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ORIGINAL ARTICLE

The effect of sylimevit, metifen, and milk thistle on the intensity of the processes of peroxidation of lipids in the body of laying hens in experimental chronic cadmium toxicosis

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Cadmium is considered a dangerous pollutant because, due to toxic stress, it causes various disorders of the functional state of the body of animals and birds. Getting into the body in small quantities, the above element accumulates for a long time in various organs and tissues, which can cause toxicosis, accompanied by disorders of biochemical processes, structure, and function of cells. The study aimed to study the effect of sylimevit, metifen, and milk thistle on the intensity of lipid peroxidation of laying hens in experimental chronic cadmium toxicosis. Thirty-two laying hens, 78 weeks old, were selected for the study. Four experimental groups were formed: control and three experimental. Chickens of the control group (C) and three experimental groups were fed with water cadmium sulfate at a dose of 4 mg/kg body weight. Chickens of the experimental group, E1 with feed, were fed the fruits of milk thistle at a dose of 2.0 g/kg of feed daily for 30 days. Chickens of the experimental group E_2 were fed metifen at a dose of 0.28 g/kg of feed once a day for 30 days. Chickens of the experimental group E_3 were fed sylimevit at a dose of 0.36 g/kg of feed once a day for 30 days. Watering chickens with cadmium sulfate water in a toxic dose contributed to the development of oxidative stress. Under conditions of intoxication of laying hens with cadmium sulfate at a dose of 4 mg/kg body weight, the most pronounced changes in the intensity of lipid peroxidation (lipid hydroperoxides increased by 40.2%, diene conjugates by 32.9%, TBA-active products by 29.3%). The use of cadmium-loaded feed to experimental chickens helped suppress the intensity of lipid peroxidation processes and reduce the formation of a large number of free radicals and reactive oxygen species, which could further lead to oxidative stress. Additional introduction to the diet of laying hens of milk thistle, metifen, and sylimevit had an inhibitory effect on the intensity of lipid peroxidation in chickens. Thus, the concentrations of primary, secondary, and final products of lipid peroxidation in the blood of laying hens of the experimental groups at all stages of the study were lower ($P<0.05–0.001$) than in control.

Keywords: Toxicology, cadmium, poultry, blood, sylimevit, metifen, milk thistle, lipid peroxidation.

Introduction

Recently, due to the increase in anthropogenic load, the environment is polluted with heavy metal ions, one of which is cadmium and its compounds. Cadmium is an element of group II of the periodic table. This element does not belong to the physiologically necessary trace elements and belongs to the first class of danger (Ali et al., 1986; Liu et al., 2008; El-Shahat et al., 2009; Al-Azemi et al., 2010). The widespread use 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) (Fregoneze et al., 1997; Gutyj et al., 2016; 2017; 2019; Vishchur et al., 2019; Lieshchova et al., 2020; Butsiak et al., 2021; Brezvyn et al., 2021; Saranchuk et al., 2021).

Sources of cadmium released into the environment are the use of phosphorus fertilizers, limestone materials, vehicle emissions (rubber of car tires and lubricants containing cadmium), as well as sediments of industrial and domestic wastewater. (Khariv et al., 2017; Lavryshyn & Gutyj, 2019). Approximately 52% of cadmium enters the atmosphere due to the combustion or processing of products containing it. About 80% of anthropogenic emissions of cadmium are related to the production of lead, zinc, copper, and cadmium. The smelting of cadmium from ores accounts for approximately 45% of the total pollution of this element. Up to 1,000 tons of cadmium are emitted into the air annually all over the world from the flue gases of power plants and industrial boilers, and 0.3 thousand tons/year during the incineration of municipal waste and wood (Lavryshyn et al., 2019; Kolombar et al., 2020; Martyshuk et al., 2020; Prychepa et al., 2021).

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Watering chickens with cadmium sulfate water in various doses contributed to the development of oxidative stress. With the development of this stress, there are significant changes in the metabolism of proteins, fats, carbohydrates, and nucleic acids. Reactive forms of oxygen, which are formed during oxidative stress, damage all biological structures (Lu et al., 2005). Active forms of oxygen are involved in the metabolic processes of the poultry body, associated with the metabolism of proteins, lipids, nucleic acids, and the synthesis of leukotrienes, prostaglandins, thromboxanes. Products formed due to the activation of lipid peroxidation processes under cadmium loading are oxidatively degraded and have cytotoxic and mutagenic effects, which leads to disruption of cell metabolism, activation of cytosolic and membrane enzymes and subsequent cell death. Active forms of oxygen are involved in the metabolic processes of the poultry body, associated with the metabolism of proteins, lipids, nucleic acids, and the synthesis of leukotrienes, prostaglandins, thromboxanes. Products formed due to the activation of lipid peroxidation processes under cadmium loading are oxidatively degraded and have cytotoxic and mutagenic effects, which leads to disruption of cell metabolism, activation of cytosolic and membrane enzymes and subsequent cell death (Ostapyuk & Gutyj, 2020).

The obtained experimental data of recent years indicate that in the pathogenesis of cadmium toxicosis are the mechanisms of activation of oxidative processes due to the intensification of the formation of free radicals (Salvatori et al., 2004; Uetani et al., 2005; Sobolev et al., 2017; 2020; 2021). One of the determining biochemical mechanisms of action of cadmium on the body of animals and birds is the imbalance between the intensity of oxidation of the structural components of cell membranes-lipids and proteins and their antioxidant regulation (Slobodian et al., 2019; Slivinska et al., 2020; 2021).

The speed and intensity of the development of lipid peroxidation processes and the formation of free radicals in animals and poultry are maintained at a certain level by a complex multilevel system of regulation-the antioxidant system. Changes in the antioxidant defense system of animals are an early nonspecific reaction of the body in response to the adverse effects of cadmium. Regulatory mechanisms balance the processes of oxidative damage to cells and tissues and restore their structure (Nazaruk et al., 2021; Fedotov et al., 2021; Vasylyev et al., 2021).

The work aimed to study the effect of silymevit, metifen, and milk thistle on the intensity of lipid peroxidation of laying hens in experimental chronic cadmium toxicosis.

Materials and Methods

Thirty-two laying hens, 78 weeks old, were selected for the study. Four experimental groups were formed: control and three experimental. Chickens of the control group (C) and three experimental groups were fed with water cadmium sulfate at a dose of 4 mg/kg body weight. Chickens of the experimental group, E1 with feed, were fed the fruits of milk thistle at a dose of 2.0 g/kg of feed once a day for 30 days. Chickens of the experimental group E_2 were fed with metifen at a dose of 0.28 g/kg of feed once a day for 30 days. Chickens of the experimental group E_3 were fed sylimevit at a dose of 0.36 g/kg of feed once a day for 30 days.

The conditions of keeping chickens and the microclimate parameters in the room for all groups were similar. The amount of feed and water consumed was taken into account during the experiment. Blood from laying hens was taken from the axillary vein in the periods: before administering drugs and cadmium sulfate on the first, seventh, fourteenth, twenty-first, and thirtieth days of the experiment. The level of TBA-active products was determined by the method of E.N. Korobeynikov (1989), the level of diene conjugates-by I.D. Stalnoya (1977); concentration of lipid hydroperoxides–GPL (Vlizlo, 2012).

The following drugs were used in the experiments:

Cadmium sulfate–*Cadmium sulfate*, an inorganic compound with the chemical formula CdSO4. Cadmium sulfate is well soluble in water, so it is well absorbed in the digestive tract into the blood and is known for its toxic effects on living organisms.

Milk thistle–*Silybum marianum,* family of Compositae, grows wild in wastelands, along roads, abandoned fields, and cultivated in medicinal gardens. The fruits of milk thistle are used for treatment. They contain 17–18% protein, 10–11% fat, 2–3% flavolignans, 0.08% essential oil, vitamins A, E, K, nutrients, quartcetin.

Metifen–*Metirhenum*; white crystalline powder, sweet to taste, with the smell of sulfur. Poorly soluble in cold water, better-in hot water (1:20). Thermostable. The drug contains phenarone and methionine.

Sylimevit-a feed additive, which includes the fruits of milk thistle, selenium, metifen, vitamins A, E, and C. We determine the doses of drugs for experimental studies in accordance with the literature and specifications. The analysis of research results was performed using the software package Statistica 6.0. The probability of differences was assessed by Student's t-test. The results of the mean values were considered statistically significant at *-P<0.05, **-P <0.01, ***-P<0.001 (ANOVA).

Results and Discussion

It is known that the intermediate stage of oxidation of polyunsaturated fatty acids to lipids by peroxide is the formation of lipid hydroperoxides, which are primarily associated with the destructive effects of LPO products in the cell. It was found that in the blood of laying hens under cadmium load, the level of lipid hydroperoxides probably increased from the 7th day of the experiment. In the blood of the control group of chickens, which were given cadmium sulfate at a dose of 4 mg/kg body weight, the level of primary products of LPO on the 14th day of the experiment increased to 7.37 ± 0.40 units. E450/ml, and at 21 days-up to 8.13 ± 0.37 units. E450/ml. On the 30th day of the experiment, the level of lipid hydroperoxides increased by 30% compared to baseline.

While feeding chickens under cadmium load with forage with milk thistle, the level of lipid hydroperoxides in their blood on the first day of the experiment increased by 1% relative to the beginning of the experiment. Subsequently, the level of lipid hydroperoxides in the blood of the experimental group E_1 compared with the control group began to decrease, namely, on the 7th day of the experiment-by 3.6%, on the 14th day of the experiment-by 10%, on the 21st day of the experiment-by 19.8% and on the 30th day of the experiment-by 14.9%, respectively (Table 1).

Blood test time (days)	**Lipid hydroperoxides (E450/ml)**			
	Groups of chicken			
	Control (Cadmium)	**Experimental 1 (Cadmium+MT**	**Experimental 2 (Cadmium+M)**	**Experimental 3 (Cadmium+S)**
At the beginning of the experiment	5.80 ± 0.14	5.88 ± 0.18	5.92 ± 0.20	5.91 ± 0.21
The first day	5.95 ± 0.20	5.94 ± 0.32	5.99 ± 0.43	5.93 ± 0.24
7 days	6.42 ± 0.23	6.19 ± 0.21	6.23 ± 0.37	6.12 ± 0.42
14 days	7.37 ± 0.40	6.61 ± 0.50	6.54 ± 0.30	6.21 ± 0.28*
21 days	8.13 ± 0.37	6.52 ± 0.29**	6.49 ± 0.25**	5.99 ± 0.34***
30 days	7.54 ± 0.20	6.42 ± 0.25**	6.45 ± 0.36**	5.93 ± 0.40***

Table 1. The level of lipid hydroperoxides in the blood of laying hens after application of milk thistle, metifen and sylimevit in chronic cadmium toxicosis (M ± m, n=8).

When metifen was administered to experimental chickens, it was found that on the first and seventh day of the experiment, the level of primary products fluctuated within the values of 5.99 ± 0.43-6.23 ± 0.37 units E450/ml. On the 14th day of the experiment, the level of lipid hydroperoxides in the experimental group E2 increased slightly compared to the previous day; however, compared to the control group of chickens, this figure decreased by 11.3%, respectively. On the 21st day of the experiment, in the blood of chickens of this experimental group, the level of lipid hydroperoxides continued to decrease and, accordingly, was 6.49 ± 0.25 units E450/ml, which was 20% lower than the control values.

When fed the feed with silymevit in the blood of chickens, the level of lipid hydroperoxides up to 14 days of the experiment increased slightly, compared with the beginning of the experiment and accordingly ranged from 5.93 ± 0.24 to 6.21 ± 0.28 units. E450/ml. On the 21st day of the experiment, there was a decrease in the studied indicator in chickens of experimental group E_3, where it decreased by 26.3% relative to the control group of chickens. On the 30th day of the experiment, the level of lipid hydroperoxides fluctuated within physiological values.

One can notice from the data given in Table 2 that the content of intermediate products of LPO in the blood of chickens of the control and experimental groups at the beginning of the experiment ranged from 7.3 ± 0.58 to 6.8 ± 0.50 μmol/L. Subsequently, under cadmium loading, the level of diene conjugates in the blood of the control group of chickens increased to 9.7 ± 0.54 μmol/L,

while in chickens of the experimental groups, this figure was much lower. Thus, on the 7th day of the experiment, the level of diene conjugates in the blood of experimental group E_1 decreased by 7.4%, in experimental group E_2-by 9.9%, and in experimental group E_3-by 8.6% compared with the control group of chickens. On the 14th day of the experiment, the lowest level of LPO secondary products was in the blood of chickens of the experimental group E_2, where it was 7.5 ± 0.60 μmol/L respectively. On the 21st day of the experiment, the level of diene conjugates in the blood of chickens of the experimental group E_2 decreased by 19.6% compared to the control group and in experimental group E_1-by 17.5%, respectively. The lowest level of diene conjugates was in the blood of chickens fed sylimevit under a cadmium load, where it decreased by 24.7% compared to the control group of chickens. On the 30th day of the experiment, the level of secondary products of LPO in the blood of all experimental groups fluctuated within the values of the initial values taken before feeding cadmium sulfate.

Blood Test Time (Days)	**Diene Conjugates (Mmol/L)**			
	Groups of Chicken			
	Control (Cadmium)	**Experimental 1 (Cadmium+MT**	**Experimental 2 (Cadmium+M)**	**Experimental 3 (Cadmium+S)**
At the beginning of the experiment	7.3 ± 0.58	7.2 ± 0.51	6.8 ± 0.50	6.9 ± 0.44
the first day	7.5 ± 0.55	7.4 ± 0.54	7.1 ± 0.52	7.2 ± 0.58
7 days	8.1 ± 0.70	7.5 ± 0.59	7.3 ± 0.61	7.4 ± 0.72
14 days	8.6 ± 0.94	7.7 ± 0.57	7.5 ± 0.60	7.6 ± 0.55
21 days	9.7 ± 0.54	7.9 ± 0.44**	7.8 ± 0.54**	7.3 ± 0.61**
30 days	9.0 ± 0.43	7.5 ± 0.61*	7.4 ± 0.57*	7.1 ± 0.38**

Table 2. The level of diene conjugates in the blood of laying hens after application of milk thistle, metifen, and sylimevit in chronic cadmium toxicosis (M ± m, n=8).

The results of the studies of Table 3 indicate an increase in the level of finished products of LPO in the blood of chickens, which were subjected to cadmium loading. It was found that the final products of LPO in the blood of chickens of the control group increased from 1.98 ± 0.04 μmol/ml to 2.56 ± 0.09 μmol/ml.

When fed milk thistle and metyfen to laying hens under cadmium load, a slight increase in the level of TBA-active products up to 14 days of the experiment, but compared with the control group of chickens, this figure decreased by 9 and 11%, respectively. On the 21st day of the experiment, the level of the final products of LPO in the blood of chickens of experimental groups E_1 and E_2 was 2.17 ± 0.08 and 2.19 ± 0.07 μmol/ml. On the 30th day of the experiment, the level of TBA-active products in the blood of chickens of the experimental group E_1 decreased by 16.4% and in chickens of experimental group E_2-by 18% relative to the control group of chickens.

Blood test time (days)	**TBA-active products (μmol / ml)**			
	Groups of chicken			
	Control (cadmium)	**Experimental 1 (cadmium+MT**	**Experimental 2 (cadmium+M)**	**Experimental 3 (cadmium+S)**
At the beginning of the experiment	1.98 ± 0.04	2.02 ± 0.04	1.99 ± 0.05	2.04 ± 0.04
the first day	2.15 ± 0.04	2.09 ± 0.05	2.06 ± 0.07	2.10 ± 0.06
7 days	2.32 ± 0.08	2.13 ± 0.08*	2.11 ± 0.08*	2.14 ± 0.07*

14 days	2.41 ± 0.09	2.19 ± 0.07*	2.15 ± 0.04*	2.10 ± 0.09*
21 days	2.39 ± 0.05	2.17 ± 0.08*	2.19 ± 0.07*	2.07 ± 0.09**
30 days	2.56 ± 0.09	2.14 ± 0.06**	2.10 ± 0.04**	2.05 ± 0.08***

Table 3. The level of TBA-active products in the blood of laying hens after application of milk thistle, metifen, and sylimevit in chronic cadmium toxicosis (M ± m, n=8).

When feeding sylimevit in the blood of the experimental group E3, the level of the final products of LPO on the 7th day of the experiment was 2.14 ± 0.07 μmol/ml on the 14th day of the experiment decreased by 13% relative to the control group of chickens. On days 21 and 30 of the experiment, the level of TBA-active products in the blood of chickens fed sylimevit ranged from 2.07 ± 0.09 and 2.05 ± 0.08 μmol/ml.

Thus, with the use of feed for laying hens under cadmium loading, experimental drugs helped suppress the intensity of LPO processes and reduce the formation of large amounts of free radicals and reactive oxygen species, which could further lead to the development of oxidative stress. Additional introduction to the diet of laying hens of milk thistle, metiphen, and sylimevit had an inhibitory effect on the intensity of sex processes in chickens' body. Thus, the concentrations of primary, secondary, and final LPO products in the blood of laying hens of the experimental groups at all stages of the study were lower ($P<0.05–0.001$) than in control.

Conclusion

Based on the results of our experimental studies, we can assume that milk thistle, metifen, and sylimevit in chronic experimental cadmium toxicosis inhibit the processes of lipid peroxidation due to the presence in their composition of antioxidants, both direct and indirect action.

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