated. First of all, changes in the total antioxidant activity (T-AOC) and the activity of the primary enzymes of the antioxidant defense system — superoxide dismutase and catalase were determined (Fig. 3).

Figure 3 shows that ingesting 100 mg/kg b. w. of $ZnHCO_3$-NPs by male rabbits improved their antioxidant defense. The total antioxidant activity of the blood serum of the experimental rabbits increased by 40.2% compared to the control values (up to 0.861 ± 0.04 mmol×Fe^{2+}, $p < 0.05$). The activity of superoxide dismutase underwent significant changes: if in control rabbits it was at the level of 10.43 ± 0.41 U/mgHb, then after the administration of $ZnHCO_3$-NPs in animals of the experimental group, it increased by 68.9% and amounted to 17.62 ± 0.84 U/mgHb ($p < 0.05$). Less pronounced changes were characterized by catalase activity — in rabbits of the experimental group, it was 18.3% higher than in the control (91.28 ± 2.43 µmol H_2O_2/min×mg protein, $p < 0.05$). We also studied changes in the state of the glutathione link of the antioxidant system under the influence of $ZnHCO_3$-NPs, which are summarized in Fig. 4.

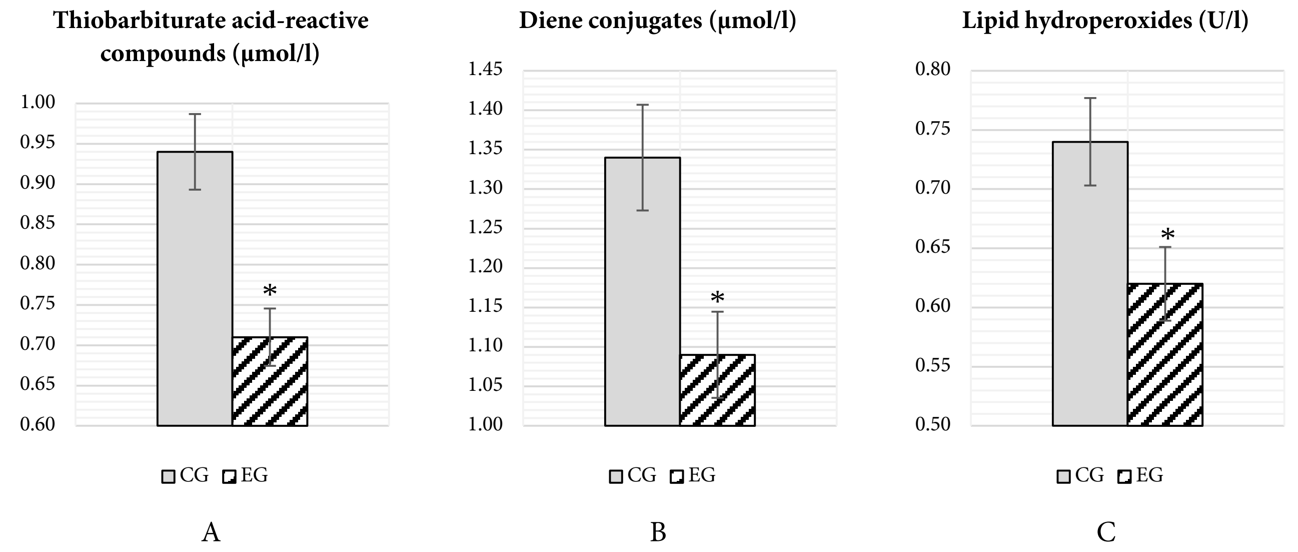

Figure 2. Dynamics of prooxidant system components in male rabbit blood under the action of zinc carbonate nanoparticles: thiobarbiturate acid-reactive compounds (A), diene conjugates (B), and lipid hydroperoxides (C). CG — control group, EG — experimental group. Significant differences ($p < 0.05$) between groups are marked in the figures with *.

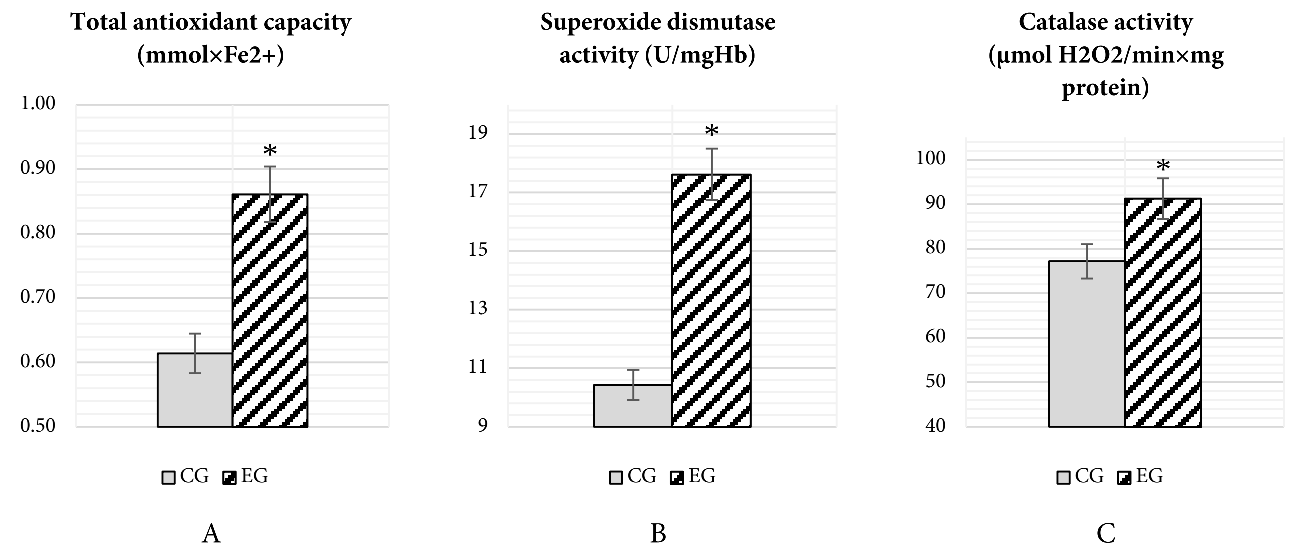

Figure 3. Dynamics of total antioxidant capacity (A), superoxide dismutase activity (B), and catalase activity (C) in male rabbit blood under the action of zinc carbonate nanoparticles. CG — control group, EG — experimental group. Significant differences ($p < 0.05$) between groups are marked in the figures with *.

In general, the obtained changes indicate a partial involvement of the glutathione system. In particular, the content of reduced glutathione increased by 15.7% compared to control rabbits (9.74 ± 0.27 µmol/l, $p < 0.05$). The activity of the enzymes of the thiol-disulfide system underwent more pronounced changes: in the control rabbits, the activity of glutathione peroxidase was at the level of 13.17 ± 0.41 µmol/min×mg protein, and glutathione reductase — 2.14 ± 0.09 µmol/min×mg protein, after the administration of $ZnHCO_3$-NPs, these indicators increased by 27.6% and 34.6%, respectively ($p < 0.05$). Thus, it can be seen that the administration of $ZnHCO_3$-NPs in male rabbits showed a complex antioxidant pharmacological effect — a clear increase in the components of antioxidant defense against a decrease in the level of peroxidation products.

Zinc-based nanoparticles have a multifaceted effect on the rabbit body, which is confirmed by the studies of numerous authors from around the world (Deguchi et al., 2021; Gur et al., 2022; Saha et al., 2024). Such an impact has a diverse manifestation of positive changes — from the health of rabbits to the quality of rabbit products (Halo et al., 2021; Masoud et al., 2025). The study of the effect of zinc oxide-chitosan nanoparticles (ZnO-CNPs) showed the presence of immunomodulatory properties, improved feed intake, total protein, and albumin content in the blood at doses of 50–100 mg/kg of rabbit body weight.

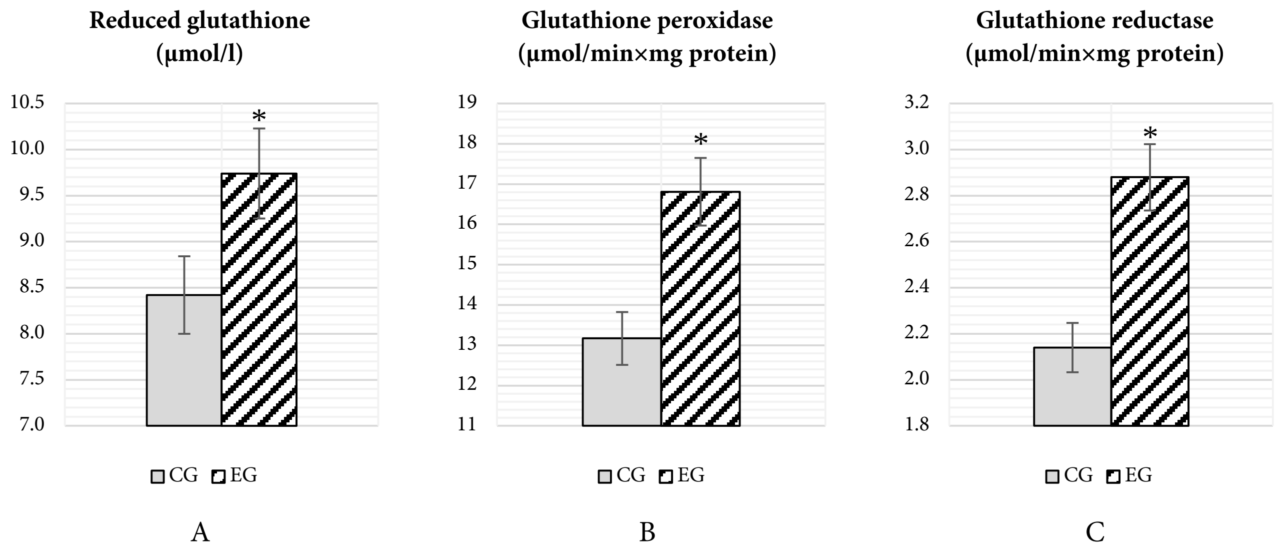

Figure 4. Dynamics of glutathione link of antioxidant system in male rabbit blood under the action of zinc carbonate nanoparticles: reduced glutathione (A), glutathione peroxidase activity (B), and glutathione reductase activity (C). CG — control group, EG — experimental group. Significant differences ($p < 0.05$) between groups are marked in the figures with *.

In addition, a decrease in the content of glucose and total cholesterol in the blood serum of rabbits was observed, indicating the presence of insulin-like effects and antilipidemic properties (Nawaz et al., 2021; Hassan et al., 2023). On the other hand, the use of these NPs is not as widespread as the addition of ZnO-NPs to rabbit diets, which can demonstrate toxic properties at the wrong dosage and depending on the duration of administration to animals (Park et al., 2017; Dahran et al., 2023).

For example, it has been shown that ZnO-NPs can affect liver function in rabbits, contributing to both excessive hepatic enzyme activity and persistent histopathological changes even when administered at low doses of 1–10 mg/kg b. w. (Moasses et al., 2024). The ability of ZnO-NPs at a dose of 60 mg/kg b. w. to reduce the reproductive toxicity of ivermectin in male rabbits, especially to reduce malondialdehyde levels and improve the activity of antioxidant enzymes — superoxide dismutase, catalase, and reduced glutathione levels, was shown (El-Shobokshy et al., 2023). It was also found that the antioxidant system in rabbits treated with 250 μl/kg b. w. improved. ZnO-NPs doped with curcumin in a model of experimental rheumatoid arthritis. Thus, the content of malondialdehyde decreased in rabbits, and indicators of redox processes improved — total antioxidant activity of blood serum, catalase, and superoxide dismutase activity (Azeez et al., 2024).

Different sources of zinc in rabbit diets have different bioavailability and pharmacological activity, so according to Hassan et al. (2021), the use of three different forms of zinc — crystalline zinc oxide as an inorganic source, zinc in combination with methionine as an organic source and zinc oxide nanoparticles — showed the advantage of the organic form and especially nanostructured zinc compared to the usual inorganic macroform. Therefore, a large number of researchers have been studying the synthesis methods, toxicity, and pharmacological properties of zinc-based NPs in recent years (Gomez-Zavaglia et al., 2022; El-Saadony et al., 2024). Taking into account the high toxicity of most zinc-based NPs, the authors of this article have created a new compound — zinc carbonate nanoparticles ($ZnHCO_3$-NPs). Previous studies have shown the low toxicity of these NPs, the absence of death in experimental animals after a single intragastric administration, and confirmed the presence of a positive effect on metabolic parameters (Koshevoy et al., 2023, 2025). At the same time, a large number of pharmacological properties of $ZnHCO_3$-NPs have not yet been investigated.

The results of the study conducted by the authors of this article proved the expressive antioxidant effect of $ZnHCO_3$-NPs on the model of male rabbits. This effect was directed at two main links: the intensity of peroxidation processes and the state of the antioxidant defense system of the rabbit organism. Thus, the data obtained are consistent with the results of El-Shobokshy et al. (2023), in particular, the introduction of $ZnHCO_3$-NPs contributed to a decrease in the content of TBA-RC, DC, and LHP in the body of male rabbits, as well as ZnO-NPs in the above study. In addition, the authors of this article found an increase in the activity of superoxide dismutase and catalase. Similar changes were observed in their studies by Abdel-Wareth et al. (2022) and Azeez et al. (2024) when ZnO-NPs were added to rabbit diets. It is important to note that although the antioxidant effect of $ZnHCO_3$-NPs and ZnO-NPs is similar, their toxicity parameters are different. Concerning $ZnHCO_3$-NPs, their prolonged administration

(30 days) and high dose (100 mg/kg b. w.) did not cause negative changes in the body of rabbits, which was confirmed by a significant increase in the total antioxidant activity. At the same time, we note the presence of a positive effect on the components of the thiol-disulfide link of antioxidant defense — at the end of the experiment, rabbits showed an increase in the activity of glutathione peroxidase and glutathione reductase, as well as the content of reduced glutathione.

According to the authors of the article, it is promising to include $ZnHCO_3$-NPs in the diets of rabbits under heat stress, since there is evidence of improvement of metabolic parameters in such animals under the influence of zinc compounds. For example, the positive effect of ZnO-NPs on the correction of the health status of animals kept under heat stress has been shown, as it is known that its effect leads to oxidative imbalance (Ebeid et al., 2023; Bashar et al., 2024; NasrEldeen et al., 2025). Rabbits treated with ZnO-NPs at a dose of 50 mg/kg b. w. for 60 days had physiological activity of alanine and aspartic aminotransferases, creatinine levels and zinc content in the blood compared to the control group (Abdel-Wareth et al., 2023a; Alrashedi, Almasmoum, and Eldiasty, 2024). The results of the same group of researchers indicate that the addition of ZnO-NPs (20–80 mg/kg) to the diet of rabbits can mitigate the negative effects of heat stress on performance and some biochemical parameters (lipid oxidation, cholesterol, liver enzyme activity) (Abdel-Wareth et al., 2022). It is important to note the positive effect of the introduction of 25–100 mg/kg wt. of ZnO-NPs on the state of androgenesis in male rabbits — it was found that, regardless of the dosage, there was an increase in testosterone levels in animals fed with ZnO-NPs (Abdel-Wareth et al., 2020, 2023b). Given the data obtained by the authors of this article, we consider it promising to further investigate the effect of $ZnHCO_3$-NPs on the state of reproductive capacity of rabbits, the possibility of using them to improve the quality of sperm and its fertilizing ability under conditions of normal temperature or heat stress.

Conclusions. The study of the properties of zinc carbonate nanoparticles under subchronic administration to rabbits by the authors of the article confirmed the hypothesis of their redox activity. Thus, after a 30-day administration of 100 mg/kg b. w. $ZnHCO_3$-NPs in rabbits of the experimental group, a decrease in the intensity of peroxidation processes was observed, which was confirmed by a decrease in the content of thiobarbiturate-active products in the blood serum, the level of diene conjugates, and lipid hydroperoxides. The antioxidant properties of the studied NPs were confirmed by a significant increase in the total antioxidant activity of blood serum, a significant increase in the activity of primary enzymes — superoxide dismutase, and, to a lesser extent, catalase activity. Regarding changes in the state of the thiol-disulfide link of antioxidant defense, the content of reduced glutathione did not increase significantly, and the activity of the enzymes glutathione peroxidase and reductase significantly exceeded the control data.

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