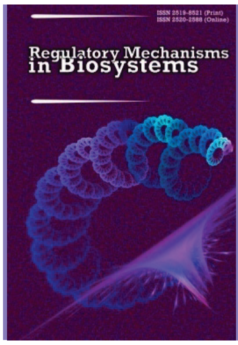

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Effect of liposomal drug based on interferon and extract from *Silybum marianum* on antioxidative status of bulls against the background of contamination of fodders by cadmium and plumbum

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The balance between anti- and prooxidants in animal organisms in general and in each cell in particular is responsible for the regulation of many metabolic processes that provide immunocompetence, growth, development and protection of animals from oxidative stress, related to inflow of cadmium and plumbum. Therefore, the objective of our study was the influence of a liposomal drug based on interferon and milk thistle (*Silybum marianum* (L.) Gaertn.) on the antioxidative status of the organism of bulls in the conditions of cadmium and plumbum loading. The experiments were performed on six-months-old Black Motley dairy cattle. The fodders in the farm were determined to contain high levels of plumbum and cadmium. The liposomal drug Lipointersyl inhibited the lipid peroxidation processes in the bulls. The drug components promoted the decrease in the level of intermediate and end products of lipid peroxidation, in particular 22% decrease in the level of diene conjugates and 20% decrease in TBA-active products. Intramuscular injection of the liposomal drug to bulls of the experimental group strengthened the antioxidant protection of their organism. On the 30th day of the experiment, blood from experimental group animals was seen to have a 9.8% increase in reduced glutathione. Assay of the enzymatic link of the glutathione system revealed that the activity of glutathione peroxidase and glutathione reductase in the blood of the animals that had been injected the liposomal drug Lipointensyl had increased by 24.0% and 27.7% respectively by the 30th day of the experiment. The experiments conducted on young cattle demonstrated that intermuscularly injected the Lipointensyl liposomal drug – against the background of cadmium and plumbum loading – promoted the activation of the glutathione system of antioxidant protection as a result of increase in the activity of its enzymatic and non-enzymatic links. The study of catalase and superoxide dismutase activities revealed that on the 30th and 40th days of the experiment, the activity of those enzymes varied within the physiological norms. Therefore, the analyzed *Silybum marianum*-based liposomal drug has antioxidant properties, it is recommended for young cattle in the conditions of contamination with heavy metals in order to prevent the development of oxidative stress.

Keywords: heavy metals; glutathione system, catalase; superoxide dismutase; diene conjugates; liposomes.

Introduction

One of the commonest types of environmental contamination is the influx of a large amount of heavy metals, which by the level of ecological threat are inferior only to pesticides. They are able to accumulate in the organisms of productive animals and could be classified to the leading environmental pollutants according to the scales of their spread and accumulation of their components in the environment (Vishchur et al., 2019; Hui et al., 2021; Razanova et al., 2022). Soil is a natural filter of technogenic contaminants, especially heavy metals that affect its biological properties (Hong et al., 2021; Wu et al., 2021). Heavy metals amass in soil at fast rates and break down slowly: breakdown time of Cd is 1,000 years, and decomposition of Pb can take up to several thousand years. In soil, heavy metals are present in water-soluble, ion-exchanging and poorly adsorbing forms. Heavy metals can be present in soil in the form of dissolved free ions, dissolved complexes with inorganic anions and organic ligands, in the compound of exchange complexes on clayey minerals and organic matter, which are held by electrostatic forces, in the form of organic-mineral heteropolar, complex-heteropolar salts, sorption complexes, etc. (Ferro et al., 2021; Zhang et al., 2021).

When the concentrations of heavy metals in soil increase, plants absorb them in larger amounts, which leads to accumulation of those contaminants in food chains (Kailasam & Peiter, 2021; Zheng et al., 2021). Heavy metals are toxic due to electric configuration, electronegativity, value of oxidative-reduced potential, ionization, affinity to some chemical groups, ability to permeate through the cellular membrane and form complexes on the surface of and inside the cell and also with the structure of a biological object (Bashchenko et al., 2020; Gul et al., 2021; Kar & Patra, 2021).

The problem of environmental pollution raises interest in studying heavy metals, particularly cadmium and plumbum as a stress factor, determining the mechanisms the organisms use to protect themselves against their toxic action (Ozturk et al., 2021; Rezapour et al., 2021). Therefore, studies by a number of scientists over the recent decades have been focusing on the effects of these pollutants on living organisms, in particular the organisms of mammals (Bomko et al., 2018; Slivinska et al., 2019). Accumulation of cadmium and plumbum in the components of the natural environment makes them more likely to enter the organisms of productive livestock and poses a threat to their health (Shiyntum & Ushakova, 2015; Akter et al., 2019; Skliarov et al., 2022). The results of many experimental studies indicate that in mammals, cadmium and plumbum have a toxic impact on a number of the organs and systems (Zeng et al., 2021; Zhao et al., 2021).

In the mechanisms of toxic actions of plumbum and cadmium, a significant role is played by the stimulation of the processes of formation of free radicals and active oxygen species, which further leads to imbalance between the concentration of oxidants and antioxidants (Tsekhmistrenko & Tsekhmistrenko, 2015). This imbalance results in oxidative stress in the organisms of intoxicated animals, including leukocytes in the blood, which are among the first to react to toxicant-caused changes in the internal environment of the organisms (Wang et al., 2021; Mylostyvyi et al., 2021; Kowalczyk et al., 2021).

To improve the wellbeing of mammals and people, plant-based drugs are often used. Other than direct action of plant drugs on the macroorganism of a vertebrate, it is possible to cause indirect effect by optimizing the composition of groups of microflora in the intestines of animals (Zazharskyi et al., 2019), or optimizing the interrelations with the microflora's parasitic organisms (Boyko & Brygadyrenko, 2019). It is generally known that the diet of herbivorous mammals is determined by their physiological specifics, health and productivity (Zazharska et al., 2018). Only a small number of plants are able to effective change the microflora (first of all species composition of bacteria and fungi) of the intestine of mammals (Zazharskyi et al., 2019, 2020). Some species of plants can strongly resist the toxic impact on both microorganisms and macroorganisms of mammals (Palchykov et al., 2019). For this reason, various forms of using such effective – as well as safe – plant-based drugs as extract from *Silybum marianum* (L.) Gaertn are becoming especially relevant.

Therefore, the objective of our study was to determine changes in antioxidant status of the organisms of young cattle in the conditions of heavy metal loading and action of the liposomal drug Lipointersyl, which contains *S. marianum* and interferon.

Materials and methods

All the procedures with the animals were performed according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Official Journal of the European Union L276/33, 2010). The experiments were carried out in the private farm Ukraina of Dubrovytsia district of Rivne Oblast, Ukraine, on 12 six-months-old Black Motley bulls. On this farm, a high content of plumbum and cadmium was determined in fodders. To perform the experiment, we formed two groups, each comprising 6 animals: the control and the experimental. Control group bulls received the standard diet. Experimental group bulls were intramuscularly injected with Lipointersyl, a liposomal drug, in the dose of 5 mL per animal. Lipointersyl contains interferon and extract from *S. marianum*. This drug was developed at the Department of Pharmacology and Toxicology of the Lviv Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies. Blood for the analysis was taken from the jugular vein at the beginning of the experiment, and also on days 10, 20, 30 and 40 after utilizing the liposomal drug.

The data were analyzed using Statistica 6.0 software (StatSoft Inc., USA). The data are presented in diagrams as x ± SD (x ± standard deviation). Differences between the values in the control and experimental groups were determined using ANOVA, where the differences were considered significant at $P < 0.05$ (taking into account Bonferroni correction).

Results

In the conditions of heavy metal loading, we determined a decrease in the antioxidant status of bulls of the control and experimental groups. The concentration of reduced glutathione in bulls of the control group fluctuated within 29.7–29.4 mg%. In animals of the experimental group, this parameter significantly increased starting already from the 10th day of the experiment (Fig. 1). In the indicated period, the concentration of reduced gluthathione increased by 10% compared with the control. Injections of bulls of the experimental group with Lipointersyl were accompanied by 32.2 ± 0.5 mg% increase in the concentration of reduced glutathione on the 30th day of the experiment, while this parameter in animals of the control group equaled 29.3 ± 0.5 mg% on average. On the 40th day of the experiment, the blood of bulls that had been receiving the liposomal drug was observed to have slight decrease in the content of reduced glutathione. However, it was significantly higher compared with animals of the control group.

Assay of the enzymatic link of the glutathione system revealed low activity of glutathione peroxidase and glutathione reductase in the blood of bulls of the control group. The activity of those enzymes was low in the blood of the control group throughout the experiment. We observed increased activity of the indicated enzymes in blood of bulls of the experimental group using the liposomal drug (Fig. 2). On the 20th day of the experiment, activity of glutathione peroxidase in the blood of animals of the experimental group increased by 13.9%, while the activity of glutathione reductase increased by 16.3% compared with the parameters of the control group of animals. We determined significant increases in the activity of glutathione peroxidase in the blood of bulls of the experimental group, equaling 24.0% on the 30th day and 21.5% on the 40th day of the experiment, compared with the control.

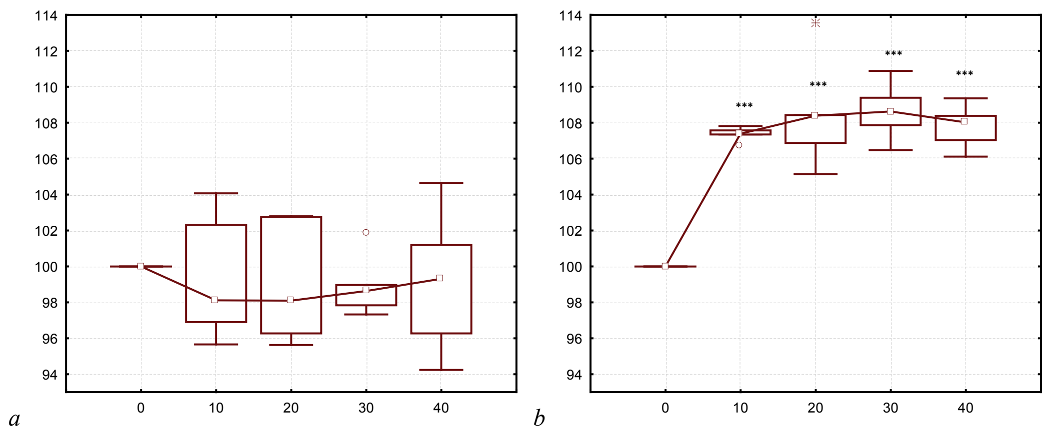


Fig. 1. Concentration of reduced glutathione (on ordinate axis, %) in blood of bulls of the control group (*a*) and bulls of the experimental group in the conditions of loading with cadmium and plumbum and action of the liposomal drug Lipointersyl (*b*): on the abscissa axis – day of the experiment, on the ordinate axis – change in the examined characteristic (%) compared with initial values of each experimental animal (taken for 100%) prior to the beginning of the experiment, small square – median, upper and lower borders of rectangle – 75% and 25% of quartile, vertical line – minimal and maximal values, circles – emissions; *** – significance of difference at $P < 0.001$ according to the results of ANOVA taking into account Bonferroni correction; n = 6

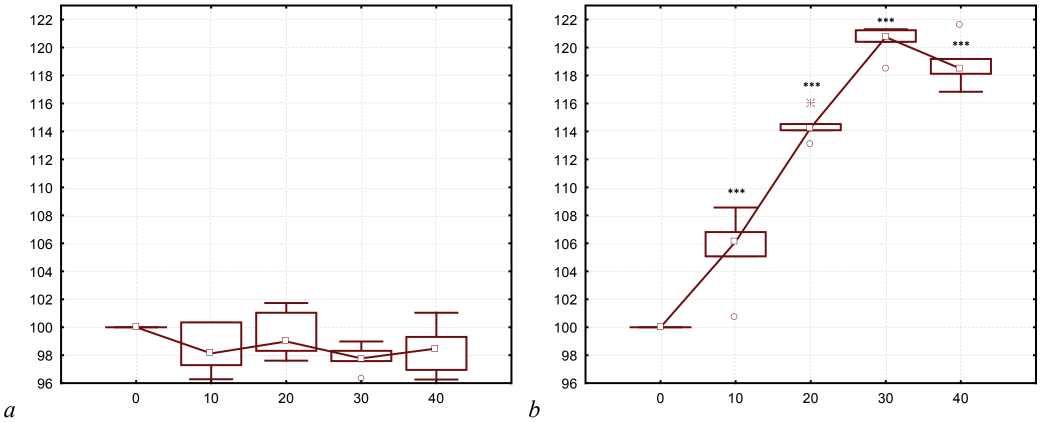


Fig. 2. Activity of glutathione peroxidase (on ordinate axis, %) in blood serum of bulls of the control group (*a*) and bulls of the experimental group in the conditions of cadmium and plumbum loading and action of the liposomal drug Lipointersyl (*b*): see Fig. 1

Similar changes were also seen during the assay of the activity of glutathione reductase (Fig. 3). On the 30th and 40th days of the experiment, the activity of the enzyme in blood of experimental group animals showed an increase by 27.7% and 27.3% respectively, compared with the control group of animals in the indicated periods of the study.

During the assay of the activity of catalase, we determined that in the conditions of plumbum-cadmium loading, the activity of this enzyme in the blood serum of bulls varied on average within 5.32–5.38 units. Lipointersyl promoted increase in catalase activity in the blood of the intoxicated animals (Fig. 4). On day 20 of the experiment, the catalase activity in blood of bulls of the experimental group showed an increase by 17.3%, compared with the control. The highest activity of catalase was in the blood serum of the experimental group on the 30th day of the experiment – 6.60 ± 0.17 conventional units, while in the control, it equaled 5.35 ± 0.12 conventional units. Activity of superoxide dismutase in blood of bulls of the control and experimental groups accounted for 0.49 ± 0.01 and 0.47 ± 0.01 conventional units/mg of protein at the beginning of the experiment, respectively (Fig. 5).

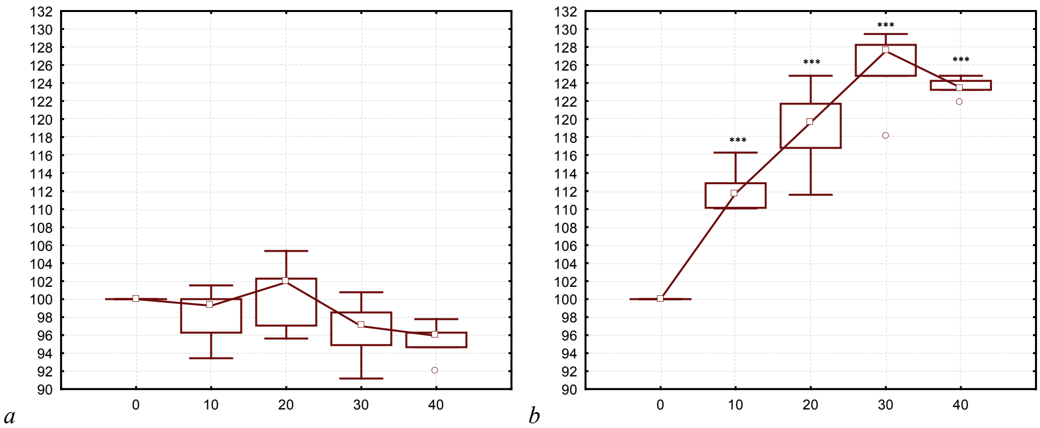


Fig. 3. Activity of glutathione reductase (on ordinate axis, %) in the blood serum of bulls of the control group (*a*) and bulls of the experimental group in the conditions of cadmium and plumbum loading and the liposomal drug Lipointersyl (*b*): see Fig. 1

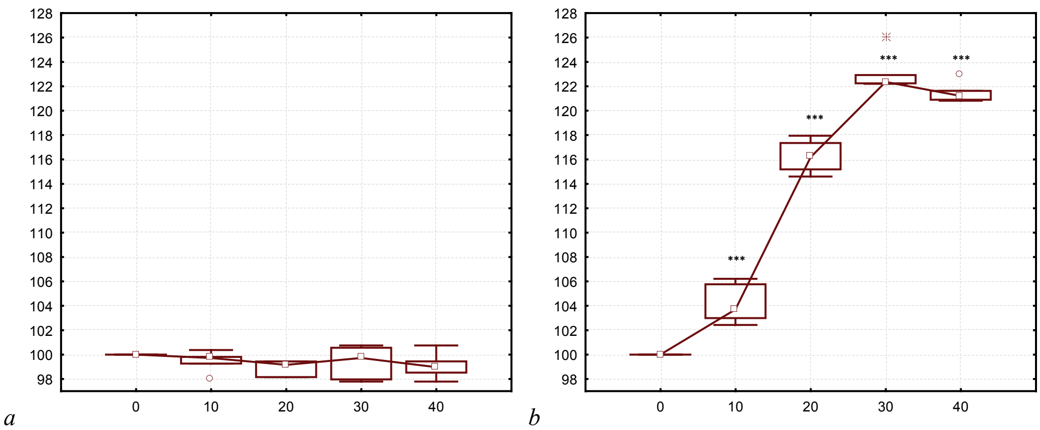
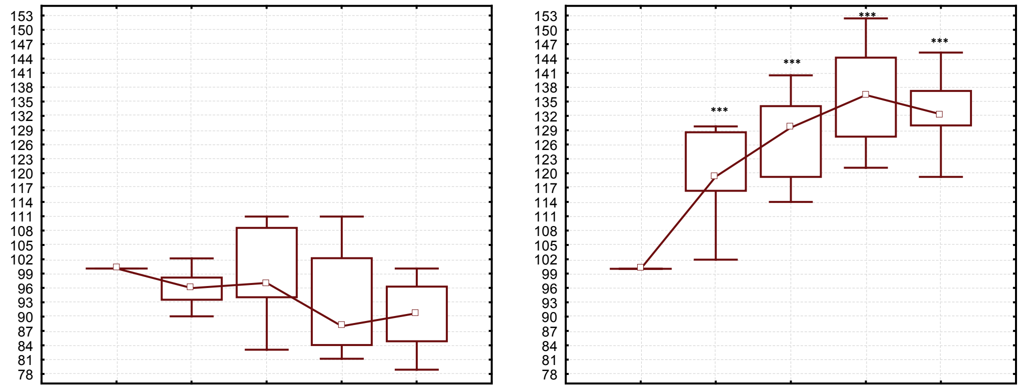


Fig. 4. Activity of catalase (on ordinate axis, %) in the blood serum of bulls of the control group (*a*) and bulls of the experimental group against the background of cadmium and plumbum loading and action of the liposomal drug Lipointersyl (*b*): see Fig. 1

During the use of the liposomal drug, we observed that the activity of superoxide dismutase in the blood of bulls of the experimental group had been increasing since the 10th day of the experiment. Significant increase in the activity of the enzyme in the blood of the experimental group of animals was seen on the 20th day of the experiment, where – compared with the control group – this parameter increased by 25%. The greatest activity of superoxide dismutase was in the blood of bulls of the experimental group on the 30th day of the experiment, equaling 0.64 ± 0.01 conventional units/mg of protein, which was 42.1% higher than the control values.

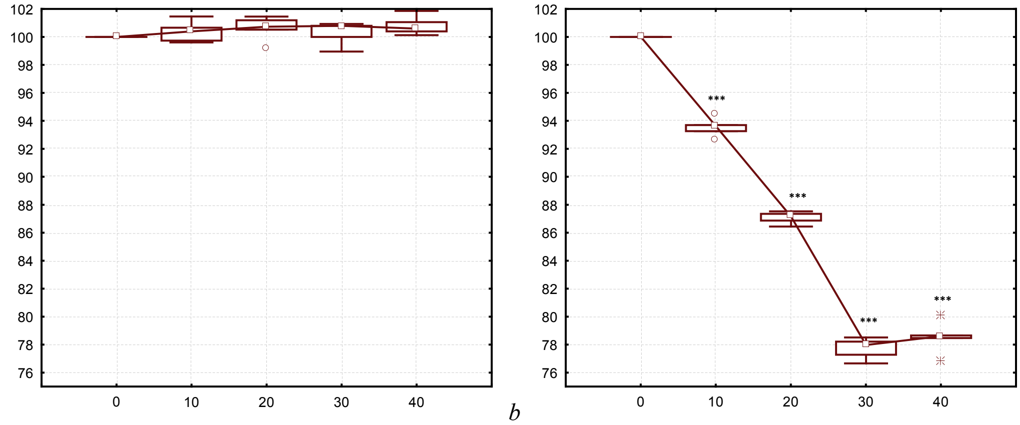
Concentration of diene conjugates in the blood of bulls of the control and experimental groups, as well as other characteristics of animals which we analyzed did not differ prior to the experiment (7.58 ± 0.14 and 7.60 ± 0.12 µmol/L). Further, the concentration of diene conjugates in the blood of bulls of the experimental group tended to decrease (Fig. 6). On the 10th and 20th days of the experiment, the concentrations of those substances in the blood of animals of the experimental group decreased by 6.6% and 13.0% respectively, compared with the control group. The lowest concentration of diene conjugates was in the blood of bulls of the experimental group (those injected with Lipointersyl) on days 30 and 40 of the experiment, when a decrease by 22.0% and 21.9%, respectively, was noted compared with the control.

Concentration of TBA-active products in the blood of bulls of the experimental and control groups at the beginning of the experiment accounted for 0.300 ± 0.006 µmol/L. We observed a decrease in the concentration of end products of lipid peroxidation in bulls that received Lipointersyl throughout the experiment (Fig. 7). The concentration of TBA-active products in the blood of bulls of the experimental group had decreased by 9.9% by day 10 of the experiment and by 14.2% by day 20, compared with the parameters of the control group. On the 30th day of the experiment, the concentration of end products of lipid peroxidation in the blood of bulls of the experimental group showed a decrease by 20.1%.



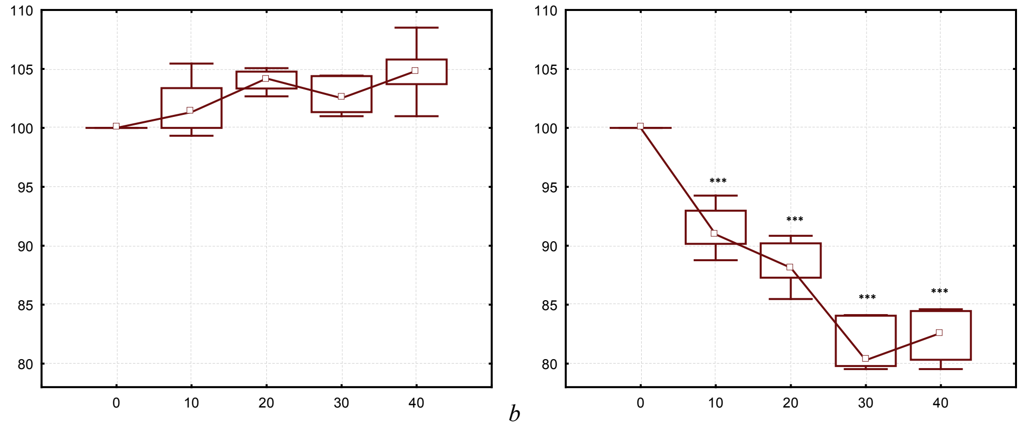
a *b*

Fig. 5. Activity of superoxide dismutase (on ordinate axis, %) in the blood serum of bulls of the control group (*a*) and bulls of the experimental group in the conditions of cadmium and plumbum loading and action of the liposomal drug Lipointersyl (*b*): see Fig. 1



a *b*

Fig. 6. Concentration of diene conjugates (on ordinate axis, %) in blood of bulls of the control group (*a*) and bulls of the experimental group in the conditions of cadmium and plumbum loading and action of the liposomal drug Lipointersyl (*b*): see Fig. 1



a *b*

Fig. 7. Concentration of TBA-active products (on ordinate axis, %) in blood of bulls of the control (*a*) and experimental group in the conditions of cadmium and plumbum loading and action of liposomal drug Lipointersyl (*b*): see Fig. 1

Therefore, injection of bulls with the liposomal drug Lipointersyl in the conditions of cadmium and plumbum loading led to decrease in intermediate and end products of lipid peroxidation.

Discussion

Prophylaxis of ecologically-caused intoxications by heavy metals, including cadmium and plumbum, is the main goal of preventive veterinary medicine. It includes carrying out a number of measures: primary prophylaxis of intoxication by cadmium and plumbum, which is first of all the adherence to the sanitary-hygienic norms (threshold limit concentrations and threshold limit levels) and performance of required measures of modernization of technological equipment, improvement of production processes (Gutyj et al., 2019; Nordberg & Nordberg, 2022).

Accumulation of heavy metals in the organisms of productive livestock may lead to significant impairments in cellular metabolism as a result of stimulation of processes of formation of free radicals that intensify lipid peroxidation, and also cause oxidative damage to proteins and nucleic acids (Dyachenko et al., 2015; Borisenko et al., 2019). In those conditions, an important role is played by the system of antioxidative protection, which includes enzymes (catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase) and non-enzymatic components (glutathione) (Bono et al., 2021). Supporting the balance between the intensity of prooxidant and antioxidant processes in the organism of agricultural animals is important for metabolic reactions and functional activity of cells in the presence of metals in the environment.

According to our assays of blood of bulls that have been held at the agricultural private farm Ukraine, we determined imbalance in the complex "system of antioxidant protection – processes of lipid peroxidation". The obtained results reveal that when fodders contain excessive threshold values, cadmium and plumbum activate the process of formation of active oxygen species and inhibit the functional activity of components of the glutathione system in the organisms of young cattle.

The staff of the Department of Pharmacology and Toxicology of the Stepan Gzhytskyi Lviv National University of Veterinary Medicine and Biotechnologies have developed the drug Metisevit, which contains selenium, methylphen and vitamins A and E. When testing this drug on young cattle in the conditions of cadmium loading, this drug promoted increase in the concentration of antioxidants of non-enzymatic link of antioxidative protection, particularly vitamins A and E throughout the experiment (Slobodian et al., 2019).

Lavryshyn et al. (2020) demonstrated efficacy of using liposomal drugs on young cattle against the background of cadmium loading (Lavryshyn et al., 2020). They determined efficacy of those drugs in the liposomal form. This medicinal form exerted a more expressed and longer effect than other forms (Gutyj et al., 2017). Liposomes play the role of so-called containers for the delivery of medicinal substances, preventing their losses during transportation (De Leo et al., 2022). Therefore, liposomes allow the drug to access the regions of the animal organism which other drug delivery carriers cannot enter (Shahid et al., 2022). For the correction of the parameters of immune system of young cattle suffering cadmium toxicosis, the author recommends using Metisevit food supplement in the dose of 0.36 g/kg of mixed feed for a month and intramuscular injection of Lipointersyl in the dose of 2 mL per animal.

Liposomes prevent the impairments of the system of antioxidative protection, since they are able to interact with liver cells and modify the membrane structure, increase their functional activity. Studies were also conducted on the ability of liposomes to detoxicate. Over a half of them enter the liver, 1/6 enter the spleen, where they become occupied by hepatocytes and macrophages (Khariv et al., 2017; Abbasi et al., 2022; Karpenko et al., 2022; Sameliuk et al., 2022).

Use of the liposomal drug Lipointersyl promoted activation of the system of antioxidative protection, which was indicated by increase in the activity of enzymatic and non-enzymatic links of the glutathione system in experimental animals, and also increase in activities of catalase and superoxide dismutase. This is likely related to the fact that the liposomal drug contains extract from *Silybum marianum* (L.) Gaertn., which contains valuable medicinal substances. Seeds of *Silybum marianum* (L.) Gaertn. contain around 200 constituents with various actions (Eita, 2022). It has large amounts of vitamins of group B, necessary for the regulation of fat metabolism, nutrition of the cardiac muscle, nervous system, skin, eyesight organs, and also fat-solving vitamins – A, F, E and K. Also, the seeds contain the following macroelements (mg/g): potassium – 9.2, calcium – 16.6, magnesium – 4.2, ferrum – 0.08; microelements (μg/g): cuprum – 1.16, manganese – 0.10, zinc – 0.71, chromium – 0.15, selenium – 22.9, iodine – 0.09, boron – 22.4 (Martyshuk et al., 2020, 2022; Dang et al., 2022). Fruits of *Silybum marianum* (L.) Gaertn. contain 17–18% of protein, 10–11% lipids, 30–40% fatty acids, 2–3% flavonoids, 1.6% carotenoids, 17–18% tocopherols, 2.0% reducing sugar and nonreducing sugars. The fruits contain essential oil, which accounts for 0.08%, oxyflavin, precursors for vitamin A, vitamins of groups B (B_1, B_5, B_{12}), D, F, E, K, resins, a small amount of saponins and traces of alkaloids, biogenic amines (tyramine, histamine), quercetin, dihydroflavonol, taxifolin, optically active dihydroconiferyl alcohol, organic acids, T factor (which increases the number of platelets in blood) (0.1%) (Shehzad et al., 2021).

Milk thistle accumulates essential biological elements, namely selenium and cuprum, which – together with vitamin E – stimulate the formation of antibodies and strengthen the immune system of the organism, and also activate the system of antioxidative protection (Yassin et al., 2021).

Especially important components in seeds of milk thistle are flavonolignan: silibin, isosilybin, silychristin, silydianin and maxifolin. Ions belong to flavonoids known as vitamin P. Those flavolignans are grouped under the common name silymarin. Pharmacolgists have experimentally confirmed that during the treatment of diseases, silymarin in the raw material of *S. marianum* was the agent that acted most efficiently. *Silymarin* was identified as 5-,7-,4-trihydroxy-3-methoxy-flavone-3-ol (3-methyltaxifolin) (Vaid & Katiyar, 2010; Tajmohammadi et al., 2018).

Extract from milk thistle displays choleretic effect and hepatoprotectory action. Extracts from fruits of milk thistle are a basic component for many drugs used to treat diseases of the bladder and liver. The experimental studies on laboratory animals revealed that liposomal milk thistle-containing drugs inhibit the formation of toxic products of peroxidation – lipid hydroperoxides, diene conjugates, TBA-active products and recover the reserve of liver lipid- and water-soluble antioxidants (Khazaei et al., 2022).

Conclusions

Intramuscular injection of the drug Lipointersyl at a 5 mL dose per animal promoted the increase in antioxidant status of the organism of young cattle against the background of cadmium and plumbum loading. This drug increased the activity of enzymatic and non-enzymatic links of the system of antioxidative protection in experimental animals. Also, we determined inhibition of the processes of lipid peroxidation in the blood of bulls of the experimental group, which was indicated by decrease in the levels of diene conjugates and TBA-active products in their blood.

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The authors declare that they have no conflict of interest.

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