

EFFECT OF LIPOSOMAL PREPARATION ON ANTIOXIDANT PROTECTION SYSTEM IN SUBCLINICAL MASTITIS OF COWS

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*The article presents the results of experimental research on the impact of complex liposomal preparation made on the basis of *Hypericum perforatum* L., on the level of products of peroxide oxidation of lipids and the state of the antioxidant protection system in cows suffering from subclinical mastitis.*

Studies have shown that in the afflicted cows there is an increase in the intensity of LPO processes. Three time intercosternal administration of liposomal preparation to the afflicted cows leads to a decrease ($P < 0,01$) in the plasma concentration of TBA-active products and lipid hydroperoxides ($7,17 \pm 0,25\%$ vs. $8,62 \pm 0,16\%$), and accordingly ($1,42 \pm 0,08\%$ vs. $1,82 \pm 0,05\%$). In this case, growth in glutathione peroxidase activity and increased level of restored glutathione in the blood of cows has been observed.

Keywords: cows, subclinical mastitis, somatic cells, products of peripheral oxidation of lipids, system of antioxidant protection.

Introduction. The profitability of modern dairy farming is directly related to milking cows [1]. World milk production is constantly growing. Over the last 20 years, milk production increased by 131.6 million tons, or 26.9%. Purposeful selection and diligent work of zootechnical workers have led to the fact that in most countries with a high culture of dairy production, the average annual dairy productivity for a cow is 8000 - 10000 kg [2, 3]. Positive dynamics of cow productivity is observed in all countries, including Ukraine. The incidence of mastitis worldwide according to available literature shows that this disease is observed in 48 cows out of every 100 heads, of which 39 have a subclinical course of the disease, and 9 animals - clinical [4].

Mastitis affects animal health and milk quality, leading to huge financial losses. Losses per cow are estimated at 108-295 in the United States, 185-375 in the European Union, and \$ 254.5 in Ukraine. Developed methods of early diagnosis, prevention and treatment of this disease through the use of various antimicrobial drugs are not always satisfactory [5].

In recent years, there has been a significant expansion of research on the use of liposomal drugs, which do not contain antibiotics, help prevent recurrence of the disease, and maximize the restoration of milk productivity [6].

In patients with mastitis of cows, there are some changes in metabolic homeostasis. Under physiological conditions, the level of lipid peroxidation (LPO) is maintained due to the balance of the system of anti- and prooxidants. The positive effect of LPO processes on living organisms (restoration of composition and maintenance of biological membranes, participation in energy processes) is provided by the antioxidant defense system, a set of enzymatic and non-enzymatic factors that protect cells from free radicals.

The aim of the article. The aim of our research was to study the activity of the antioxidant defense system and the intensity of lipid peroxidation in cows suffering from subclinical mastitis under the action of a liposomal preparation, made on the basis of *St. John's wort* (*Hypericum perforatum* L.).

Materials and methods. The study was conducted in LLC "Molochni Riku" Brody district of Lviv region on cows 2-3 lactations, which on the principle of analogues were divided into two groups: control (healthy animals) and experimental, 5 animals each. The experimental group was formed from animals with subclinical mastitis (SM).

Subclinical mastitis was determined by the reaction of the secretion from each quarter on a milk control plate with 2% solution of mastidine. Cows of the experimental group in the affected quarters of the udder intracisternally three times with an interval of 24 hours was administered liposomal drug - the first day 10 cm³, the next two days - 5 cm³. Milk before administration of the drug was milked by hand, disinfected teat. After drug administration, the mammary gland was massaged from the bottom up to

distribute it evenly. The cows were transferred to manual milking. Half of the therapeutic dose was prophylactically administered to healthy quarters of the breast.

Liposomal preparation made on the basis of plant raw materials is an antibacterial preparation developed in the laboratory of immunology of the Institute of Animal Biology of NAAS. The composition of the drug includes: novoimanin - extract of St. John's wort (*Hypericum perforatum* L.), vitamins A, D3, E, lecithin, twin. [7, 8]. The drug is active against gram-positive bacteria, including *Streptococcus pyogenes* and *Streptococcus agalactiae*. The anti-inflammatory effect is due to the presence of flavonoids in the drug. It has the ability to heal the wound surface and stimulates tissue regeneration [10-12].

For biochemical studies of cows, blood was taken from the jugular vein before morning feeding on the 1st day (before drug administration) and on the 3-rd and 9-th day after its use.

Determined the content of lipid hydroperoxides (Mironchik AK, 1982), TBA-active products (Korobeynikova EN, 1989), glutathione peroxidase activity (GP) by the rate of oxidation of glutathione in the presence of tertiary butyl hydroperoxide (Moin VM, 1986) and the content of reduced glutathione in red blood cells (Butler E., 1982). The studies were performed according to the methods described in the guide [9]. The obtained digital data were statistically processed using Microsoft Excel software for personal computers, using conventional methods of variation statistics with the determination of mean values (M), their quadratic error (m) and the significance of differences by Student's t-test.

Research results. The antioxidant system provides adaptive stability of the animal body and regulates the reactions of the LP due to the functioning of the system of enzymatic and non-enzymatic mechanisms of control over the content of reactive oxygen species, free radicals and lipid peroxidation products. One of the negative consequences of lipid peroxidation is the formation of TBA – active products, which are the end products of LPO.

Studies have shown that the disease of cows in the subclinical form of mastitis leads to an increase in sick cows content of TBA–active products, as well as lipid hydroperoxides – an intermediate product. Thus, on the first day of the experiment (before drug administration) the level of TBA–active products was 26,57% ($P < 0,01$), and lipid hydroperoxides by 32,85% ($P < 0,001$) higher than in control animals.

Table 1. The content of products of lipid peroxidation in the blood plasma of cows. ($M \pm m$; $n=5$)

Parametes	Control groups animal	Experimental groups animal		
		before treatment	3-rd day of treatment	9-th day from the beginning of treatment
TBA-active products, $\mu\text{mol/l}$	$6,83 \pm 0,31$	$8,62 \pm 0,16^{**}$	$8,13 \pm 0,22^*$	$7,17 \pm 0,25^{\circ\circ}$
Lipid hydroperoxides, unit E/ml	$1,37 \pm 0,06$	$1,82 \pm 0,05^{***}$	$1,63 \pm 0,09$	$1,42 \pm 0,08^{\circ\circ}$

Note: $^{\circ\circ}$ – $P < 0,01$ – probability in animals of this group compared with the indicators before drug administration (1st day of the experiment); * – $P < 0,05$, ** – $P < 0,01$, *** – $P < 0,001$ – the difference is significant compared to the control group.

After intracisternal administration to cows of the studied liposomal drug on the 9th day there was a significant decrease in the content of TBA–active products ($7,17 \pm 0,25\%$ vs. $8,62 \pm 0,16\%$, $P < 0,01$) and lipid hydroperoxides ($1,42 \pm 0,08\%$ vs. $1,82 \pm 0,05\%$, $P < 0,01$) compared with the level recorded before treatment with the drug.

Therefore, the drug studied by us has an inhibitory effect on the intensity of both intermediate and final products of lipid peroxidation in patients with subclinical mastitis of cows.

Glutathione peroxidase activity is important for characterizing the relationship between prooxidant and antioxidant processes in animal cells. It is known that glutathione peroxidase catalyzes the conversion of hydrogen peroxide and lipid hydroperoxides to the corresponding oxo compounds, performing a detoxifying function in cells. Thus, this enzyme inhibits the processes of free radical

oxidation and protects plasma membranes, intracellular structural components and biomolecules from damage [9].

It was found that the plasma GP-activity and the content of reduced glutathione in the erythrocytes of blood of cows with subclinical form of mastitis were lower than in clinically healthy animals (table. 2). In particular, GP-activity was lower by 15,15%, and the level of reduced glutathione – by 9,3% ($P < 0,05$), than in control animals.

Table 2. Glutathione peroxidase activity and the content of reduced glutathione in the blood of cows ($M \pm m$; $n=5$)

Parametes	Control groups animal	Experimental groups animal		
		before treatment	3-rd day of treatment	9-th day from the beginning of treatment
GP act. in erythrocytes, Nm GSH/min x mg protein	23,30 \pm 1,47	19,77 \pm 0,57	22,91 \pm 0,86	23,14 \pm 1,03°
Reduced glutathione in erythrocytes, μ m/ml	0,43 \pm 0,01	0,39 \pm 0,01*	0,41 \pm 0,01	0,43 \pm 0,01°

Note: ° – $P < 0,05$ – probability in animals of this group compared to the indicators before drug administration (1st day of the experiment); * – $P < 0,05$ – the difference is significant compared to the control group.

After the introduction of the experimental drug, in sick cows, compared to the control group of animals, a probable increase in glutathione peroxidase activity and an increase in the content of reduced glutathione. On the 9-th day of treatment in sick cows, they were at the level of clinically healthy animals.

Therefore, the introduction of liposomal drug in subclinical mastitis in cows leads to a decrease in the intensity of lipid peroxidation and to an increase in certain indicators of the glutathione link of the antioxidant system.

Conclusion. The disease of cows with the subclinical form of mastitis leads to a significant increase in the content of TBA-active products and lipid hydroperoxides, and a decrease of 15,15% in GP-activity and 9,3% in the level of reduced glutathione in the blood of animals.

Intracisternal administration of the liposomal drug induces a decrease in the content of TBA-active products and lipid hydroperoxides and an increase in glutathione peroxidase activity and the content of reduced glutathione to the level of clinically healthy cows.

Research prospects. It is planned to conduct a comprehensive functional study of immunocompetent cells, under the conditions of using a new complex liposomal preparation based on plant raw materials.

ВПЛИВ ЛІПОСОМАЛЬНОГО ПРЕПАРАТУ НА СИСТЕМУ АНТИОКСИДАНТНОГО ЗАХИСТУ ПРИ СУБКЛІНІЧНОМУ МАСТИТІ КОРІВ

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*У статті наведені результати експериментальних досліджень щодо впливу комплексного ліпосомального препарату, виготовленого на основі звіробію продірявленого (*Hypericum perforatum L.*), на рівень продуктів пероксидного окиснення ліпідів та стан системи антиоксидантного захисту у корів, хворих на субклінічну форму маститу.*

Дослідження показали, що у хворих корів відбувається підвищення інтенсивності процесів ПОЛ. Інтрацистернальне введення хворим коровам тричі ліпосомального препарату, призводить до зниження ($P < 0,01$) концентрації у плазмі крові ТБК-активних продуктів та гідроперекисів ліпідів ((7,17 \pm 0,25% проти 8,62 \pm 0,16%), і відповідно (1,42 \pm 0,08% проти 1,82 \pm 0,05%). При цьому

зафіксовано зростання у крові корів глутатіонпероксидазної активності та вмісту відновленого глутатіону.

Ключові слова: корови, субклінічний мастит, соматичні клітини, продукти пероксидного окиснення ліпідів, система антиоксидантного захисту.

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