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LOW-MOLECULAR COMPONENTS OF COLOSTRUM PERFORM THE FUNCTION OF ANTIDOTE IN COPPER SULPHATE TOXICATION OF THE BODY

Ivanov Ievgen
PhD, Associate professor,
Department of Molecular Biology and Biotechnology
V. N. Karazin Kharkiv National University. Ukraine

Kozheshkurt Valentyn
PhD, Senior lecturer, Department of physical and biomedical electronics and integrated information technology
V. N. Karazin Kharkiv National University. Ukraine

Bondar Anastasia
Assistant lecturer, Department of Molecular Biology and Biotechnology
V. N. Karazin Kharkiv National University. Ukraine

Summary. *The important action of low-molecular-weight components of colostrum (LCC) as an antidote for intoxication of the body with metal ions was studied. We used the analysis of the dynamics of body weight and body temperature, the indicators of redox systems (the number of lipid hydroperoxides and the activity of glutathione peroxidase), as well as the number and morphotype of bone marrow cells. It was shown that the administration of LCC at a dose of 0.1 mg / 100 g of body weight against the background of intoxication was accompanied by the restoration of body weight and body temperature to the control value, and the indicators of the redox system shifted towards antioxidants, which also corresponds to the control. At the same time, the functional characteristics of the bone marrow remained practically unchanged.*
Key words: *bovine colostrum, low-molecular-weight enzymes, bone marrow cells, metal ions intoxication, copper sulfate.*

Introduction. Heavy metal ions are among the most common environmental pollutants [[1],[2]]. The danger and peculiarity of the effect of heavy metal ions on biological systems is that they accumulate in water, soil, microorganisms, plants and are transmitted through food chains. Since most of them are essential, biological systems are able to adapt to them and this can lead to their bioaccumulation and toxic effects. The accumulation of heavy metal ions in the body can also occur if the mechanisms of their elimination are disrupted, as in the case of copper ions, which leads to Wilson's disease [[3],[4]]. In this regard, a pressing biomedical problem is the development of biological antidotes. We believe that low molecular weight

components of cow's colostrum can be used as an antidote. This statement is based on the fact that the low-molecular-weight components of colostrum include a large set of biologically active compounds that take part in the systemic regulation of metabolism. In order to test this assumption, *Wistar* rats were intoxicated by three consecutive injections of copper sulfate at a dose of 1 mg / 100 g of body weight, followed by administration of low-molecular-weight components of colostrum per os at a dose of 0.1 mg / 100 g of body weight 24 hours after the last copper sulfate.

Methods. The experiment included three groups of animals, 5 males per group: the control group, which received saline instead of copper sulfate solution; the intoxication group, which received copper sulfate; and the experimental group, which received low molecular weight components of colostrum (LCC). Colostrum was obtained at the "Alfa" farm (Ukraine) from cows on the Ukrainian milky-pitted breed with a second milk yield. Defatting was carried out by centrifuging colostrum at 3000 *g* for 20 minutes at room temperature. After fat removal, all proteins with a molecular weight greater than 10 000 Da were removed from the defatted colostrum by membrane filtration. After this, the samples were dried on a rotary apparatus. Intoxication of experimental animals was carried out by three injections of copper sulfate with an interval of 48 hours, which was 5 days from the start of the experiment, at a dose of 1 mg / 100 g of body weight. The resulting samples of low molecular weight proteins were dissolved in physiological solution and administered to experimental animals *per os* at a dose of 0.1 mg / 100 g of the body weight. After 24 hours, the animals were taken into the experiment.

Blood serum was obtained, the content of lipid hydroperoxides was determined in it according to the method [[5]] and the activity of glutathione peroxidase according to the method [[6]]. Bone marrow was isolated from the rat femurs and total number and morphotypes of bone marrow cells were determined. The obtained results were statistically processed by Mann-Whitney method using the Statistica 8.0 software (StatSoft Inc., USA). Differences between the control and experimental groups were considered significant at $p<0.05$ comparing with a control variant.

Results. The ability of LCC to provide detoxification functions was evaluated by such integral indicators as changes in the weight and body temperature of experimental animals. It turned out that animals after intoxication with copper sulfate lost weight 2-3 days after the last injection of copper, they did not grow over the next 12 days and after this delay period they recovered quite quickly, and after 18-20 days from the start of the experiment they did not differ from the control (Fig. 1 a). During the period of growth loss and retardation, a decrease in body temperature by one degree was observed in these animals compared to the control group of animals (Fig. 1 b).

In the event that the animals received low-molecular-weight components of colostrum (LCC) per os, their body weight growth was also slightly slower than in the control, but was superior to the group receiving copper sulfate and they recovered faster than the group after intoxication (Fig. 1 a). At the same time, the body temperature of animals receiving LCC against the background of intoxication group did not differ from that of the control (Fig. 1 b).

Therefore, LCC are able to eliminate the negative inhibitory effect of copper sulfate on metabolism and can be considered as a potential antidote.

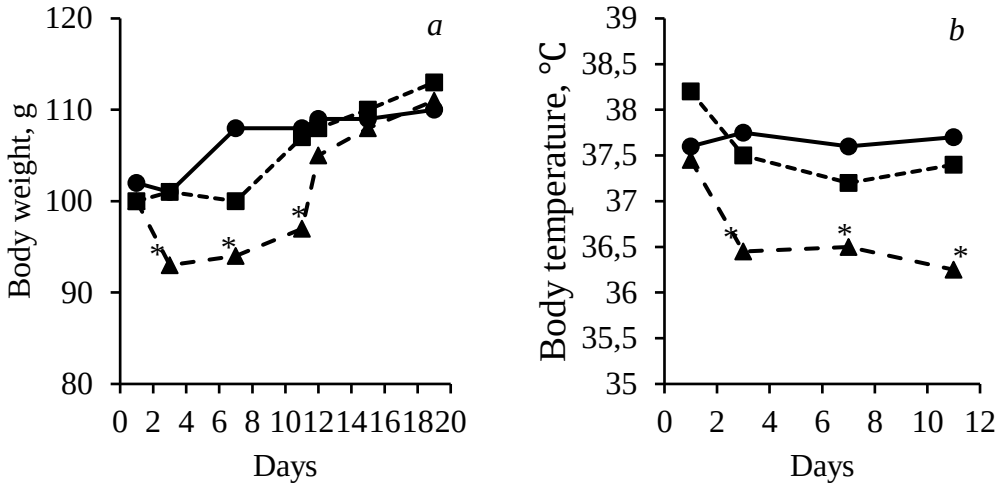

Fig. 1. Body weight (a) and body temperature (b) in the control group of animals (▲), in animals after intoxication with copper sulfate (■) and in animals with toxicosis and after receiving low-molecular components of colostrum at a dose of 0.1 mg / 100 g body weight (●). The average values from 5 experiments and standard errors are presented; those variants for which $P < 0.05$ are marked with an asterisk.

The antitoxic effect of LCC can be realized in various ways and on the basis of at least two strategies: increasing the rate of removing from the body copper ions and cytotoxic compounds formed in the body against the background of their action and/or activating resistance and adaptive mechanisms. In a series of experimental studies, it was shown that the body's response to the action of various toxic factors depends not only on the dose and duration of their action (temporal characteristics), but also on the functional characteristics of the body at the moment of action [[7],[8]]. In the next series of experiments, the effect of LCC on such basic indicators as indicators of the body's redox system and functional characteristics of the bone marrow was tested.

It is known that copper ions can exhibit a pro-oxidant effect, which is associated with an increase in the products of free radical reactions [[8]] and this mechanism can lead to subsequent toxic effects. It was found that 24 hours after the last injection of copper sulfate to the animals, the content of lipid hydroperoxides in the blood serum, the amount of which was evaluated by the resulting malonaldehyde (MDA), was increased by 75 % compared to the control (Fig. 2 a). This increase in the content of free radical reaction products occurred against the background of inhibition (by 36 %) of one of the "central" antioxidant enzymes – glutathione peroxidase (Fig. 2 b).

Consequently, intoxication of the body with copper sulfate was accompanied by a shift in the equilibrium in the redox system towards pro-oxidants.

If, against the background of intoxication, animals received LCC at a dose of 0.1 mg / 100 g of body weight, then the content of lipid hydroperoxides in the blood serum did not differ from the control. The activity of glutathione peroxidase in this case even exceeded that of intact control animals by 30 % (Fig. 2 b).

Consequently, the mechanisms for eliminating the toxic effect of copper ions by low-molecular-weight components of colostrum can be implemented through the regulation of the body's redox system.

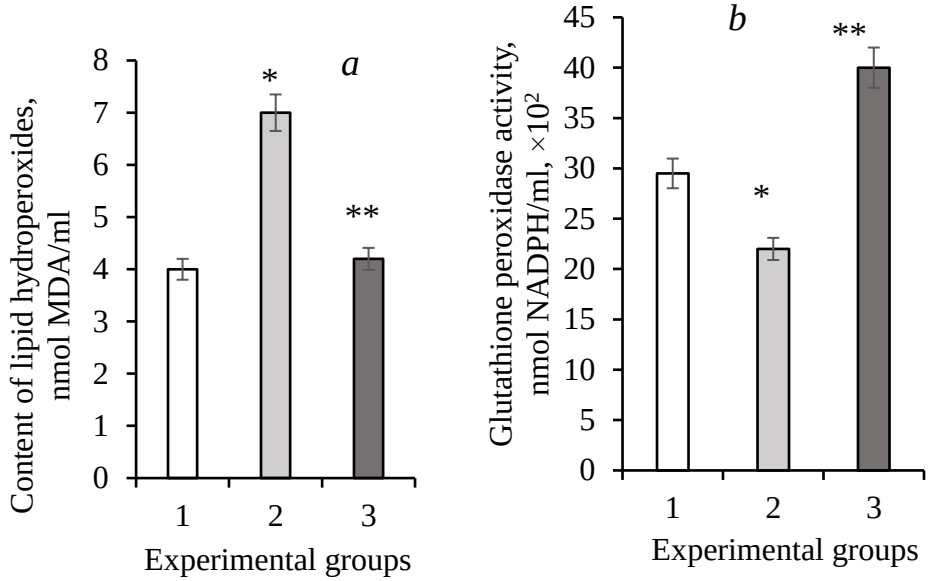

Fig. 2. MDA content in blood serum (a) and glutathione peroxidase activity in blood serum (b) in control animals (1), animals with toxicosis (2) and animals with toxicosis that were administered LCC at a dose of 0.1 mg / 100 g of body weight (3). The means of five animals in each group and their standard errors are presented. One asterisk indicates differences for which P < 0.05 compared to the control variant, and two asterisks – compared to the intoxication group.

Since LCC include a variety of biologically active compounds, they can have a systemic effect on all or most of the body's regulatory systems [[9]]. In this regard, the response of the immune system to the antitoxic effect of LCC is of greatest interest.

It was found that the administration of LCC to animals with Cu-induced liver fibrosis at a dose of 0.1 mg / 100 g of body weight slightly (40%) increased the number of cells in the bone marrow (14.3×10^6 cells / ml to 20.3×10^6 cells / ml), while the ratio between cell types (neutrophils, metamyelocytes, lymphocytes, eosinophils, basophils and myelocytes) remained at the level of control values. Consequently, LCC did not make pronounced changes in the functional characteristics of the bone marrow of animals after intoxication.

Medications can be developed based on LCC, that can eliminate the toxic effects of heavy metal ions and possibly other toxicants.

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