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ANTIOXIDANT PROTECTION IN WHITE RATS ON THE BACKGROUND OF DRINKING WATER USE WITH EXCESSIVE NITRATE CONCENTRATION

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Background. *The problem of nitrate pollution in terms of its scale and consequences has reached a global level. Approximately one-third of groundwater bodies for which information is currently available exceed the maximum allowable concentrations of nitrates in drinking water.*

Objectives. *The aim of the research is to study the features of antioxidant protection in the body of white rats on the background of drinking water with excessive concentrations of nitrates for 30 days regarding the age.*

Methods. *The experiment was performed on 2 groups of outbred white female rats of different age (mature and immature), 30 animals each, divided into 5 subgroups, which differed in the quality of drinking water they consumed independently. In a 30-day study, nitrates were given to rats with drinking water at a dose of 50, 150, 250, 500 mg of sodium nitrate per liter. The animals of the control group drank water from the city water supply. The effect of nitrates on superoxide dismutase (SOD), catalase (CAT) and ceruloplasmin (CP) in the serum of rats was evaluated.*

Results. *The research has shown increased SOD, CAT and CP in blood serum on the background of drinking water with a nitrate concentration of 150 to 500 mg/l in the experimental groups of animals of both ages. According to the research, the most significant changes in parameters are caused by drinking water with nitrates at a concentration of 500 mg/l. More significant changes were in immature animals then the mature ones.*

Conclusions. *Excessive concentrations of nitrates in drinking water adversely affect the body of experimental animals causing activation of the studied parameters of the antioxidant system.*

KEYWORDS: drinking water; nitrates; superoxide dismutase; catalase; ceruloplasmin.

Introduction

The problem of nitrate pollution in terms of its scale and consequences has reached the global level. It consists in total pollution of the biosphere, deterioration of plant agricultural products and drinking water, negative impact on the human body, especially young children and farm animals. Approximately one third of the groundwater bodies, for which information is currently available, exceeds the maximum permissible concentrations (MPC) of nitrates in drinking water that is equal to 50 mg/dm^3. Monitoring of nitrate levels in groundwater in different districts of Ternopil region showed that the maximum concentration ranges from 1.1 to 7.3 times or from 55 to 365 mg/l and even more [1, 2]. This is due to the widespread use of nitrogen-containing organic and inorganic fertilizers in agriculture. However, unfortunately, residents of houses have been using such water for drinking for many years, because nitrates, even their excessive concentrations are not felt or tasted by humans.

Nitrates are non-toxic, but entering the gastrointestinal tract they are reduced to nitrites, which due to their oxidizing properties are ten times more toxic than nitrates. Nitrites are absorbed into the blood, and consequently reach tissues. The main mechanism of nitrite toxicity is the oxidation of Fe^{2+} to Fe^{3+}, the conversion of hemoglobin into methemoglobin (MetHb) [3].

The use of nitrates with drinking water affects the nervous and cardiovascular systems, development of embryos; it can cause colorectal cancer, thyroid disease and neural tube defects [4, 1]. When systematically ingested, they can lead to disorders of the endocrine and central nervous system, blood circulation, etc. [5].

In addition, it is established that nitrites in the presence of amines can form N-nitrosamines, which have carcinogenic activity. Nitrates can also inhibit the enzyme systems involved in tissue respiration. As a result, a large number of free radicals is formed. At the same time, at

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the cellular level the processes of free radical oxidation of phospholipids of biological cell membranes take place that lead to development of oxidative stress [6].

This can be overcome by reducing the intensity of lipid peroxidation (LPO) in the body with antioxidant system (AOS) which controls and inhibits all stages of reactions of free radicals capable of damaging the cell from their initiation to formation of hydroperoxides and malonic dial [7].

This protective system combines a number of substances of different nature; each of them acts in close contact with its other structural elements, harmoniously complements, and in many cases enhances the action of each other. The main antioxidants of blood plasma are superoxide dismutase (SOD), catalase (CAT) and ceruloplasmin (CP) [8, 9]. When an organism is in unfavorable living conditions, in particular ecological, or when it is exposed to adverse factors the activity of these antioxidants increases sharply [10].

Therefore, the aim of the research was to study the features of antioxidant protection in the body of white rats on the background of drinking water with excessive concentrations of nitrates for 30 days regarding the age.

Methods

The study was conducted on 60 outbred white female rats which were kept on the standard diet of the vivarium of I. Horbachevsky Ternopil National Medical University. Animals were divided into 2 age categories: the 1st category – 30 mature animals weighing 180-200 g and the 2nd – 30 immature animals weighing 60-80 g. The experimental animals of each age category were divided into 5 groups with 6 rats in each. The groups were selected by randomization. The animals of control group drank drinking water from the city water supply. The rats of the 1st group drank drinking water with nitrate content of 50 mg/l, the 2nd – 150 mg/l, the 3rd – 250 mg/l, the 4th – 500 mg/l. To create model aqueous solutions, drinking water from city water supply was used; taking into account background concentrations, sodium nitrate ($NaNO_3$) was added into it to create aqueous solution with nitrates at a dose of 50, 150, 250, 500 mg per liter. The experiments were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Research Purposes (Strasbourg, 1986) [12] and the norms of biomedical ethics and the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001).

The animals were taken out of the experiment by bloodletting under thiopental-sodium anesthesia in 30 days after the beginning of the experiment.

The activity of catalase (CAT), ceruloplasmin (CP) and superoxide dismutase (SOD) was determined in the serum by means of a semi-automatic biochemical analyzer Humalyzer 2000 using standard kits by HUMAN diagnostics (Wiesbaden, Germany). The kits used for evaluation of CAT, CP and SOD were adapted for their determination in animal serum. The results were statistically processed using computer programs STATISTICA 10.0 and Excel 2010. The choice of statistical research method was based on the correct distribution of the studied features. Further pairwise comparison of groups was performed using the Mann-Whitney U-test, the statistical significance $p<0.05$.

Results

The results of our studies showed that when drinking water with different concentrations of nitrates, the AOS of the experimental animals of both ages underwent significant changes.

The key enzyme of AOS is SOD. It was found that the level of SOD, CAT and CP in the 1st experimental group of both age categories did not differ significantly from the data in the control group.

The increase in SOD activity in other groups of experimental rats of both ages compare to the controls was directly proportional to the concentration of nitrates in water. The level of SOD in the 2nd group of mature animals increased significantly by 43.7%, in the 3rd – by 56.2% and in the 4th – by 62.5% (Fig. 1).

In the immature animals, a more significant increase was evidenced. Thus, in the 2nd group the activity of SOD significantly increased by 61.1%, in the 3rd – by 94.4%. However, the activity of SOD increased the most in the 4th group – by 116.6% (Fig. 2).

SOD always works in conjunction with CAT, which converts hydrogen peroxide to water and molecular oxygen. When determining the activity of CAT in the serum of mature animals it increased by 16.0% in the 2nd group, in the 3rd – by 30.5% ($p<0.05$), and in the 4th group – by 97.2% ($p<0.05$) compare to the control (Fig. 3).

In the immature animals, insignificant increase in the activity of this parameter was found in the 2nd and 3rd groups (by 6.7% and 18.3%, respectively). However, in the animals

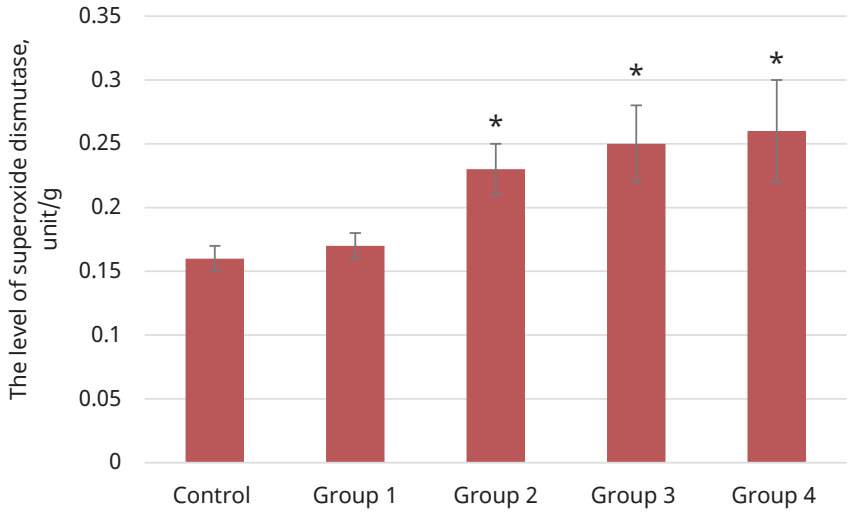

Fig. 1. The level of superoxide dismutase in the serum of mature experimental animals when drinking water with different concentrations of nitrates (unit/g).
*Note: * – the differences are significant comparing to the control group (p<0.05). Error bars indicate ± standard deviation of the mean after three replicates.*

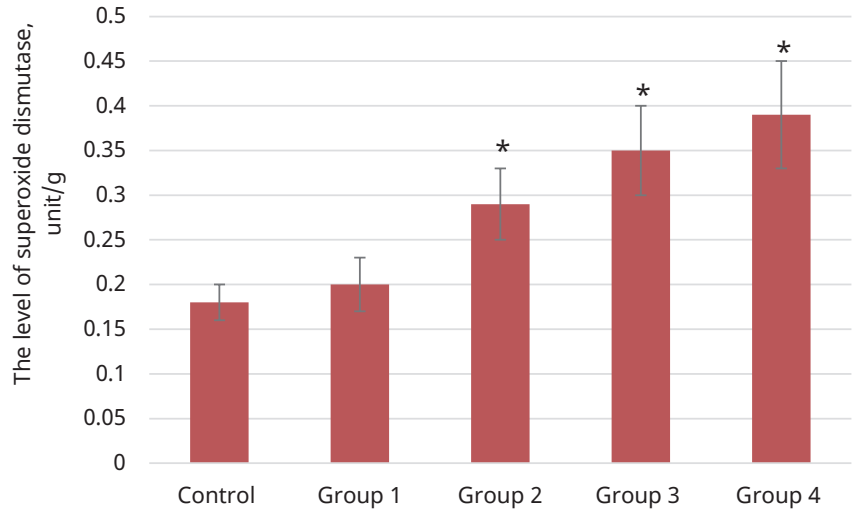

Fig. 2. The level of superoxide dismutase in the serum of immature experimental animals when drinking water with different concentrations of nitrates (unit/g).
*Note: * – the differences are significant comparing to the control group (p<0.05). Error bars indicate ± standard deviation of the mean after three replicates.*

of the 4th group the parameter increased by 28.8% (p<0.05) compare to the control group (Fig. 4).

The nonenzymatic part of AOS was assessed for the presence of CP in the blood serum, which is also one of the main antioxidants in the human's body. A main feature of this protein is its high stability to the toxic action of reactive oxygen species which allows it to maintain biological activity under conditions of their intensive generation [7, 8].

Increased level of CP in both age categories was established; it differed not much. In the 2nd group of mature animals the parameter increased by 22.1%, in the 3rd group – by 23.4%, in the 4th group – by 53.3% (p<0.05) (Fig.5).

The number of SR in the immature animals of the 2nd group increased by 28.4%, in the 3rd – by 39.0% (p<0.05), in the 4th – by 61.0% (p<0.05) (Fig. 6).

Discussion

According to the literature the antioxidant substance in the cell is present at low concentrations and significantly reduces or prevents oxidation of the oxidizable substrate. The most

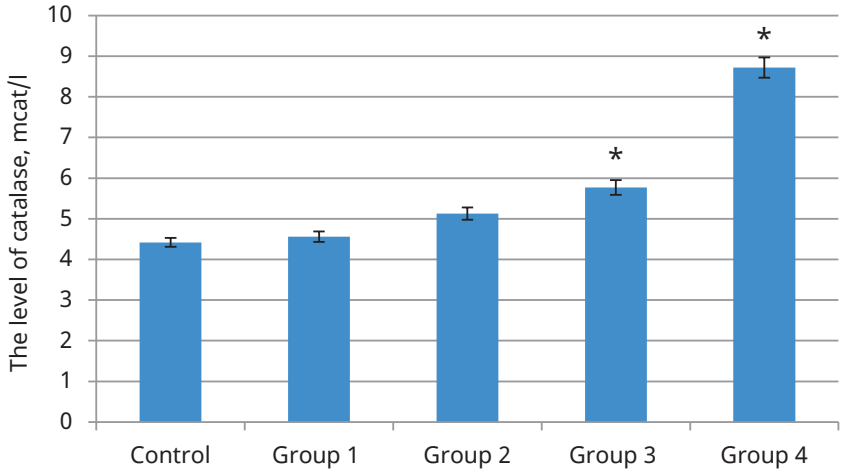

Fig. 3. The level of catalase in the serum of mature animals when drinking water with different concentrations of nitrates (mcat/l).
*Note: * – the differences are significant comparing to the control group (p<0.05). Error bars indicate ± standard deviation of the mean after three replicates.*

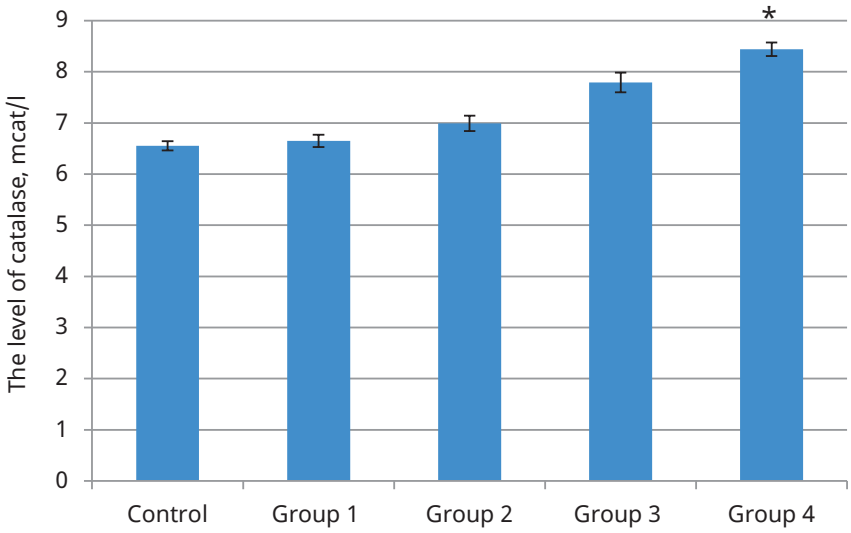

Fig. 4. The level of catalase in the serum of immature animals when drinking water with different concentrations of nitrates (mcat/l).
*Note: * – the differences are significant comparing to the control group (p<0.05). Error bars indicate ± standard deviation of the mean after three replicates.*

efficient enzymatic antioxidants contain glutathione peroxidase, catalase and superoxide dismutase. SOD is one of the most potent intracellular enzymatic antioxidants, and it catalyzes the conversion of superoxide anions to dioxygen and hydrogen peroxide. The hydrogen peroxide is removed by catalase. This enzyme is present in the peroxisome of aerobic cells and is very efficient in promoting the conversion of hydrogen peroxide to water and molecular oxygen [13]. An increase in the activity of CAT and SOD is usually observed in cases of environmental pollutants since SOD-CAT system represents the first line of defense against oxidative stress [14, 15]. According to Krishnamoorthy and Sangeetha [16], sodium nitrite (300 mg/kg body weight) significantly increased the lipoperoxidation and the activities of liver marker enzymes such as alanine aminotransferase, aspartate aminotransferase, acid phosphatase and lactate dehydrogenase.

Analyzing the dynamics of changes in the activity of AOS in the serum of rats, significantly higher SOD against CAT and CP were established. The SOD interrupts the chains of oxygen-dependent free radical reactions, causing dismutation of the superoxide anion radical with the release of hydrogen peroxide, which may

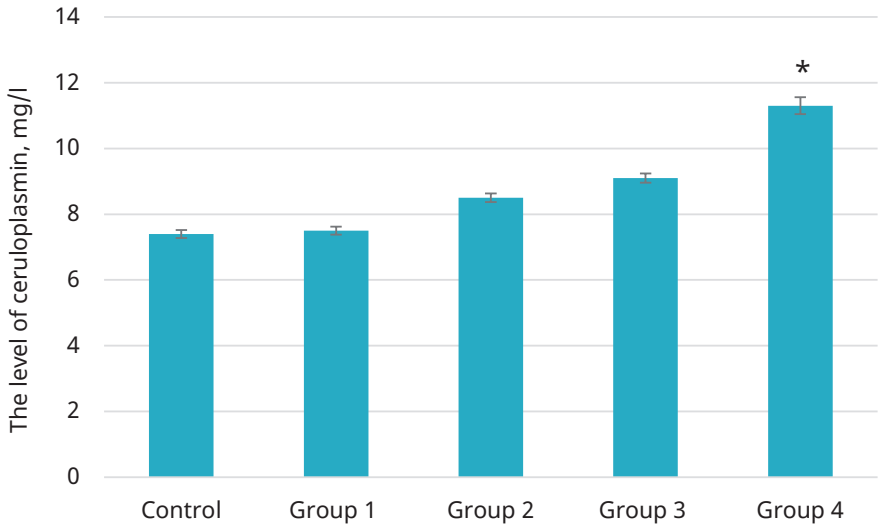

Fig. 5. The level of ceruloplasmin in the serum of mature animals when drinking water with different concentrations of nitrates (mg/l).
*Note: * – the differences are significant comparing to the control group (p<0.05). Error bars indicate ± standard deviation of the mean after three replicates.*

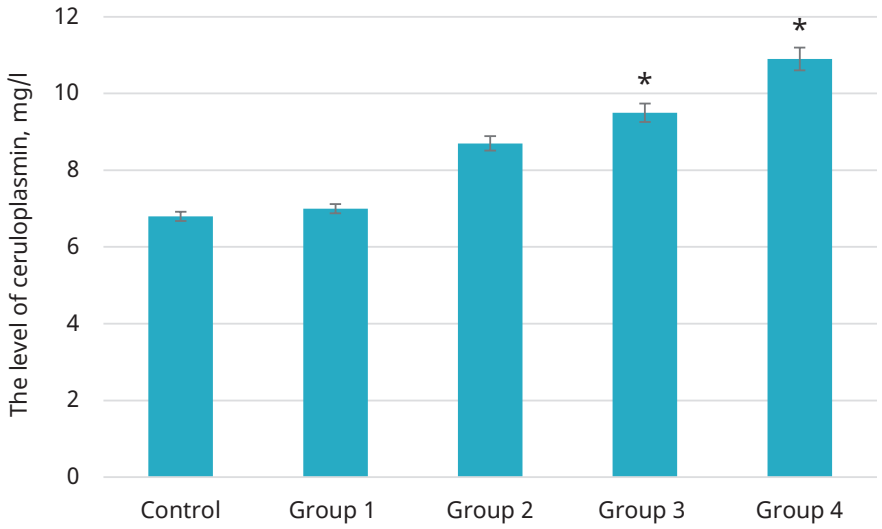

Fig. 6. The level of ceruloplasmin in the serum of immature animals when drinking water with different concentrations of nitrates (mg/l).
*Note: * – the differences are significant comparing to the control group (p<0.05). Error bars indicate ± standard deviation of the mean after three replicates.*

precede formation of the most toxic hydroxyl radical (OH^-) and triplet oxygen [10]. Increased activity of SOD is an additional damaging factor and may cause pathological processes as a result of increased cytotoxic action of hydrogen peroxide, formed as a result of dismutation of superoxide [8]. The increase of CAT in serum does not exclude the presence of cytolysis on the background of drinking water with different concentrations of nitrates, because CAT is an intracellular enzyme and its increased activity in the blood occurs in cases of increased permeability of cell membranes.

CP neutralizes free radicals and protects cell membranes due to its ability to inhibit lipid peroxidase. It prevents activation of the floor, formation of toxic iron ions and damage to cell membranes. This enzyme acts as an "extracellular superoxide dismutase" neutralizing superoxide anions in the extracellular environment, blood, amniotic and cerebrospinal fluid, muscle, kidney and myocardium. CP increases with iron deficiency and excessive

copper, in response to hypoxia, insulin, thrombin, estradiol, proinflammatory cytokines [11].

Increased oxidation processes are extremely crucial in the genesis of many pathological conditions, including inflammatory lesions of various tissues. In case of increased intake of xenobiotics, depletion of antioxidant depots and influence of negative factors, oxidative stress develops; it is characterized by prooxidant and antioxidant disbalance and development of oxidative damage [17].

Conclusions

The study allowed drawing the conclusion that excessive concentrations of nitrates in drinking water adversely affected the body of experimental animals causing activation of the studied parameters of the antioxidant system. As a result, the increased activity of SOD, CAT and CP in blood serum against consumption of drinking water with concentration of nitrates from 150 to 500 mg/l within 30 days in the experimental groups of both age categories has been revealed. The most pronounced changes in the parameters were regarding drinking water with nitrates at a concentration of 500 mg/l. The changes were more pronounced in immature animals then in the mature ones.

Conflict of Interests

The authors declare no conflict of interest.

Acknowledgements

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Autho's Contributions

Maryana Danchyshyn – investigation, data curation, formal analysis, writing – original draft, writing – reviewing and editing; *Olena Lototska* – conceptualization, methodology, writing – reviewing and editing.

ОСОБЛИВОСТІ АНТИОКСИДАНТНОГО ЗАХИСТУ В ОРГАНІЗМІ БІЛИХ ЩУРІВ НА ТЛІ ВЖИВАННЯ ПИТНОЇ ВОДИ З ПОНАДНОРМАТИВНОЮ КОНЦЕНТРАЦІЄЮ НІТРАТІВ

М. В. Данчишин, О. В Лотоцька

ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО МОЗ УКРАЇНИ, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Проблема забруднення довкілля нітратами за своїми масштабами та наслідками вийшла на глобальний рівень. Приблизно в одній третині підземних водних об'єктів, щодо яких наразі доступна інформація, перевищено гранично допустимі концентрації вмісту нітратів у питній воді.*

Метою *цієї роботи стало вивчення особливості антиоксидантного захисту в організмі білих щурів на тлі вживання питної води з понаднормативною концентрацією нітратів впродовж 30 днів в залежності від віку.*

Матеріали. *Експеримент проводили на 2 групах безпородних білих самок-щурів різного віку (статевозрілих та статевонезрілих) по 30 особин у кожній, розділених на 5 підгруп, які відрізнялися за якістю питної води, яку вони споживали самостійно. У 30-денному дослідженні нітрати давали щурам у питній воді в дозі 50, 150, 250, 500 мг нітрату натрію на літр. Тварини контрольної групи пили воду з міського водопроводу. Оцінювали вплив нітратів на активність супероксиддисмутази (СОД), каталази та церулоплазміну (СР) в сироватці крові щурів.*

Результати. *У результаті досліджень виявлено підвищену активність СОД, каталази та СР у сироватці крові на тлі вживання питної води з концентрацією нітратів від 150 до 500 мг/л у піддослідних групах обох вікових категорій. Встановлено, що найбільш виражені зміни показників викликає вживання питної води з нітратами у концентрації 500 мг/л. Більш виражені зміни були у статево незрілих тварин.*

Висновки. *Присутність нітратів у питній воді у понаднормативних концентраціях негативно впливає на організм піддослідних тварин, викликаючи активацію досліджуваних показників антиоксидантної системи.*

КЛЮЧОВІ СЛОВА: питна вода; нітрати; супероксиддисмутаза; каталаза; церулоплазмін.

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