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Scientific and Experimental Substantiation of Possibility of Application of the Original Drug Bio-effective W in Bovine Leucosis.

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ABSTRACT

The purpose of this research was to study the effectiveness of the original drug on the basis of lichen compounds of birch (Bioeffective W) in bovine leucosis. The data obtained in the experiment in a dairy farm office of Shaverky of a limited liability company (LLC) "Moksha" of Krasnoslobodsky district of the Republic of Mordovia (Russia) in the period from September to December 2013 with hematological form of bovine leucosis and seropositive animals. On the principle of analogues were formed four groups of cows: one of them – a control group (not injected drugs) and 3 main experimental groups, there were 5 animals in each group. In the second experimental group the cows were injected with a drug Ligfoll intramuscularly in the rump at a dose of 5 ml (for the 1-st, 4-th, 7-th, 10-th and the 13th day of the experiment), then once a week within a month (according to the methodological recommendations on the use of the drug). Animals in the third and fourth experimental groups were injected with Bioeffective W in the dose of 10 and 20 ml, respectively (on 1-st, 4-th, 7-th, 10-th and the 13th day), then once a week within a month. Blood from the experimental animals were studied on the 15-th, 30-th and 60-th days after administering of the drugs. Studies have shown that the drug Bioeffective W contributed to the increased level of leucocytes, lymphocytes and the percentage of lymphocytes in the blood of animals, and at the same time, Bioeffective W showed erythropoietic properties. This property of the drug was more impressive than in Ligfoll, dose dependencies were not revealed.

Key words: bovine leucosis, Ligfol, original drug Bioeffective W, blood, lymphocytes.

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INTRODUCTION

Among all the infectious diseases of bovine in the Russian Federation the leukemia ranks first and is registered in most subjects. Leukaemia and other malignant diseases of a farm animals, which are included in the list of quarantine and especially dangerous diseases currently represent one of the most pressing veterinary, biological and social problems [1]. The products obtained from animals with leukemia contain abnormal metabolites of tryptophan, lysine and other cyclic amino acids, have strong carcinogenic properties and are therefore harmful to human health [2, 3].

The anthropogenic transmission of leukemia virus of cattle was recognized as the world's leading way of transmission. Leukemia is most frequent in animals with high milk yield and a weakened protective-adaptive reactions of the body and lower blood levels of hormones of a thyroid and pancreas glands.

Currently in the Russian Federation as the basis of diagnosis of leukemia is serologic method is used the reaction of immunodiffusion (RID). Other diagnostic methods (enzyme-linked immunosorbent assay method, method of biosample, polymerase chain reaction, and others) are sensitive too and detect animals earlier after infection with the virus of bovine leukemia, but a practical wide distribution has not received. Hematological, clinical and pathological methods help to identify animals in hematological and neoplastic stages of development of the leukemic process [2, 4 - 6].

Treatment in this disease has not been developed.

There are known methods of prevention of bovine leucosis with the use of drugs Ligfoll and immunometabolic drug - the Amber biostimulant. However, the effectiveness of these drugs for the prevention of the lymphocytosis in bovine leukemia insufficiently substantiated in a production environment, the procedure is time consuming and expensive [7, 8].

Thus, the bovine leukemia represents the actual social, medical and General biological problem requiring scientifically-based practical solutions. The problem of the occurrence of virus-induced genesis of cancer and leucosis, the assessment of risk factors and development of methods of prevention and treatment is an enormous problem for veterinary Virology and epizootology, and far from its solution.

MATERIALS AND METHODS OF RESEARCH

The data presented in this paper on the feasibility of Bioeffective W in the complex treatment of leukemia in bovine, obtained in a dairy farm office of Shaverky LLC “Moksha” Krasnoslobodsky district in the period from September to December 2013 in cattle with hematological form of leukemia and seropositive animals.

All livestock on the farm provided enough fodder. The system of livestock is stall-pasture. All animals receive preventive vaccinations against: amkare, anthrax, leptospirosis, paratyphoid fever, ringworm, esherihiosis. Allergic studies: tuberculosis; serological: brucellosis, leucosis.

A dairy farm office of Shaverky LLC “Moksha” since 2004 was affected with leucosis cattle, and was restricted. On the principle of analogues, there were formed four groups of cows (control and 3 experimental). All groups had 5 animals. Cows of the second group were injected intramuscularly Ligfoll in the rump at a dose of 5 ml (for 1-th, 4-th, 7-th, 10-th and the 13-th day), then once a month. Animals in the third and in the fourth experimental groups were injected with Bioeffective W with a dose of 10 and 20 ml respectively of the scheme of application of the 2-nd group).

Hematological investigations were carried out in the veterinary clinic of the Agricultural Institute of Mordovia State University named after N. P. Ogarev on the automatic hematological analyzer for veterinary MICROCC-20Vet (HTI, USA). In each of the sample were obtained 20 indicators. The analyzer automatically produces the blood sample, its dilution, mixing, lysis, feeding and cleaning.

The resulting digital research material was subjected to statistical analysis using standard parametric methods, the reliability was determined by t-Student's criterion with the use of the software package

Microsoft Excel (2000) and STAT 3.

BRIEF DESCRIPTION OF THE DRUG LIGFOLL

Ligfoll possesses strong adaptogenic stress-corrective properties. One of the targets of the Ligfoll was to help animals to cope with disease by themselves, including such heavy disease as cancer.

The main properties of Ligfolla are adaptogenic, antioxidant, antitumor and regenerative. These effects are directly linked to the presence in its composition of modified humic substances obtained by hydrolysis of the natural lignin. The antitumor effects of Ligfolla at the present moment is debatable. Ligfoll, reducing the virulence of the causative agent of the disease in the incubation period, causes the positive dynamics of reduction of absolute lymphocytosis in the peripheral blood of hematologic main indicator of the development of leukemic process (delays the development of clinical manifestations).

BRIEF DESCRIPTION OF THE DRUG BIOEFFECTIVE W

It is known that lichen compounds show a variety of biological effects: antiviral, antibacterial, antifungal, antiprotozoal, antimutagenic, antioxidant, antitumor, antinociceptive, antipyretic and anti-inflammatory; at the same time the lichen compounds are considered low toxic.

The availability of lichens and atraric acid makes them one of the most valuable medicinal materials. The substance used in the present research on the basis of atraric acid (Bioeffective W) has shown effectiveness in preclinical studies on cell lines of malignant tumors (mammary adenocarcinoma). New anticancer drug had not only a pronounced cytostatic and cytotoxic effect, but it does not have a negative impact on the body.

RESULTS AND DISCUSSION

The hematological indices are statistically processed and the most informative of them are summarized in table 1.

Table 1, Hematologic indicators of cows after application of Ligfol drugs and Bioeffective W.

№	Indicators	Initial data	Groups of animals	The time of the study			
				Before the onset	15 day	30 day	60 day
1.	WBC, 10^9/l (10^3/mcl)	12.2±1.1	control	11.0±2.4	9.2±1.5	11.3±1.4	11.6±1.5
			1	11.0±1.2	10.3±1.1	12.9±2.1	14.7±2.1
			2	12.2±2.1	13.1±1.4	15.7±0.8	14.9±0.5
			3	14.1±2.6	15.1±1.9*	14.6±2.6	15.1±1.3
2.	LYM, 10^9/l (10^3/mcl)	7.0±0.6	control	5.9±1.1	4.7±0.7	6.6±0.9	5.5±0.3
			1	6.2±0.9	5.8±0.9	7.8±1.8	9.6±1.7
			2	6.4±0.7	7.5±1.0	10.1±0.6	9.4±0.8
			3	9.0±1.7*	8.8±0.9*	8.1±1.4	9.4±0.7
3.	LYM %	57.5±1.8	control	53.9±4.2	51.0±1.7	58.0±1.7	50.9±5.9
			1	55.2±3.1	56.8±7.5	57.2±6.5	63.8±4.7
			2	54.7±3.2	56.8±3.3	64.5±1.5	63.2±4.4
			3	64.6±2.7	59.4±1.5*	57.7±4.8	62.4±1.9
4.	GRA, 10^9/l (10^3/mcl)	2.6±0.4	control	2.8±1.2	2.2±0.4	2.6±0.45	3.9±1.4
			1	2.6±0.2	2.5±0.7	3.3±0.6	3.1±0.7
			2	3.2±1.3	2.9±0.5	3.3±0.3	3.1±0.5
			3	2.1±0.5	2.9±0.6	3.7±1.1	3.2±0.4
5.	RBC, 10^{12}/l (10^6/mcl)	5.3±0.1	control	5.3±0.6	6.0±0.4	5.3±0.2	5.7±0.3
			1	5.3±0.3	6.2±0.3**	6.2±0.5	7.0±0.5
			2	5.1±0,1	6.5±0.4**	7.1±0.2	6.7±0.3
			3	5.4±0,04	6.3±0.4**	6.4±0.7	7.0±0.3
6.	HGB, g/l	79.2±2.2	control	78.4±8.5	89.0±5.5	80.0±3.1	89.6±4.1
			1	79.0±4.4	93.3±2.1**	102.0±6.9	113.2±7.2
			2	77.8±2.5	99.6±6.5**	110.6±3.1	109.0±5.2
			3	81.2±2.1	97.0±5.2**	101.0±8.5	110.3±6.0

7.	The average concentration of hemoglobin in the erythrocyte, g/l (MCHC)	347.7±3.3	control	351.0±4.6	309.0±6.8	321.0±4.7	320.6±4.3
			1	336.4±11.1	303.8±4.9**	321.0±3.9	314.8±1.7
			2	350.2±5.7	303.6±2.9**	308.2±2.4	319.2±5.2
			3	352.3±2.0	309.8±2.6**	316.2±2.3	305.3±2.1
8.	PLT, 10^9/l (10^3/mcl)	241.1±23.3	control	241.0±35.2	172.8±45.7	147.5±48.9	243.0±39.9
			1	285.2±31.4	253.0±41.1	231.8±34.2	250.0±39.4
			2	182.0±26.8**	178,6±21.2	208.2±48.0	160.4±22.6
			3	253.8±68.4	178.7±20.8**	638.4±261.4	272.5±31.6

*≤0.05 comparing to the control.

**≤0.05 comparing to the initial level.

Analyzing the indicators characterizing the state of the white blood cells it was revealed that in the control group of animals the number of leukocytes decreased to 9.2±1.5 x 10^9/l by 15-th day in subsequent periods of the study, and was 11.3±1.4, and to 11.6±1.5 x 10^9/l on the 30-th and 60-th day respectively. Animals in the 1 experimental group had the number of white blood cells to the 15-day decreased to 10.3±1.1x10^9/l, in subsequent periods of the study it was increased and amounted to 12.9±2.1 and 14.7±2.1x10^9/l on the 30-th and 60th day respectively. In animals of the 2-nd experimental group the number of leukocytes by the 15-th and 30-th days of the study was increased to 13.1±1.4 and 15.7±0.8x10^9/l, respectively, and by the 60-th day decreased to 14.9±0.5 x 10^9/l. In animals of the 3-rd experimental group the number of leukocytes was significantly increased, compared with control, by 15-th day to 15.1±1.9x10^9/l, by 30-th day it was a marked decrease to 14.6±2.6x10^9/l and it increased by 60-th day to 15.1±1.3x10^9/l. Therefore, treatment of animals with these drugs resulted in a marked reduction in the number of leukocytes in the blood of experimental animals, but rather increased their level that can be clearly seen in animals when applying the drug Bioeffective W. Also it was noted the dependence of this process with the dose the drug used.

The level of lymphocytes in animals of the control group to the 15-day was 4.7±0.7x10^9/l, and then for 30-th day had increased to 6.6±0.9x10^9/l, then decreased by 60-th day and was 5.5±0.3x10^9/l. In animals of the 1st experimental group, the percentage on the 15-th day was 5.8±0.9x10^9/l, and then increased in subsequent periods of the study, and was 7.8±1.8 and 9.6±1.7x10^9/l, respectively by 30-th and by 60-th day. In animals of the 2-nd experimental group on the 15-th day of the study it was 7.5±1.0x10^9/l, and was increased by 30-th day to 10.1±0.6x10^9/l, then decreased and on the 60-th day it was 9.4±0.8x10^9/l. In animals of the 3-rd experimental group on the 15-th day of the study it was found that the number of lymphocytes was significantly higher compared with control, and was 8.8±0.9x10^9/l, slightly decreased by 30-th day to 8.1±1.4x10^9/l, and by 60-th day increased to 9.4±0.7x10^9/l. Analyzing data for this indicator, it is noted that the application of Ligfoll and Bioeffective W did not lead to a reduction in the number of lymphocytes in the blood, but rather a marked increase in their number, the most constant and vivid in the 60-th day of the experiment.

While analyzing the percentage of lymphocytes it was noted the following. In the control group, on the 15-th day this rate was 51.0±1.7%, then increased, and by 30-th day was 58.0±1.7%, by 60th day was 50.9±5.9 percent. In animals of the 1st experimental group the percentage of lymphocytes on the 15th day of the study was 56.8±7.5%, in subsequent terms of study rose to the 30-th and 60-th days, and was 57.2±6.5 and 63.8±4.7%, respectively. In animals of the 2-nd experimental group on the 15-th day this indicator was equal 56.8±3.3 per cent, to 30-th day increased to 64.5±1.5%, and on the 60-th day decreased and accounted for 63.2±4.4 percent. In animals of the 3-rd experimental group on the 15-th day it was like the control animals, to the 30-th day decreased to 57.7±4.8% and to 60-th day increased and was equal to 62.4±1.9 percent. Thus it is noted that the use of drugs does not lead to a decrease in the percentage of lymphocytes in the blood of experimental animals, but rather marked increase in animals which were injected with Ligfoll and Bioeffective W (especially in a dose of 10 ml).

The level of granulocytes in the blood of the animals of the control group on the 15-th day was 2.2±0.4x10^9/l, then increased to 30-th day to 2.65±0.4 to 60-th day to 3.9±1.4x10^9/l. In animals of the 1-st experimental group on the 15-th day it was equal to 2.5±0.7x10^9/l, and for the 30-th day had increased to 3.3±0.6x10^9/l, then decreased and on the 60-th day it was equal to 3.1±0.7x10^9/l. In animals of the 2-nd experimental group on the 15-th day of the study it was equal to 2.9±0.5 x 10^9/l, for the 30-th day increased to 3.3±0.3x10^9/l, and decreased to 3.1±0.5 x 10^9/l. In animals of the 3-rd experimental group on the 15-th day the level of granulocytes in the blood was equal to 2.9±0.6x10^9/l, was increased by the 30-th day to 3.7±1.1x10^9/l, for the 60-th day decreased to 3.2±0.4x10^9/l. Therefore, specific differences in the changes of this index in

animals receiving the drug and in the control animals were not revealed.

Analyzing the indicators characterizing the state of red blood it was established that in the control group of animals the number of red blood cells to a 15-day research increased to $6.0\pm0.4x10^{12}$/l, for 30-th day decreased to $5.3\pm0.2x10^{12}$/l and to 60-th day increased and was $5.7\pm0.3x10^{12}$/l. In animals of the 1st experimental group on the 15-th day ($p\leq0.05$ compared with baseline) it was $6.2\pm0.3x10^{12}$/l, by 30-th day was $6.2\pm0.5x10^{12}$/l, and by 60-th days increased to $7.0\pm0.5x10^{12}$/l. Animals of the 2-nd experimental group on the 15-th day of a study it was $6.5\pm0.4x10^{12}$/l, on the 30-th day increased to $7.1\pm0.2x10^{12}$/l, and by the 60-th day decreased to $6.7\pm0.3x10^{12}$/l. In animals of the 3-rd experimental group, the percentage on the 15-th day it was significantly higher compared to the outcome, and amounted to $6.3\pm0.4x10^{12}$/l, then gradually increased and on the 30-th and 60-th days, respectively, was equal to 6.4±0.7 and $7.0\pm0.3x10^{12}$/l. Overall, the changes in this indicator marked an increase in animals of all experimental groups, but when used in the animals treated with the Bioeffective W, it has been clearly expressed. A direct dependence on the dose of a used drug was not evaluated.

The level of hemoglobin in the blood, in animals of the control group on 15-th day of study was equal 89,0±5.5x10g/l, on the 30-day decreased up to 80.0±3.1 g/l, and by 60-day increased to 89.6±4.1 g/l. In animals of the 1st experimental group on the 15-th day was significantly higher compared with the outcome (93.3±2.1 g/l), then increasing, on the 30-th and 60-th day, was equal 102.0±6.9 g/l and a rate of 113.2±7.2 g/l, respectively. In animals of the 2-nd experimental group on the 15-th day of study the percentage was significantly higher compared with the outcome (99.6±6.5 g/l), then increased, and by 30-days was equal to 110,6±3.1 g/l. At 60-day was noted down to 109,0±5.2 g/l. In animals of the 3-rd experimental group the percentage on the 15-day studies was significantly equal in comparison with the outcome 97.0±5.2 g/l, then gradually increased in subsequent periods (30 and 60 days) was equal to 101.0±8.5 and 110.3±6.0 g/l, respectively. Therefore, the marked increase in the level of hemoglobin in the blood of animals of all experimental groups treated with Ligfoll and Bioeffective W (especially in a dose of 10 ml).

The average concentration of hemoglobin in erythrocytes in animals of the control group on the 15th day of the studies ranged from 309,0±6.8 g/l, to 30-day increased to 321.0±4.7 g/l, to 60-day decreased slightly and amounted 320.6±4.3 g/l. In animals of the 1st experimental group on the 15-day it was significantly higher compared with the outcome (was 303.8±4,9 g/l), to 30-day increased to 321.0±3.9 g/l, and to the 60-day decreased slightly and amounted to 314.8±1.7 g/l.. In animals of the 2-nd experimental group the figure was changed as follows: on the 15-th day were significantly lower compared with the outcome (to 303.6±2.9 g/l), then gradually increasing, on the 30-th and 60-th day was 308.2±2.4 and 319,2±5.2 g/l.. In animals of the 3-rd experimental group on the 15-day studies, the average concentration of hemoglobin in the red blood cell was at the level of outcome (309.8±2.6 g/l), at 30-day increased to 316.2±2.3 g/l, then decreased slightly and was equal to 305.3±2.1 g/ l.. Analysis of the findings shows a pronounced decrease on the 15-th day of the study in animals of all experimental groups.

The level of platelets (thrombocytes) in the control group of animals on the 15-day of a study was equal to $172.8\pm45.7x10^{9}$/l, then decreased on the 30-th day to $147.5\pm48.9x10^{9}$/l, and sharply increased to $243.0\pm39.9x10^{9}$/l on the 60-day. In animals of the 1st experimental group on the 15-th day was equal $253.0\pm41.1x10^{9}$/l on the 30-th day had declined to a $231.8\pm34.2x10^{9}$/l and by 60-th day increased to $250,0\pm39,4x10^{9}$/ l.. The level of platelets in animals of the 2nd experimental group on the 15-th day remained at the control level ($178,6\pm21.2x10^{9}$/l), to 30-day increased up to $208.2\pm48.0x10^{9}$/l and on the 60-day it was observed a decrease to $160,4\pm22,6x10^{9}$/l. In animals of the 3-rd experimental group on the 15-th day the platelet count was significantly lower in comparison with the outcome (of $178.7\pm20.8x10^{9}$/l), on the 30-day there was a sharp increase in this figure to $638.4\pm261.4x10^{9}$/l ($p\geq0.05$), on the 60-th day decreased to $272.5\pm31.6x10^{9}$/l. Hence in animals after use of the drug Bioeffective W at a dose of 20 ml there was a higher platelet count.

Analyzing the obtained data on indicators of the white blood cells of animals one can conclude that Ligfoll and Bioeffective W cause a mild leukocytosis and lymphocytosis. However it was noted that in animals after the application of Bioeffective W this process is stronger and he is more resistant.

Analysis of parameters of red blood of animals suggests that Ligfoll and Bioeffective W cause increased levels of erythrocytes, hemoglobin and platelets, but in animals after application Bioeffective W this

process is also stronger and more resistant.

A marked variety of biological effects of Ligfoll and Bioeffective W are difficult to explain in a common mechanism of action. Overall, judging by these research data, the mechanisms of biological effects of Ligfoll and a new drug - Bioeffective W at the present moment are controversial [9-13].

CONCLUSION

The injection for animals of Ligfoll and a new drug called Bioeffective W lead to the moderate leukocytosis and lymphocytosis in the experimental cows. The use of these drugs resulted in a strong stimulation of Erythro - and thrombocytopoiesis. Identified biological effects are more impressive when using Bioeffective W. A strong dependence of biological effects on the dose of the drug was not found. Overall, the studies indicate that the drug on the basis of lichen compounds of birch shows marked hematopoietic properties. The possibility of its use in bovine leukemia remains a controversial issue.

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