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Influence of liposomal drug on the intensity of proteins oxide modification processes in subclinical mastitis of cows

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The article contains the experimental studies of the liposomal drug based on plant raw materials — hypericum (*Hypericum perforatum* L.) effect on the intensity of oxidative modification of proteins (OMP) in the blood and milk of cows with subclinical mastitis. Studies have shown that cows with signs of subclinical form of mastitis in the serum have an increase in the content of aldehyde-derived OMP_{370} and ketone-derived OMP_{430}, respectively, 1.3 and 1.2 times relative to similar indicators in healthy animals. In the milk of sick cows, the content of derivatives OMP_{370} and OMP_{430} was1.99 and 2.29 times higher, respectively, than in animals of the control group. At the beginning of the study sick cows' milk was recorded a significantly low value of the activity of the enzymatic link of antioxidant protection — superoxide dismutase. At the same time, a 2.6-fold ($P<0.001$) increase in the number of somatic cells was noted compared to their number in the milk of clinically healthy cows. Intracisternal injection of liposomal drug to cows caused a decrease in the intensity of oxidative processes. In the blood of sick cows the content of aldehyde derivatives OMP_{370} on the 9th day of the experiment was 23.1% ($P<0.05$) less than before the drug, and in milk the content of OMP_{370} decreased by 61,8% ($P<0.01$). Similar changes were observed with respect to the level of ketone derivatives. In particular, on the 9th day of the experiment, the content of OMP_{430} decreased by 11.7% ($P<0.05$) compared with its value in the blood of sick animals before the introduction of the study drug, and in milk it decreased by 64.2% ($P<0.01$). During the treatment on the 9th day of the experiment, the number of somatic cells in milk decreased by 41.8% ($P<0.01$). In the course of treatment on the 3rd and 9th day there was a tendency to increase superoxide dismutase activity in the milk of sick cows compared with the beginning of the experiment. Thus, intracisternal injection of liposomal drug to cows with subclinical mastitis leads to a decrease in aldehyde and ketone derivatives of proteins oxidative modification in serum and milk. At the same time, an increase in the activity of the enzymatic link of antioxidant protection and a decrease in the number of somatic cells in the milk of cows were recorded.

Key words: cows, subclinical mastitis, somatic cells, oxidative modification of proteins, liposomal drug

The problem of cattle mastitis in Ukraine is defined by researchers as the main issue of the livestock industry. Because ofthe widespread spread of udder diseases among cows, dairy farming and processing industry suffer significant economic losses due to reduced dairy productivity, deteriorating quality of milk and dairy products [11].

Milk undergoes significant physicochemical changes during mastitis which seriously affects the technological processes of its processing into dairy products, especially reducing their quality [10].

Any pathological processes in the body are accompanied by the activation of free radical processes in the tissues and organs of a sick animal. Free radicals in-

clude compounds that contain unpaired electrons and have a much greater reactivity to their non-radical counterparts [8, 13]. All functionally important free radicals that are formed in the body contain oxygen.

These compounds are combined by the term "reactive oxygen species" (ROS). The main forms of ROS generated in a living organism are: superoxide radical, hydroxyl radical, nitric oxide, peroxyl radical, hydrogen peroxide and others. The main forms of ROS are primarily normal components of cellular metabolism and perform certain biological functions. Their reactive aggressiveness is restrained by a powerful antioxidant system. However, with the development of pathological processes, this balance is disturbed in the direction of uncontrolled synthesis of ROS, which ends with the formation of oxidative stress.

It is established that under conditions of oxidative stress and excessive ROS generation, the processes of proteins uncontrolled modification develop, causing protein fragmentation, their denaturation, as well as the formation of primary amino acid radicals, which then enter into secondary interaction with neighboring amino acid residues. All these in totalcreate a difficult situation of the damaging effect of ROS on protein macromolecules. This leads to the loss of proteins biological activity and disruption of metabolic, in particular regenerative processes [4].

In view of the various functions of proteins in animals, as well as taking into account the data of literature sources on the primary damage of protein molecules ROS [1, 6], the study of peroxidation processes, especially proteins, is important.

Destruction of cellular proteins in the process of oxidative modification of proteins (OMP) in proteosomes leads to cell death [12], i.e. a direct link between the processes of OMP and many diseases is established [15]. It is believed that the level of OMP compared to the level of lipid peroxidation (LPO) is a more informative marker of the presence of oxidative stress in the body [16].

Both LPO and OMP are normal functional processes in the body that involve vital functions. Moreover, the processes of OMP are largely associated with protective and adaptive reactions of the organism. However, the violation of protective reactions leads to a decrease in the body's resistance. Thus, oxidative modification of proteins plays a key role in the molecular mechanisms of oxidative stress, which is an important part of many pathological processes in the body in various diseases and requires further study. The aim of the study was to determine the effect of liposomal drug on the intensity of oxidative modification of proteins in blood and milk, as well as on superoxide dismutase activity in the milk of cows with subclinical mastitis.

Materials and Methods

We hereby declare all ethical standards have been respected in the preparation of the submitted article. Permission to use animals approved by the State Agrarian and Engineering University in Podillya in accordance with the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes.

Experimental studies were conducted at LLC "Lany-Vinkovechchyna" (Khmelnytsky region) on cows which were divided into two groups: control (healthy animals) and experimental, 7 animals in each group, based on the analogue's principle. The experimental group was formed from animals with subclinical mastitis (CM) [2].

Subclinical mastitis was determined by the reaction of 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 were injected intracisternally liposomal drug three times with an interval of 24 hours — the first day 10 cm^3, the next two days — 5 cm^3. Before the drug injection cowswere milked by hand, the teats were disinfected. After drug injection, the udder was massaged from the bottom to the top for its even distribution. The cows were transferred to manual milking. Half of the therapeutic dose was prophylactically injected to healthy quarters of the udder.

A liposomal drug made on the basis of plant raw materials is an antibacterial preparation developed in the laboratory of immunology of the Institute of Animal Biology NAAS. The composition of the drug: novoimanin — extract from *Hypericum perforatum* L., vitamins A, D_3, E, lecithin, twin. 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 [3].

For biochemical studies, cows were bled from the jugular vein before morning feeding on the 1st day (before drug injection), on the 3rd and 9th day after its use.

The level of oxidative damage of proteins was evaluated by the content of aldehyde (OMP_{370}) and ketone derivatives (OMP_{430}) of oxidative modification of proteins in reaction with 2,4-dinitrophenylhydrazine [7]. Superoxidedismudase activity in cows' milk was determined by the method described by E. E. Dubinina [5].

To control the recovery of milk quality, we used the analyzer AMB 1-02 designed to measure the conditional milk stickiness and calculate the concentration of somatic cells in it [9]. Statistical data processing was performed using *Microsoft Excel* software.

Results and Discussion

Free radicals with high reactivity are formed as a result of metabolic transformations of substances under the action of pathogenic factors in the body of animals. Formed in the body, they interact with the components of the cell, cause damage to cell membranes, thus accompanying the development of the pathological process. Protein degradation is a more reliable mark-

er of oxidative tissue damage than lipid peroxidation products (LPO) because protein oxidative modification (OMP) derivatives are more stable.

As a result of our research, it was found that before treatment animals with signs of mastitis have blood content of oxidative modification of proteins, namely: aldehyde-derived OMP_{370} and ketone-derived OMP_{430} that is 1.3 and 1.2 times higher than the blood of control cows groups (table 1). The introduction of the studied liposomal drug caused a decrease in the intensity of oxidative processes, as indicated by a decrease of 23.1% (P<0.05) of aldehyde derivatives OMP_{370} in the experimental group cows blood on the 9th day of the experiment than before the introduction of liposomal drug. Similar changes were observed with respect to the level of ketone derivatives. Thus, on the 9th day of the experiment, the content of OMP_{430} decreased by 11.7% compared with the value of this indicator in the blood of sick animals before the introduction of the study drug (P<0.05). These data indicate the inhibitory effect of the components of the studied liposomal drug on the intensity of oxidative modification of proteins in the blood of cows with subclinical mastitis [14].

Table 1. The content of aldehyde and ketone starting oxidative modification of proteins in the serum of cows (M±m; n=7)

Parameters	Control group	Experimental group		
		before treatment	3rd day of treatment	9th day from the beginning of treatment
OMP_{370}, nmol/mg protein	21.34±1.64	27.46±1.23**	24.53±2.05	21.45±1.96*
OMP_{430}, nmol/mg protein	32.18±1.32	38.65±2.08**	37.6±2.06	32.92±2.35*

Note. * — P<0.05 — probability in animals of this group compared to the indicators before drug injection (1st day of the experiment); ** — P<0.05 — the difference is significant compared to the control group data.

Research has shown that before treatment cows with signs of mastitis have the content of derivatives OMP_{370} and OMP_{430} in milk 1.99 and 2.29 times higher compared with similar indicators of cows in the control group (table 2).

The content of both aldehyde and ketone derivatives of oxidative modification of proteins in cows' milk probably decreased in the dynamics of treatment, which is also an important diagnostic indicator of normalization of the intensity of oxidation processes and confirms the effectiveness of the study drug. Thus, the content of aldehyde derivatives OMP_{370} in the milk of the experimental group cows on the 3rd and 9th day of the experiment was 36.8 and 61.8% (P<0.01), respectively, less than before the drug injection. Similar changes were observed with respect to the level of ketone derivatives. In particular, on the 3rd day of the experiment, the content of OMP_{430} in milk decreased by 40.6%, and on the 9th — by 64.2% (P<0.01) compared with the value of this indicator in sick animals before the introduction of the study drug.

On the 3rd and 9th day during treatment, there is a tendency to increase superoxide dismutase activity in the milk of the experimental group animals compared with the indicator at the beginning of the experiment. The obtained results confirm that the components of the drug cause a regulatory effect on the intensity of oxidation and maintenance of pro- and antioxidant balance in the body of sick cows.

Table 2. The content of derivatives of oxidative modification of proteins and superoxide dismutase activity in cow's milk (M±m; n=7)

Parameters	Control group	Experimental group		
		before treatment	3rd day of treatment	9th day from the beginning of treatment
OMP_{370}, nmol/mg protein	7.78±1.84	15.47±2.25**	10.12±1.54	6.25±1.16*
OMP_{430}, nmol/mg protein	6.14±1.72	14.06±2.08**	8.65±1.43	5.32±0.96*
SOD, unit of action/mg min.	11.96±1.86	11.84±2.34	12.34±1.65	13.47±1.85

Note. In this and the next table * — P<0.01 — probability in animals of this group compared to the indicators before drug injection (1st day of the experiment); ** — P<0.001 — the difference is significant compared to the control group data.

Before treatment a significant increase in the number of somatic cells of 2.6 times (P<0.001) is observed in the milk of the experimental animals group, compared with clinically healthy animals, which is associated with the subclinical form of mastitis (table 3). The introduction of the studied liposomal drug revealed a decrease in the number of somatic cells from the third day of treatment, and on the 9th day of the experiment the number of somatic cells in the secretion of the mammary gland of the experimental group cows was lower by 41.8% (P<0.01) than in the beginning of the experiment, and differences compared to animals in the control group were not significant. These data indicate a normalizing effect of the study drug components on the content of somatic cells in the milk of cows suffering from a subclinical form of mastitis.

Table 3. The content of somatic cells in the milk of cows (M±m; n=7)

Parameters	Control group	Experimental group		
		before treatment	3rd day of treatment	9th day from the beginning of treatment
SC, thousand/cm³	259.3±40.05	667.9±64.9**	573/7±52/08**	388/7±44/97*

Thus, the use of liposomal drug contributed to a prolonged decrease in the intensity of oxidative processes, as well as the number of somatic cells in milk, due to both normalizing and stimulating effect of drug components on the activity of these protective mechanisms in cows suffering from subclinical mastitis.

Conclusion

Intracisternal injection of the studied liposomal drug to animals led to a decrease in the content of products of oxidative modification of proteins (P<0.05–0.01). At the same time, the use of the drug caused a decrease in the number of somatic cells in the milk of cows by 41.8% (P<0.01) with a simultaneous increase in superoxide dismutase activity compared to pre-treatment values.

Prospects for Further Research

It is planned to conduct a comprehensive functional study of immunocompetent cells, under the conditions of using a new complex liposomal drug in the treatment of cows with a clinical form of mastitis.

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Вплив ліпосомального препарату на інтенсивність процесів окисної модифікації протеїнів за субклінічного маститу корів

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Наведені результати експериментальних досліджень впливу ліпосомального препарату, виготовленого на основі рослинної сировини, звіробою продірявленого (*Hypericum perforatum* L.), на інтенсивність процесів окисної модифікації протеїнів (ОМП) у крові та молоці корів, хворих на субклінічну форму маститу. Дослідження показали, що у корів з ознаками субклінічної форми маститу в сироватці крові констатовано підвищення вмісту альдегідопохідних $ОМП_{370}$ і кетонопохідних $ОМП_{430}$ — відповідно, в 1,3 і 1,2 раза щодо аналогічних показників у здорових тварин. При цьому у молоці хворих корів вміст похідних $ОМП_{370}$ та $ОМП_{430}$ був в 1,99 і 2,29 раза більший, ніж у тварин контрольної групи. На початку дослідження у молоці хворих корів зафіксовано вірогідно низьке значення показника активності ензимної ланки антиоксидантного захисту — супероксиддисмутази. Водночас констатовано збільшення у 2,6 раза ($P<0,001$) кількості соматичних клітин порівняно з їх кількістю у молоці клінічно здорових корів. Інтрацистернальне введення коровам ліпосомального препарату спричиняло зниження інтенсивності окисних процесів. У крові хворих корів вміст альдегідних похідних $ОМП_{370}$ на 9-ту добу експерименту був на 23,1% ($P<0,05$) менший, ніж до початку введення препарату; в молоці вміст$ОМП_{370}$ зменшився на 61,8% ($P<0,01$). Аналогічні зміни констатовано щодо рівня кетонових похідних. Зокрема, на 9-ту добу експерименту вміст $ОМП_{430}$ знизився на 11,7% ($P<0,05$) порівняно з його значенням у крові хворих тварин до введення досліджуваного препарату, а в молоці став меншим на 64,2% ($P<0,01$). За проведеного лікування на 9-ту добу експерименту кількість соматичних клітин у молоці знизилась на 41,8% відносно початку досліду ($P<0,01$). У процесі лікування на 3- і 9-ту доби виявлено тенденцію до підвищення супероксиддисмутазної активності у молоці хворих корів порівняно з показниками на початку досліду. Отже, інтрацистернальне введення ліпосомального препарату коровам, хворим на субклінічну форму маститу, призводить до зниження альдегідних та кетонових похідних окисної модифікації протеїнів у сироватці крові та молоці. При цьому зафіксовано підвищення активності ензимної ланки антиоксидантного захисту та зниження кількості соматичних клітин у молоці корів.

Ключові слова: корови, субклінічний мастит, соматичні клітини, окисна модифікація протеїнів, ліпосомальний препарат

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