5.3 Correcting efficiency of milk phospholipides under conditions of animal poisoning by cadmium

Today, due to the intensive development of industry, the environment has significantly increased the content of heavy metals. Among toxic metals - cadmium, mercury, arsenic, lead, included in the group of highly dangerous ecotoxicants. The wide distribution of cadmium in minerals, along with its use in industrial production, determines the gradual increase in the content of this element in the environment (air, soil, water). Anthropogenic cadmium load can induce a complex of factors that can cause disturbances at almost all levels of the ecosystem and directly in the human body, as its integral part. Heavy metals affect the body, which leads to the emergence of various pathological conditions, not only due to acute intoxication, but also in conditions of entry into the body at relatively low concentrations [549–551].

The toxic effect of cadmium is associated with its effect on the structural state of cell membranes. Biological membranes are that cellular structure, changes and damages of which cause disturbances in the structure and functioning of the cell as a whole. The effects that develop as a result of the action of cadmium can be either specific or non-specific (the absence of a strict dependence on the structure of the substance). Due to the nonspecific action of cadmium ions, the structural integrity of the membranes can be violated, which leads to deformation, lysis of the cell and its death. The selective effect of cadmium on membrane-bound enzymes, transport systems, and receptor complexes is mediated mainly through its interaction with protein SH-groups [551, 552].

Today, we have proved that the use of a liposome form of a phospholipid-containing dietary supplement “FLP-MD” in animals under conditions of toxic effects on the cadmium organism is characterized by high prophylactic and therapeutic effects, which is explained by the membrane-stabilizing properties of its components, stimulation of antioxidant processes in organs and tissues and prevention of metabolic disorders the status of their body [553–555].

Cytotoxicity of Cadmium and its manifestation. There are three main ways in which cadmium enters the body: through the gastrointestinal tract with food (feed) and water, through the lungs with air, and through the skin. Cadmium that enters through the lungs is more easily absorbed by the body – 10–30%. When entering through the gastrointestinal tract, the absorption rate is only 4–7% (0.2–5 μg Cd per day). The average daily rate of cadmium withdrawal from the body is insignificant, approximately 0.001% of its total content. Slow excretion of cadmium, mainly in urine, is explained by the absence of a specific biochemical mechanism of metal removal from the body associated with reabsorption processes in the proximal tubules, which ensures the formation of nephrotoxicity mechanisms without its additional supply [556–559].

When cadmium enters the body through the gastrointestinal tract, its absorption mainly occurs in the small intestine. The process of absorption of cadmium ions in the intestine is characterized by its rapid accumulation in the mucous membrane with subsequent slow entry into the circulation system. When cadmium enters the cavity of the small intestine, its digestive functions may be impaired. As a result of absorption, the metal is absorbed in the intestine, and then enters the liver with portal blood flow and is intensively absorbed by hepatocytes [559–562].

Cadmium metabolism in the body is characterized by the following features:

1) The lack of an effective mechanism for homeostatic control;

2) Retention (cumulation) in the body with a long half-life (average 25 years);

3) The predominant accumulation in the kidneys and liver;

4) intense interaction with other divalent metals both in the process of absorption and accumulation.

It was found that cadmium combines with negatively charged groups of membranes and thus modifies the charge of their surface. At the same time, a significant increase in the microviscosity of the lipid phase of the membrane is recorded without changing its polarity. This situation is associated with the fact that the metal "cross-links" the negatively charged groups of phospholipids located nearby and, thus, limits their mobilit [562–568].

Due to the presence of UFA, phospholipids of cell membranes are most susceptible to the oxidation reaction initiated by free radicals that form in the cell. Activation of the process of formation of free radicals leads to an increase in the intensity of production of highly reactive secondary radicals, easily diffuse over considerable distances, and activation of lipid peroxidation [551, 559].

An important mechanism of damage to biological membranes is the hydrolysis of phospholipids due to activation of phospholipases (especially phospholipases A2). The result of the action of phospholipase A2 on lipids of biological membranes is the release of arachidonic acid. The latter, in turn, is a substrate of cyclooxygenase. The conversion of arachidonic acid with the participation of cyclooxygenase leads to the formation of eicosanoids (prostaglandins, thromboxane, prostacyclins) - substances that activate the development of inflammatory processes in tissues. Under the influence of another enzyme (5-lipoxygenase), arachidonic acid is converted into leukotrienes and eicosatetraenoic acids. They are neutrophil chemoattractants - substances that regulate vascular permeability [552, 556].

Biological effects of heavy metals on membranes:

1) As a result of the growth of membrane permeability, the rate of entry of ions and substrates into the cell and the exit of metabolic products from it changes. This leads to metabolic disorders in the cell, electrical properties of the membranes;

2) The structural organization and functional activity of the cell are disrupted, cell death is possible;

3) The formation of a number of active substances that are involved in the pathogenesis of the toxic process.

One of the main mechanisms by which most heavy metals realize their toxic effect is the activation of free radical oxidation, which is accompanied by damage to macromolecules and supramolecular complexes, including biological membranes. A variety of biologically important molecules can be drawn into the processes of free radical oxygen transformations. The formation of oxygen reduction products in living systems creates the possibility of their interaction between themselves and other

molecules or ions, the appearance of toxic products and the launch of pathological processes such as LPO [556].

Lipoperoxidation processes play an important role in the mechanisms of toxic action of cadmium ions [549, 550]. This element, unlike other heavy metals, does not directly generate free radicals in cells. However, numerous data indicate the generation of superoxide radical, hydroxyl radical and NO - radicals in the cells as a result of the indirect action of cadmium. The generation of cadmium hydrogen peroxide is shown, which becomes a significant source of free radicals as a result of the Fenton reaction. Cadmium can replace ferum and copper in a number of proteins like ferritin, which, in turn, leads to an increase in the concentration of Fe^{2+} and Cu^{2+}.

The study of the prooxidant effect of cadmium on liver cells showed that the mechanism of induction of lipid peroxidation by metal is associated with the displacement of ions that initiate the oxidation process from cellular structures [559]. In addition, it was found that animal intoxication with cadmium promotes the production of oxygen radicals in the mitochondria and microsomes of hepatocytes, and also leads to a decrease in the content of reduced glutathione (RGL) in the liver. The obtained similar results also indicate that under the influence of cadmium, oxygen radicals are produced in the cells. The introduction of cadmium chloride to animals leads to a significant activation of lipid peroxidation in the cerebral cortex and myocardium, however, there is no change in the activity of antioxidant enzymes in these organs.

Studies of the activity of a number of antioxidant protection enzymes (AO) - protection (catalase (Cat), superoxide dismutase (Cu, Zn-SOD, Mn-SOD) of the liver and kidneys after chronic intake of cadmium with drinking water indicate their sensitivity to the action of this element. In vitro experiments have shown that a decrease in the content of TBA-active products as a result of vitamin E supplementation does not restore the activity of AO-defense system enzymes under the action of cadmium. It is believed that cadmium binds to the imidazole group His-74 protein. This is caused by the disruption of the decomposition of hydrogen peroxide. Cadmium acts as an

inhibitor of the activity of the Mn-dependent SOD of the mitochondria of the liver, probably due to the replacement of Mn (II) by cadmium ions [551].

It was shown that acute intoxication of animals with cadmium leads to an increase in the activity of enzymes of the AO-defense system: Cu, Zn-SOD, Cat, glutathione peroxidase (GP), glutathione reductase (GR) and glutathione transferase (GT). In particular, the influx of metal into the animal organism causes a significant increase in the LPO intensity (the intracellular level of TBA-active products increases) and inhibition of the activity of antioxidant enzymes (SOD, Cat, GP, GR). Activation of free radical oxidation due to the action of heavy metals is associated with the depletion of the reserve of natural antioxidants (ascorbic acid and tocopherol) in cells along with a change in the activity of antioxidant enzymes (Cat and SOD) [551].

The administration of large doses of cadmium or arsenic salts to animals leads to overproduction of superoxide radicals and the accumulation of metabolites of the oxidative reaction. A previous incubation of cells with methionine and cysteine, protects them from oxidative stress caused by the action of mercury, cadmium, and copper, which indicates the leading role of inactivation of SH-groups of proteins in the induction of free radical oxidation by metals [552, 556].

It is known that active radicals can cause oxidative modification of not only lipids, but also proteins. Tryptophan, tyrosine, histidine and cysteine are actively oxidized. In addition, the hydroxyl radical (·OH), as a rule, causes aggregation of proteins, and in combination with O_2 and O_2 – fragmentation. Fragmentation of proteins can also cause lipid radicals. Attack of ·OH aromatic and sulfur-containing amino acid residues is accompanied by their irreversible changes. Oxidative modification of enzymes of the AO-defense system leads to changes in their activity: O_2·inhibits the activity of Cat and GP; H_2O_2 causes inactivation of SOD and cytochrome P-450. Thus, free aggressive radicals affect various target structures of cells: membrane lipids, free amino acids, polysaccharides, receptor molecular complexes, transport proteins, etc. [559, 562].

The result of this is a change in the functional condition of the cell, a mutation of the genetic code, at the macroorganism level, leads to mutagenesis and neoplasms in the remote periods after the action of the toxicant, necrosis, cell lysis and accelerated

apoptosis. That is why drugs with membrane-active properties acquire significant relevance in cadmium intoxication of animals, stimulate the development of reparative processes in damaged cell structures [552, 556].

Again, the liposomal form of dietary supplement “FLP-MD” contains a mixture of various classes of phospholipids isolated from milk (butterdish), which have a natural fatty acid spectrum for animals, a mixture of unsaturated fatty acids (oleic, linoleic, linolenic) and antioxidants (vitamins A and E) [558–562].

For clinical trials, outbred male rats, weighing 180–200 g, contained in a standard vivarium diet were used. Animals were divided into 4 groups of ten animals each. The duration of the experiment was 20 days. Group I included control animals (intact); Group II – healthy animals were orally administered dietary supplement “FLP-MD” (13.5 mg/kg body weight) for 14 days; Group III – for animals, cadmium chloride was administered orally at a dose of 1.0 mg per 1 kg of body weight for 14 days (once a day), corresponds to 1/50 LD50 (single dose); Group IV – animals were given dietary supplement " FLP-MD ”(13.5 mg/kg body weight) for 6 to and 14 days after daily administration of cadmium chloride at a dose of 1.0 mg / kg body weight.

During slaughter, by decapitation, in laboratory animals, for the study of biochemical parameters, samples of the liver and small intestine were taken to obtain homogeneous preparations, as well as blood, from which serum was further obtained.

Corrective properties of liposomes based on milk phospholipids for cadmium poisoning of animals. Cadmium, which is recognized as one of the significant pollutants of the biosphere, upon entry into the body of animals causes a number of toxic effects, affecting various organs and systems. The main target organs for cadmium intoxication are the gastrointestinal tract, liver and kidneys. It is known that one of the main mechanisms by which most heavy metals realize their toxic effect is the activation of free radical oxidation, accompanied by damage to all major classes of biological macromolecules and supramolecular complexes, including biological membranes. There is evidence that disruption of lipid peroxidation processes also plays an important role in the mechanism of toxic effects of cadmium ions on the body. It is

believed that cadmium causes a significant increase in lipid peroxidation and a decrease in the activity of antioxidant enzymes: GP, SOD, Cat [549–552, 555].

The determination of the content of lipid peroxidation products for the action of harmful factors of various origin is highly informative, determines its use in order to diagnose the effectiveness of treatment and prevention of diseases of various etiologies.

Constant monitoring of the content of reactive oxygen species, free radicals, etc. clearly regulates the reaction of lipid peroxidation and is aimed at maintaining homeostasis in the body. Failure of such a control, which may occur as a result of exogenous or endogenous exposure, leads to intensification of lipid peroxidation, an increase in the concentration and accumulation in the body of lipid peroxidation products, which are characterized by a high reactivity with respect to systemic damaging effects on cells. Indicators that determine the level of lipid peroxidation and the state of the antioxidant system that characterize the functioning of the body's protective and adaptive mechanisms, and therefore, can be sensitive tests for the effects of various exogenous factors.

So, under the conditions of administration of cadmium chloride to blood rats, a significant increase in the concentration of creatinine (on average by 21%) and glucose (by 30%) was established. It is likely that the regular intake of cadmium chloride in the body of animals within 14 days causes metabolic disturbances in the renal parenchyma, glomerular filtration and tubular reabsorption. In addition, under the conditions of the introduction of cadmium into the body, a pancreatic function may be impaired, which leads to a weakening of the glycogen-forming and glycogen-fixing functions of the liver and is manifested by an increase in the concentration of glucose in the blood. At the same time, this condition is accompanied by a characteristic increase in enzyme activity: AST - by an average of 22% and GGT – by 40%, which indicates the hepatotoxic effect of cadmium. Oral administration to animals of the liposomal form of dietary supplement “FLP-MD”, against the background of cadmium intoxication, leads to partial normalization of GGT activity and serum glucose concentration, and AST activity and creatinine content do not differ from control values. It should be noted

that the introduction of the liposomal form of dietary supplement “FLP-MD” to control animals does not lead to changes in the biochemical parameters of blood serum.

The activation of LPO intensity was evaluated by the accumulation of the final product, TAC-active products. It was found that the introduction of cadmium chloride into the animal organism leads to the accumulation of TBA-active products in the epithelial cells of the small intestine (on average by 300%), in liver cells (by 50%) and blood serum (by 36%). Under the conditions of the introduction of the liposomal form of dietary supplement “FLP-MD”, along with the intake of cadmium chloride, the level of accumulation of TBA-active products remains increased (by 203% compared to control values) only in blood serum.

The results of determining the activity of SOD and Cat indicate multidirectional changesin their activity under experimental conditions.

Under the conditions of cadmium administration, the activity of SOD in liver cells decreases, on average, by 50%, and in epithelial cells of the small intestine – by 37%. At the same time, the activity of Cat in the studied organs under these conditions does not change. However, in the blood serum, Cat activity decreases – by 77%, while the activity of SOD does not change. The introduction of the liposomal form of dietary supplement “FLP-MD”, along with the intake of cadmium chloride, indicates the normalization of SOD activity indicators in the studied objects. At the same time, Cat activity in liver and blood serum preparations even exceeds the control values by 39 and 25%, respectively. Since Cat is involved in oxidative phosphorylation and oxygen transfer to intracellular structures, an increase in the activity of this enzyme may indicate the mobilization of the body's defenses.

The content of RGL under the conditions of cadmium administration decreases, on average, by 26%, 12 and 18% in preparations of the liver, mucous membrane of the small intestine and blood. Under these conditions, the activity of the studied enzymes – HT and GP – decreases. The greatest changes are observed for blood, respectively, at 61 and 37%.

Under the conditions of the introduction of cadmium chloride against the background of the liposomal form of dietary supplement "FLP-MD", less pronounced

changes in the studied parameters are observed. Although the content of RGL in blood serum and liver remains reduced, respectively, by 18 and 27% relative to the control, as in the conditions of cadmium administration, the activity of the enzymes HT and GP decreases to a lesser extent. Thus, the activity of HT and GP changes in blood serum by 43 and 19%, respectively, and in the liver by 9 and 17% relative to control values.

The obtained results indicate the intensification of lipid peroxidation against the background of a decrease in the potential of enzymatic and non-enzymatic AO-defense units in the blood, liver and mucous membrane of the small intestine under the conditions of 14-day intake of cadmium chloride in animals at a dose of 1.0 mg/kg body weight. The accumulation of the final product of LPO – TBA-active products is observed in all the studied objects. Oral administration of cadmium chloride to rats leads to the activation of lipid peroxidation in the cells of internal organs. Intoxication of animals with cadmium chloride causes an increase in the content of oxygen radicals in cells, including mitochondria and hepatocyte microsomes. It is known that cadmium ions have a pro-oxidant effect: active radicals can cause oxidative modification of not only lipids, but also proteins and nucleic acids.

When LPO is activated, the state of the AO-system of the body becomes important. The activity of Cat and SOD, which are involved in the neutralization of active oxygen compounds, varies in different ways in the internal organs when cadmium is introduced into the body of rats. SOD activity, especially in the liver, decreases, which can be explained by damage to the enzyme molecule or an increase in the concentration of hydrogen peroxide as its inhibitor. Confirmation is the results that indicate that in the liver and small intestine, according to the studied conditions, the activity of Cat almost does not change, and the activity of the GP, enzyme, also participates in the neutralization of hydrogen peroxide, decreases. The glutathione peroxidase system is one of the universal systems for the decomposition and neutralization of peroxides, which prevents the initiation of secondary lipid oxidation reactions and is involved in the inactivation of xenobiotic oxidative metabolism products.

Glutathione transferase plays a leading role in the biotransformation and detoxification of xenobiotics of various origins by catalysis of their conjugation reactions with restored GL [555, 569–570]. A decrease in the activity of this enzyme, along with a decrease in the content of RGL, as well as a decrease in the activity of GP, Cat, and SOD, testifies to the inhibition of the functioning of the OA-defense systems of the body under the conditions of cadmium chloride administration.

Simultaneously with cadmium, the liposomal form of dietary supplements “FLP-MD” enters the body of animals, leading to an improvement in the state of pro-antioxidant balance. A less pronounced accumulation of TBA-active products in the studied biological objects is observed. The activity of SOD almost does not differ from the control values, and the activity of Cat even exceeds their value, which may indicate the mobilization of the body's defenses.

Thus, the obtained results allow us to recommend the specified liposomal form of dietary supplement “FLP-MD” as a means of drug protection of the animal’s body during cadmium poisoning.

So, the cytotoxic effects of heavy metals, including cadmium, are based on various mechanisms, which include the following: impaired energy metabolism and intracellular calcium homeostasis activation of free radical processes in cells; damage to cell membranes. The use of membrane stabilizing drugs can be used in the complex treatment of cadmium intoxication.

The study of the correcting properties of milk phospholipids in the form of liposomal dietary supplement "FLP-MD" was carried out in vivo experiments under the action of cadmium chloride.

Studies of the biochemical parameters of blood serum, epithelial cells of the liver and small intestine of rats indicate a damaging effect (the presence of destructive processes in the cells) of cadmium on the cellular structures of the animal organism under experimental conditions. Preventive administration to animals of the liposomal form of dietary supplement “FLP-MD” based on milk phospholipids is effective because it leads to less pronounced changes in the values of the studied biochemical parameters of blood serum, liver and small intestine relative to control values.

According to the toxic effect of cadmium on the body, the main factor loads are introduced by indicators characterizing: the functioning of the glutathione system in the tissues of the liver, small intestine and blood serum, the functioning of the body systems (indicators of blood serum), as well as the processes of pro-antioxidant balance of the body. The state of the object of research under the conditions of the action of cadmium for using the liposomal form of dietary supplement "FLP-MD" is approaching the control group. That is, the use of poisoned animals liposomal form of dietary supplement "FLP-MD" exhibits a pronounced corrective effect. First of all, this is due to the composition of the dietary supplement “FLP-MD”, since its main component is phospholipids, which, on the one hand, undergo oxidation due to the action of oxygen radicals and, on the other hand, stabilize cell membranes. The liposomal form of dietary supplement “FLP-MD” also includes vitamins with membrane-active and antioxidant properties.

Thus, simultaneously with the introduction into the body of rats of cadmium chloride for fourteen days, the use of the liposomal form of dietary supplement "FLP-MD" with the original combination of lipids, mainly phospholipids, which are fatty acid in their composition, obtained from natural and cheap raw materials (oilers) correspond to membrane lipids of animal cells, a mixture of mono- and polyunsaturated fatty acids obtained from linseed oil, and fat-soluble vitamins – α-tocopherol and retinol acetate, stimulates the development of e reparative processes in damaged tissues and restores the metabolic status of the organism of animals exposed to cadmium ions, and also has a stabilizing effect on the pro-antioxidant balance.