

Assessment of the Antioxidant Properties of Plant and Chemical Origin Dietary Supplements in the Model Test System

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Abstract: For the test model system, the chemiluminescence method has been chosen which makes it possible to evaluate the processes of free-radical oxidation and objectively characterize dietary supplements having pro or antioxidant properties. The dietary supplements of plant origin (erakond, milk thistle and carsil) and of chemical origin (santochin, emecidine, iodine-matrix compounds) are used to care metabolism. Dietary supplements have antioxidant properties, also, slow oxidation processes and can be essential for correction of metabolism. The intensity of many enzymatic and non-enzymatic reactions determines the formation of free radicals which are formed in pathology and as a rule, increase the oxidation processes disrupting the structural compartments of the cell (cell membrane organelles, nucleus, etc.). For the regulation of metabolism, we offered doses or dietary supplements matrices that change the rate of free-radical oxidation and reduce the development of pathological changes in the body. Then, the reactions of free-radical oxidation in the body were studied. In this study, we studied synthetic and plant nutritional supplements and also, determined the activity of oxygen forms and the sum of luminosity on model systems of different kinds. Also, the above mentioned dietary supplement was activated with the help of various substances for the activity of lipid oxidation. As a more complex model for the study of chemiluminescence, complex compounds were also studied. As an example, iodine is a pro-oxidant but associated with pectin changes its properties. Another dietary supplement is an Erakond-antioxidant which enhances its properties with pectin or chitosan. The proposed dietary supplement model assessment allows us to make an objective conclusion about the properties of the substance and its changes with complexing compounds.

Key words: Chemiluminescence, model system, santohin, oxymethyluracyl, erakond, pectin, milk thistle, carsil, dietary supplement

INTRODUCTION

Now a days, researchers have been intensively studying the processes of chain reactions in the cell and their significance in the initiation of pathology. Free radicals provide oxidation of Foreign compounds have a microbicidal effect influence metabolism, accumulation and biotransformation of substances and also, immunity (Bagautdinov *et al.*, 2008; Bagautdinov,

2008, 2009; Baymatov *et al.*, 2009; Gil'dikov and Loseva, 2018). Free radicals are chemically active, they are capable of producing chain reactions of oxidation (Bagautdinov *et al.*, 2008; Bagautdinov, 2008, 2009; Baymatov *et al.*, 2009). On the one hand free radical oxidation, providing normal life activity is necessary for metabolism on the other hand, its decreasing or increasing is a universal aspect of the pathogenesis of many pathologies (Bagautdinov *et al.*, 2008, 2009;

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Bagautdinov, 2009; Gil'dikov and Loseva, 2018; Dzhangiryants, 2002). For example, formed ketones, aldehydes and other toxic compounds cause significant harm to the body (Baymatov *et al.*, 2009) by damaging biological membranes, enzymes and hormones and reduce protective functions (Bagautdinov, 2009; Baymatov *et al.*, 2009).

The main task at the present day is to create a universal technology for full animal nutrition. It implies a comprehensive study of components and dietary supplements for feeds that positively affect the body's functional state and productivity (Gil'dikov *et al.*, 2017; Gil'dikov and Loseva, 2018). The quality of feed components and their interaction is reflected in the metabolic processes and functions of the body. The presence of active compounds, tannins organic acids, flavonoids, anthocyanins, hydroxycinnamic and phenolic carboxylic acids, macronutrients in dietary supplements makes it possible to use them for increasing productivity in animals (Bagautdinov, 2008, 2009; Korotaeva *et al.*, 2008). In the literature, there is some information about the content of antibacterial, anti-inflammatory, radio-protective, immune-stimulating and antioxidant components in dietary supplements.

The main goal of the research is identification of the antioxidant activity of plant and chemical origin preparations in model test systems and animal organisms.

The goal is reflected in the following tasks; Establish antioxidant activity in model systems of chemical origin dietary supplements (santohin, oxymethyluracilum, emicidine, iodine-matrix compounds) and plant origin dietary supplements (erakond, milk thistle and carsil).

Show morphological and functional changes in rats after the application of antioxidants in liver pathology. To test antioxidant santohin at a hepatitis on pigs in the production conditions.

MATERIALS AND METHODS

The present studies were carried out on model test systems of dietary supplements evaluated by the chemiluminescence method on the HL-003 device. The chemiluminescent method is based on the detection of extremely weak luminescence of biological fluids and homogenates and also when comparing the control to samples activated by various chemical substances (for example by mexidol). In model test systems, lipid peroxidation processes were initiated with ferrous salts (Fe^{+2}) with formation of ROS (O , OH^{*-} , O_2H^{*-} , OCl , NO^* , H_2O_2). For the research in the model system, solutions of the claimed substances were taken in different

concentrations, their chemiluminescence was determined more than 10, respectively (Baymatov *et al.*, 2009). That's why, the concentration of iodine and pectin and other test ingredients in the sample was taken according to the recommended therapeutic dose then it was reduced by 10 times and the latter also. Such three concentrations of solutions served as a criterion for estimating the magnitude of the light-sum of chemiluminescence. To assess the effect of drugs on reactive oxygen species, they were added to the chicken yolk which is now accepted as the standard of animal origin lipids (Dzhangiryants, 2002). The chemiluminescence kinetics was altered by ferrous salts iron in the reference lipid. Spontaneous luminescence, fast illuminating flash amplitude which depended on the lipid hydroperoxides content were isolated. Antioxidant activity was determined by the duration of the latent period of luminescence, the magnitude of the light-sum of chemiluminescence, i.e., the ability of lipids to undergo oxidation (Baymatov *et al.*, 2009).

Addition of mexidol to the model system caused dose-dependent inhibition of luminescence and was the standard for comparing the studied drugs which confirmed the dependence of intensity of chemiluminescence and free radical oxidation in the reference and the studied drug (Klebanov *et al.*, 1988; Stauff and Schmidkuntz, 1962; Stauff *et al.*, 1963; Stauff and Wolf, 1964; Uchida and Peters, 1983; Vassil'Ev and Vichutinskii, 1962). The obtained data were processed statistically and reduced to the tabular data.

RESULTS AND DISCUSSION

The data obtained showed that santohinum in small and medium doses, does not lead to weakening of reactive oxygen species generation in the blood (Fig. 1).

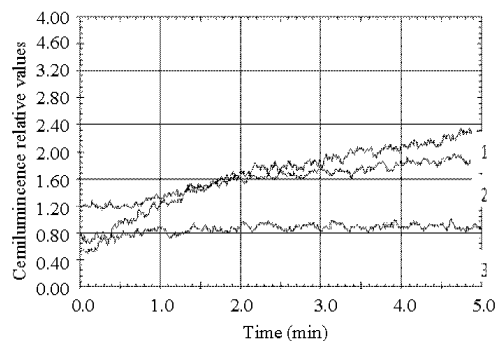


Fig. 1: Spontaneous chemiluminescence of blood changed by luminol dependent: 1) Control; 2) 0.01 mL of santohinum is added and 3) 0.01 mL of mexidol

Table 1: Comparison of Mexidol and Santochinum in model system (in % from control)

Drugs	Concentration	Cholesterol in reactive (mg/mL)	Cholesterol in lipids oxygen species model	Whole blood chemiluminescence
Control	0.000	100	100	100
Antioxidant	0.001	32.4±2.8*	24.4±2.1*	48.2±3.2*
Mexidol	0.010	10.1±0.2*	9.5±0.9*	
Santochinum	0.010	81.5±7.6*	76.2±3.6*	98.6±5.4
	0.020	76.4±5.3*	60.1±7.1*	
	0.050	68.5±3.8*	54.3±3.2*	
	0.100	51.4±3.6*	46.3±1.8*	

NB: Mean values from 5 measurements are listed here, p<0.05 is marked by*

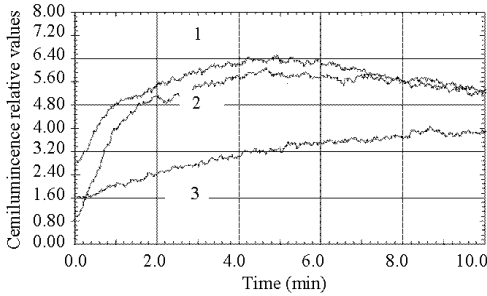


Fig. 2: Chemiluminescence of blood stimulated by luminal-zymosan: 1) Control; 2) With 0.01 mL of santochinum and 3) 0.01 mL of mexidol

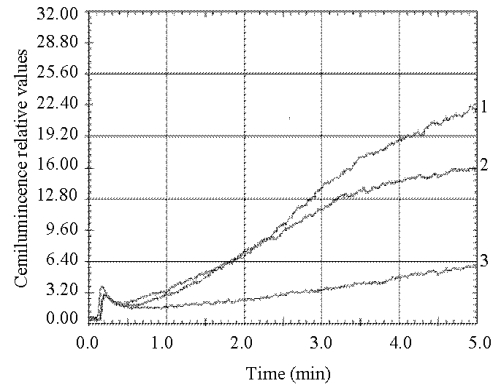


Fig. 4: Chemiluminescence biological reference lipids; 1) Control; 2) 0.1 mL of santochinum is added and 3) 0.05 mL of mexidol

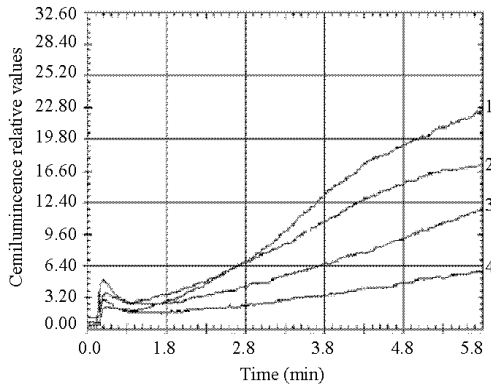


Fig. 3: Chemiluminescence (ferrum induced) of reference lipid; 1) Control, 2) 0.001 mg/mL of mexidol; 3) 0.01 mg/mL of mexidol and 4) 0.1 mg/mL of mexidol

It can be seen both during spontaneous and stimulated luminescence. Whereas many other synthetic antioxidants including mexidol have an inhibition effect which is undesirable in inflammatory processes (Fig. 2 and 3). Mexidol in different concentrations causes dose dependent inhibition of iron-induced chemiluminescence of yolk lipoproteins.

Santochinum inhibited chemiluminescence connected with lipid peroxidation but as for antioxidant intensity was approximately 2 times less effective than mexidol (Fig. 4). Numeric values of these differences are shown in Table 1.

The comparison of chemiluminescence curves show that the inhibition of chemiluminescence in models on which we studied reactive oxygen species and in reference lipids with addition of mexidol turned out to be higher than with addition of santochinum. The latter inhibits lipid peroxidation processes in a greater degree and reactive oxygen species generation in a lesser degree may take place because of the oil solution.

Figure 5 chemiluminograms of induced chemiluminescence in the model system of rats blood serum are made with the help of software “Power graph” intended for registration, illustration, processing and storage of analog-digital transformed data (Russia):

From the obtained data, it can be seen (Fig. 5) that in the model system of rats blood serum of the control group the light-sum of the luminescence is 160.16±12.3 of standard units. Addition of emicidine and mexidol solutions in a dose of 5 mg to the model system led to a significant decrease in the light-sum level by 20.37 and 50.46%, respectively. The difference between the antioxidant capacities of mexidol in comparison with emicidine is 37.78%.

Then we needed to neutralize the pro-oxidant properties of iodine but to preserve its biological activity. First, we determined the dose-dependent

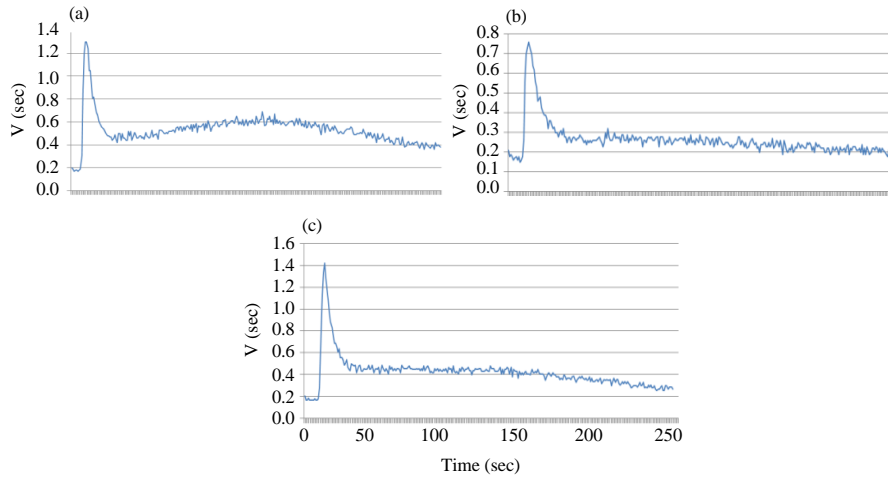


Fig. 5: a) Induced chemiluminescence of rats blood serum in the control group; b) Induced chemiluminescence of rat blood serum with the addition of 5 mg of mexidol and c) Induced chemiluminescence of rats blood serum with the addition of 5 mg of emicidine

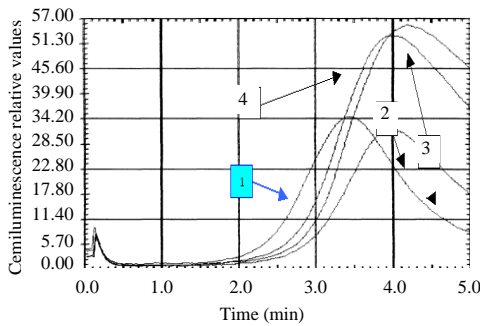


Fig. 6: Chemiluminescence of iodine solutions in model system: 1) Control; 2) 4-iodine water 0.15 $\mu\text{g}/\text{mL}$ (2); 3) 0.015 $\mu\text{g}/\text{mL}$ and 4) 0.0015 $\mu\text{g}/\text{mL}$

chemiluminescence of iodine solutions. Figure 6 shows the chemiluminescence record of the model test system with addition of ferrum salts (1-control) and iodine water. The obtained data show that iodine at a 0.015 $\mu\text{g}/\text{mL}$ concentration of distilled water causes an approximately 2-fold increase in the light-sum amount of chemiluminescence compared to the control samples. Then similar studies were conducted on pectin (Fig. 7).

Polysaccharide pectin in model systems, not in all concentrations has a pronounced antioxidant activity effect on the indices of chemiluminescence.

As can be seen from Fig. 7, only a concentration of 0.005 mg/L has an inhibitory effect on the processes of free radical oxidation. After we got the complexing compound iodine-containing dietary supplements, it was added at concentrations of 0.015, 0.0015 and 0.15 $\mu\text{g}/\text{mL}$ in the incubation medium (Fig. 8).

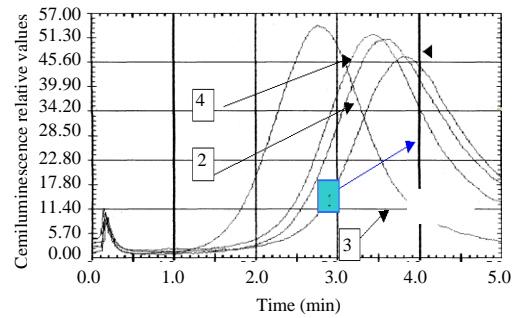


Fig. 7: Chemiluminescence of pectin solutions in different concentrations; 1) Control; 2) 4-addition of pectin in the concentration of 0.005 mg/mL (2); 3) 0.05 mg/mL and 4) μ 0.5 mg/mL

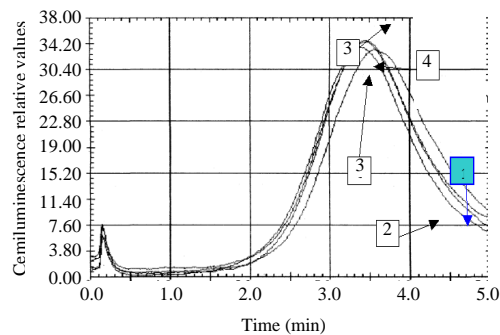


Fig. 8: Chemiluminescence of iodine-pectin compound: 1) Control; 2) 4-concentration of 0.001 $\mu\text{g}/\text{mL}$ (2); 3) 0.015 $\mu\text{g}/\text{mL}$ and 4) and 0.15 $\mu\text{g}/\text{mL}$

Figure 9 compares the chemiluminescence of iodine solution and iodine-pectin in different concentrations the

Table 2: Mexidol activity and dietary supplement in different concentrations (in%)

Product	Concentration (mg/mL)	Chemilumi-nescencein reactive oxygen speciesmodel	Chemiluminescence in lipids	Whole blood chemiluminescence
Control	0.000	100	100	100
Mexidol	0.001	32.41±2.82*	24.47±2.19*	48.2±3.21*
Oxymethyluracyl	0.001	43.12±1.12*	32.33±1.83*	156.3±11.55*
Milk thistle	0.010	73.10±6.45*	54.66±4.26*	168.3±14.47*
Carsil	0.010	54.32±3.43*	48.93±2.23*	124.7±12.34*

NB: the data are reliable p<0.05* in comparison to control

Table 3: Chemiluminescence (ferrum-induced) (relative units) and TBA-reactive substances (mol/g of tissue) in rats

Groups of experimental animals	Indices	
	Chemiluminescence	TBA
Intact animals (control)	110.5±7.2	205.5±1.5
Tetrachloromethane intoxication	145.7±5.4*	227.5±0.5*
Correction by carsil	135.3±10.3*	211.6±4.8*
Correction by milk thistle	130.6±3.2**	218.4±5.7**
Correction by milk thistle	125.5±100.2**	212.7±8.6**

NB:*Reliable in comparison with control (p<0.05)

Table 4: Luminol-dependent chemiluminescence of rats blood

Animal groups	Type of research	Chemiluminescence light-sum (relative units)
Intact animals (control)	Spontaneous chemiluminescence	1.1±0.7*
	Induced	30.4 ± 5.6**
Tetrachloromethane intoxication	Spontaneous chemiluminescence	9.9±2.5*
	Induced	60.9±8.3**
Correction by carsil	Spontaneous chemiluminescence	6.6±0.3*
	Induced	38.2±6.5**
Correction by milk thistle	Spontaneous chemiluminescence	8.1±0.3*
	Induced	45.5±1.3**
Correction by milk thistle	Spontaneous chemiluminescence	6.9±2.1*
	Induced	49.8±6.1**

NB: Reliable p<0.05 in relation to control* with tetrachloromethane intoxication

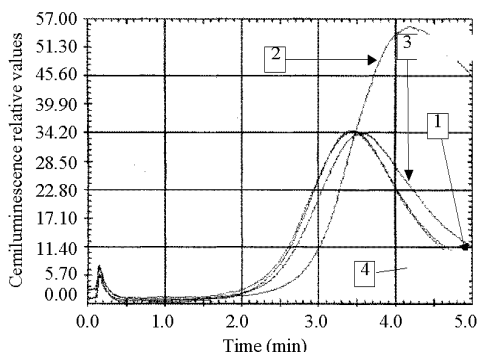


Fig. 9: Comparative chemiluminescence of iodine and complexing compound; 1) Control; 2) Iodine solution (0.015 µg/mL; 3) Pectin solution (0.05 µg/mL) and 4) Iodine-pectin (0.015 µg/mL)

biologically active substances of the dietary supplement. The solution of iodine has a pro-oxidant effect (curve 2), iodine in combination with the organic matrix (4) and pectin (3) reduces the light-sum of chemiluminescence by 1.5 times. Table 2 shows the indicators that characterize dietary supplements as antioxidants. We assessed the effect of mexidol, milk thistle, carsil and oxymethyluracyl on the indices of chemiluminescence in various model systems. Chemiluminescence in models generating reactive oxygen species and in reference lipids with the addition of milk thistle, carsil and oxymethyluracyl was inhibited and was close to the activity of the control drug mexidol. Oxymethyluracyl showed the highest properties for quenching free radical oxidation and chemiluminescence index in comparable concentrations with mexidol. Oxymethyluracyl, simultaneously does not inhibit reactive oxygen species in neutrophils, stimulating them in contrast to mexidol.

The antioxidant properties of the milk thistle and derived from it carsil were lower than in the drugs in Table 1. As a result, we increased the concentration of milk thistle and carsil solutions tenfold. However, it was a model system, one cannot ignore the doses of substances introduced into the body. The latter drugs have therapeutic doses sufficiently high in comparison with mexidol, so, due to this in their practical application they can compare their antioxidant activity. In the second series, experiments were performed on rats and the therapeutic efficacy of milk thistle, carsil and oxymethyluracyl was determined by correcting metabolic disorder. At the same time, the chemiluminescence index was measured to determine the antioxidant activity of the drugs and their therapeutic effect. Table 3 gives the light-sum of chemiluminescence rat liver homogenates and the concentration of TBA-active products (Table 4).

As can be seen from the table, milk thistle, carsil and oxymethyluracyl reduce chemiluminescence and increase TBA-reactive substances in the body tissues. Chemiluminescence of heparinized blood of control rats with luminol is typical and has a steep rise and a gradual decrease during the experiment. Spontaneous and zymosan induced chemiluminescence changes the curve steepness, the time it reaches the maximum value intensity and light-sum. With the help of zymosan, it is possible to identify the indices on which the properties of phagocytic cells depend

Table 5: Light-sumchemiluminescence of researcheddietary supplement (n = 10)

Chemiluminescence indices researched ingredients doses	Light-sum, st.un.* min	Spontaneous luminescence, standard units	Flash, standard units	Maximum luminescence, standard units	Inclination, standard units/min
Kohtpojib	104.8±4.20	1.44±0.11	19.41±1.48	42.74±1.48	3.02±0.36
Dietary supplement ‘erakond’					
The dose is 10 times lower than the regulated content	102.20±4.45	1.17±0.43	27.75±0.81***	29.12±1.25***	5.48±0.67**
Recommended dosage	89.41±2.25**	1.51±0.10	18.56±0.43	33.46±0.98***	3.46±0.22
The dose is 10 times higher than the regulated content	79.90±2.23***	1.81±0.29	32.10±0.50***	18.12±0.55***	5.13±0.34***
Dietary supplement ‘erakond-pectin’					
The dose is 10 times lower than the regulated content	89.57±1.61**	3.30±0.26***	21.39±0.18	30.25±0.41***	2.75±0.22
Physiologic cincentration	71.90±2.08***	3.97±0.51***	28.18±0.39***	17.90±0.85***	4.13±0.41
The dose is 10 times higher than the regulated content	54.94±1.69****	3.93±0.22****	26.57±1.23**	13.54±0.96***	7.71±0.50***
Dietary supplement ‘erakond-chitosan’					
The dose is 10 times lower than the regulated content	97.22±4.48	2.37±0.61	22.31±0.83	34.41±2.21**	6.48±1.09**
Recommended dosage	75.80±2.93***	3.04±0.71*	29.45±0.89***	29.83±0.97***	6.17±0.13***
The dose is 10 times higher than the regulated content	33.59±1.40***	2.04±0.17**	22.64±1.60	39.82±0.43	6.75±0.44***

NB: (**difference (p = 0.05) (***)difference (p = 0.01) (****)difference (p = 0.001)

their chemotactic activity. In the necessary states when non-specific immunity factors are to be reduced it is necessary to introduce inductors of phagocytosis such as zymosan. This drug is valuable because it simultaneously stimulates the production of phagocytes by reactive oxygen species with an increase in luminol-dependent chemiluminescence of blood. The magnitude of the light-sum of the spontaneous luminol-dependent chemiluminescence of induced blood in rats varies at the ratios 3, ..., 3.5 (the second index is divided into the first one). Under the influence of xenobiotics that change the liver function, the ratio of spontaneous and induced luminol-dependent chemiluminescence of blood increases to 5, ..., 8.

The experimental data showed that oxymethyluracyl proved to be a rather effective inhibitor of free radical oxidation and at the same time it improved the reparative regeneration of liver which was shown by morphological studies on these animals. Also, chemiluminescence of animals blood serum was studied which according to the recommended methods was induced by ferrum salts. Figure 10 shows a sample of the curve of chemiluminescence with a flash in the blood serum of laboratory animals in normal state.

The data on the properties study of milk thistle, carsil and oxymethyluracyl in Table 5 prove the restraining effect of solutions on the activity of lipid peroxidation in the blood of experimental animals.

It has been established that the complexes under analysis exhibit antioxidant activity inhibit the generation of reactive oxygen species ($O_2, OH^{\cdot}, O_2H^{\cdot}$) and have a regulatory effect on the rate and intensity of the

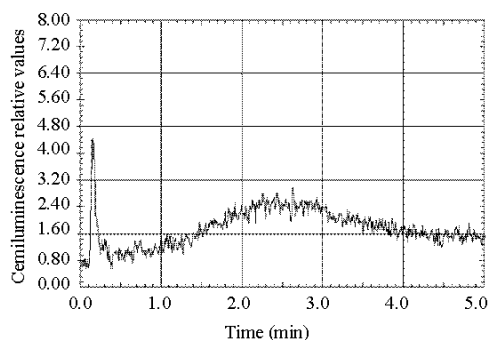


Fig. 10: A sample of the curve of chemiluminescence with a flash

oxidation processes (Table 5). It can be seen that the ‘Erakond’ dietary supplement when injected into the incubation medium of the model test system, suppresses the generation of active forms of oxygen: the indicator of the light-sum of chemiluminescence in the control is 104.8±4.20 standard units and when injected 0.1 mL solution of “Erakond” 89.41±2.25 standard units (p = 0.01). The luminescence light-sum decreased by 15.0% in relation to the control. With an increase in the concentration of “Erakond” in the model test system (10 times higher than the recommended dose), the integralchemiluminescenceindex decreases by 24.0% which shows the dose-dependent effect.

Then, the antioxidant activity of the ‘Erakond-pectin’ complex (Table 5) was evaluated in the test system where the formation of the reactive oxygen species was generated. The presented data show that the dietary supplement being analyzed has an inhibitory effect on the generation of free radicals. The light-sum index of

the chemiluminescence complex introduced at the recommended amount was 71.90 ± 2.08 standard units against 104.8 ± 4.20 standard units in the control having reduced the investigated parameters by 31.0%. With a 10-fold increase in the concentration of the test substance in the incubation medium, the light-sum of the luminescence was 54.94 ± 1.69 standard units which is 47.5% lower comparing to the control.

When studying the antioxidant activity of dietary supplements 'Erakond-chitosan', it was found that the test compound also exhibits antioxidant properties, showing the effect associated with the dose of the substance.

CONCLUSION

Available methods for assessing chemiluminescence and free radical oxidation in animals and determining the antioxidant activity of drugs and dietary supplements have become available with the advent of portable instruments. The method of chemiluminescence deserves the most serious attention, since, the registration of chemiluminescence is a reflector of the interaction of various biological substances that generate chain reactions which is a reflection of the functional state of the organism. In connection with the obtained data, we find it necessary to conduct further research and substantiate the feasibility of using model systems to identify the properties of various drugs (dietary supplements) which have antioxidant activity. Actual and promising as our studies with iodine have shown are complex-forming matrices that can improve the properties of dietary supplements.

In this case, pectin and chitosan had exactly the very characteristics and allowed the 'Erakond' dietary supplements to be transformed into a complexing compound with the new properties of 'Erakond-pectin' and 'Erakond-chitosan'. In the test system containing a dosage of dietary supplements 10 times higher than physiological a minimal quantum yield of chemiluminescence was observed against the control and the light-sum of the luminescence was reduced by 68.0%. In this research, both model systems and experimental pathological models were used and the effect of milk thistle, carsil and oxymethyluracyl on free radical oxidation, reactive oxygen species and lipid peroxidation was revealed. We have found that dietary supplements *in vitro* and *in vivo* have the ability not only to influence chain reactions but also to influence the processes of reparative regeneration. In rats with a modeled liver

pathology with tetrachloromethane, free radical oxidation was corrected by administration of these drugs and their effectiveness was determined.

The effectiveness of dietary supplements activity makes it possible to choose a drug with known properties and to use it in pathological conditions to correct the disordered functions of the body.

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