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SOME QUESTIONS OF PHARMACEUTICAL DEVELOPMENT OF INTRAVAGINAL DOSAGE FORM CONTAINING HUMULUS LUPULUS EXTRACT

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The paper presents the results of the studies on development of the process flow scheme of production and study of antioxidant activity of an intravaginal dosage form. Research by the chemiluminescence method has demonstrated direct antioxidant effect, reduction of free radical formation processes in model systems.

Keywords: biodegradable collagen repair patches containing Humulus lupulus extract, pharmaceutical development, antioxidant properties

The development of formulations for dosage forms of vaginal use is an urgent direction in pharmaceutical technology due to the fact that the wide spread of inflammatory diseases of the female sexual sphere affects the microecobiocenosis of the vagina, women's reproductive functions and, in general, demographic profile in the Russian Federation.

To reduce the manifestations of possible adverse reactions of local antibacterial therapy and to increase the time of remission of inflammatory gynecological diseases, we recommend a comprehensive, multi-level approach and a rationally selected dosage form [2].

A promising dosage form for prevention and treatment of gynecological diseases is intravaginal biodegradable collagen repair patches (BCP). A successful combination of technological and consumer properties, such as simplicity of composition and technology, stability during storage, combination of adhesion and moisture absorption, uniform distribution in the vaginal fluid, natural excretion without additional flushing and douching, justifies the relevance of the development of BCP.

Biodegradable collagen repair patches have osmotic activity and biopharmaceutical advantages, in particular, high bioavailability associated with significant bioadhesion, which

makes it possible to prolong the action of biologically active substances (BAS) [1,4,9].

Prospects for using herbal-based medicines in gynecological practice is obvious due to the multi-level impact on the causes of inflammatory diseases.

The source of obtaining intravaginal dosage forms for pharmaceutical development is Humulus lupulus, which is common in the territory of the Republic of Bashkortostan. Previous studies have shown the presence of a diverse complex of hydrophilic and lipophilic biologically active substances in the studied raw materials, among which polyphenolic compounds (flavonoids, catechins, phenolcarboxylic acids, oxycoric acids and phytoestrogens of particular significance), bitter glycosides (derivatives of acylfloroglucides – humulon, lupulon, etc.), essential oils and terpenoids are significant in quantitative and qualitative composition [1,3]. Such a variety of chemical compositions specifies the complex pharmacological action (anti-inflammatory, antioxidant, antimicrobial, capillaroprotective, phytoestrogenic, analgesic) of Humulus lupulus [3,5,7].

The object of our previous study was a liquid extract of Humulus lupulus multiple fruits as an active pharmaceutical ingredient (API) with identified pharmaceutical and technological properties.

Study purpose – development of a process flow scheme for production of BCP with a liquid extract based on Humulus lupulus multiple fruits and assessment of its antioxidant activity.

MATERIALS AND METHODS

For experimental confirmation of the formulation and process flow scheme of the production of BCP based on 2% acetic acid solution of collagen, a liquid extract of Humulus lupulus multiple fruits was used.

Studies have shown that the optimal extractant for maximum extraction of BAS from raw materials and having the best technological properties for development of BCP was 50% ethyl alcohol with the extraction module "raw material: extractant" 1:10, the extraction method is bismaceration. Standardization of liquid extract of Humulus lupulus multiple fruits was carried out on the qualitative and quantitative content of the most significant BAS groups (the amount of flavonoids (according to rutin), the amount of APG, extractives).

The presence of phenolic and lipophilic biologically active substances in a liquid extract of Humulus lupulus multiple fruits suggests the presence of antioxidant activity

The antioxidant properties of the studied medications were evaluated by their effect on iron-induced chemiluminescence (CHL) using the domestic hardware and software complex CHLM-003, for which we used standard test systems where the process of free radical oxidation (FRO) proceeded [6]. In the model systems used, the processes of formation of reactive oxygen intermediates (ROI) and reactions of lipoperoxidation processes (LPP) take place.

RESULTS AND DISCUSSION

The process flow scheme of production of BCP based on liquid extract of Humulus lupulus multiple fruits for vaginal use consists of several stages – production preparation, raw material preparation, the stage of obtaining a semi-product, the actual process of freeze-drying of patches, the stages of dispensing, packaging and labeling (Fig. 1).

The study of the effect on iron-induced chemiluminescence, simulating the formation of ROI, was carried out instrumentally. The results of the experiments were evaluated by the degree of change in the luminance indicators in the

addition of the studied samples and recalculated in % of the control. The test samples were diluted in saline, 1.0 ml of the test solutions were selected and added to the test systems.

As a control, the model systems were used without adding medicine solutions (with the addition of saline in the same volumes).

Methods for performing the experiment and preparing the reagents are described in the instructions for the hardware and software complex CHLM-003.

Study of the influence of the studied samples on the antioxidant system parameters

As the first system where the ROI was generated, 20 ml of phosphate buffer (20 mM KH_2PO_4, 105 mM KCl) with adding the solution of luminol (10–5 M) and sodium citrate (50 mM). The pH value of the solution obtained was brought up to 7.45 units by titration with a saturated solution of potassium hydroxide. Into 20 ml of the model system 1 ml of each of the studied solutions was introduced: 1 – water solution of BCP with

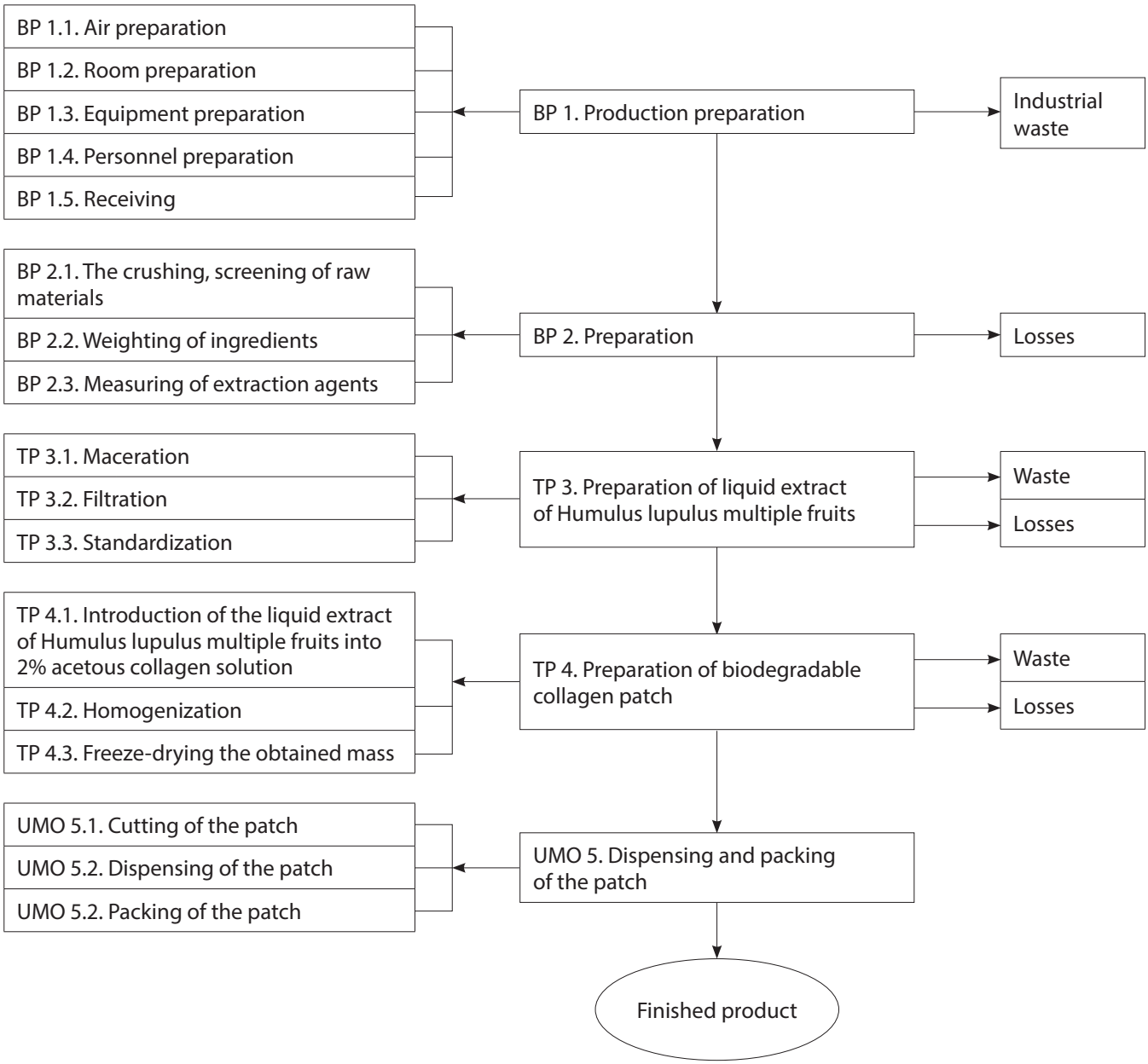

FIG. 1. *Technological stages of production of the BCP on the basis of liquid extract of Humulus lupulus multiple fruits*

liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C), 2 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C), 3 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C), 4 – liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10). For initiation of reactions that are accompanied by generating ROI, 1 ml of 50 mM solution of Fe^{2+} salts was introduced. Registration of the luminescence was continuing for 5 minutes with constant remixing.

Chemiluminescence of model systems was characterized by spontaneous luminescence, rapid flash, and then developing a slow flash. The main most informative characteristics of chemiluminescence were the amplitude of the maximum glow and the light sum of the glow, determined by the intensity of radiation.

Study of the influence of the studied samples on the formation of lipoperoxidation products

For the study, a model system was used where the reactions of formation of final peroxidation products occur. The influence of the studied samples on the lipid peroxidation processes (LPP) was studied in lipids of chicken yolk, which have a composition that is similar to blood lipids. Lipids were obtained by homogenizing the chicken yolk in a phosphate buffer in ratio of 1:5 and by the following 20-fold dilution, 20 ml was taken. Into 20 ml of the model system 1 ml of each of the studied solutions were introduced: 1 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C), 2 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C), 3 – water solution of BCP with liquid

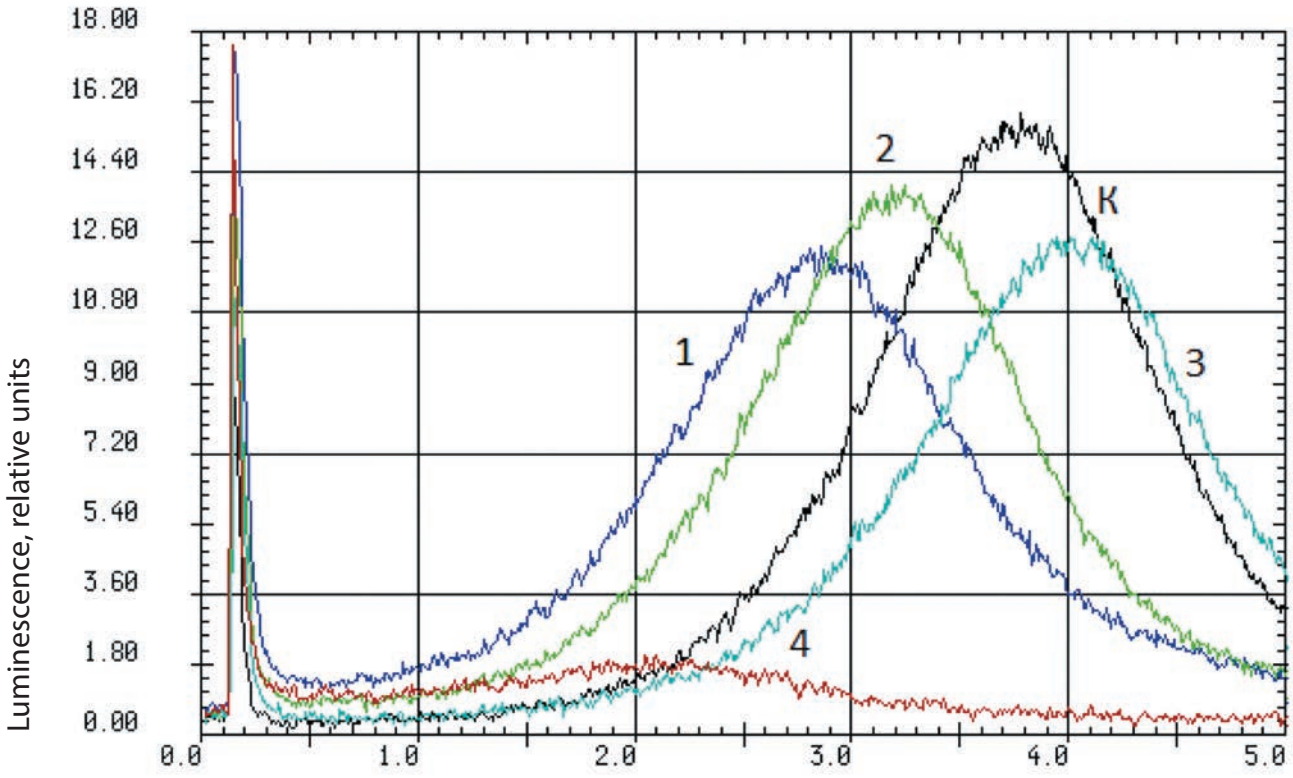

FIG. 2. *Influence of BCP with liquid extract of Humulus lupulus multiple fruits and liquid extract of Humulus lupulus multiple fruits on the FRO processes in the ROI model system. Designations: K – control, 1 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C), 2 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C), 3 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C), 4 – liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10).*

extract of Humulus lupulus multiple fruits (0.8 g; t = 40°C), 4 – liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10).

Adding 1 ml of 50 mM Fe^{2+} solution to the system led to initiation of processes of oxidation of unsaturated fatty acids that are part of lipids, and to formation of end products of lipoperoxidation, which was accompanied by luminescence. The level of spontaneous luminescence characterized the intensity of lipid peroxidation processes before the introduction of the catalyst; the amplitude of the rapid flash reflected the rate of oxidation of Fe^{2+} ions and formation of ROI and lipid hydroperoxides; the duration of the latency period correlated with the antioxidant activity of the studied medication. The value of the light sum of the luminescence determined the ability of lipids to undergo oxidation.

Data on the influence of the studied samples on CHL in the model system generating ROI are shown in Fig. 2.

Quantitative characteristics of the influence of the studied samples on FRO are presented in Table 1.

Adding of aqueous solutions of BCP with liquid extract of Humulus lupulus multiple fruits of different concentrations into the model system

Table 1

INFLUENCE OF BCP WITH LIQUID EXTRACT OF HUMULUS LUPULUS MULTIPLE FRUITS AND LIQUID EXTRACT OF HUMULUS LUPULUS MULTIPLE FRUITS ON THE LIGHT SUM OF CHL IN THE MODEL SYSTEM GENERATING ROI

№	Sample of the studied products	Light sum		Spontaneous luminescence, abs.	Flash abs.	Maximal luminescence	
		abs.	% in relation to control			abs.	% in relation to control
1.	Control	26.63	100	0.45	8.96	15.91	100
2.	Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C)	25.64	96.2	0.70	17.47	12.49	78.5
3.	Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C)	25.98	97.6	0.52	13.55	14.09	88.6
4.	Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C)	22.35	83.9	0.51	11.16	12.77	80.2
5.	Liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10)	6.45	24.2	1.21	16.53	2.25	14.1

*Note. The intensity of the luminescence of model systems without addition of the studied substances is assumed to be 100%. The average data of 6 measurements is вызушашув. * – significant differences ($p < 0.05$) are marked.*

shortened the latent period, for BCP with liquid extract of Humulus lupulus multiple fruits at concentrations of 4.0 and 2.0 and fresh liquid extract of Humulus lupulus multiple fruits the slow flash started and went out early. All samples of solutions of BCP with liquid extract of Humulus lupulus multiple fruits of different concentrations reduced the parameters of the light sum of the luminescence of CHL in the model system depending of a dose, the most effective solution was BCP with liquid extract of Humulus lupulus multiple fruits at a concentration of 0.8 g.

Freshly prepared liquid extract of Humulus lupulus multiple fruits, which is the basis for obtaining BCP, almost 3.5–4 times reduced the most significant factor of CHL in comparison with solutions of BCP with liquid extract of Humulus lupulus multiple fruits and control.

Further, a model system was used for the study, where the processes of final lipoperoxidation occur. Data on the influence of the studied samples on CHL in this model system are presented in Fig. 3.

Introduction of samples into the egg yolk lipoprotein model system was also accompanied by decrease in the parameters of the light sum and amplitude of maximum luminescence (Fig. 2).

Quantitative characteristics of studied parameters are presented in table. 2.

The results of the study showed that all samples of solutions of BCP with liquid extract of Humulus lupulus multiple fruits of different concentrations reduced the light sum of the luminescence and maximal luminescence of CHL in the model system depending of a dose, the most effective solution was BCP with liquid

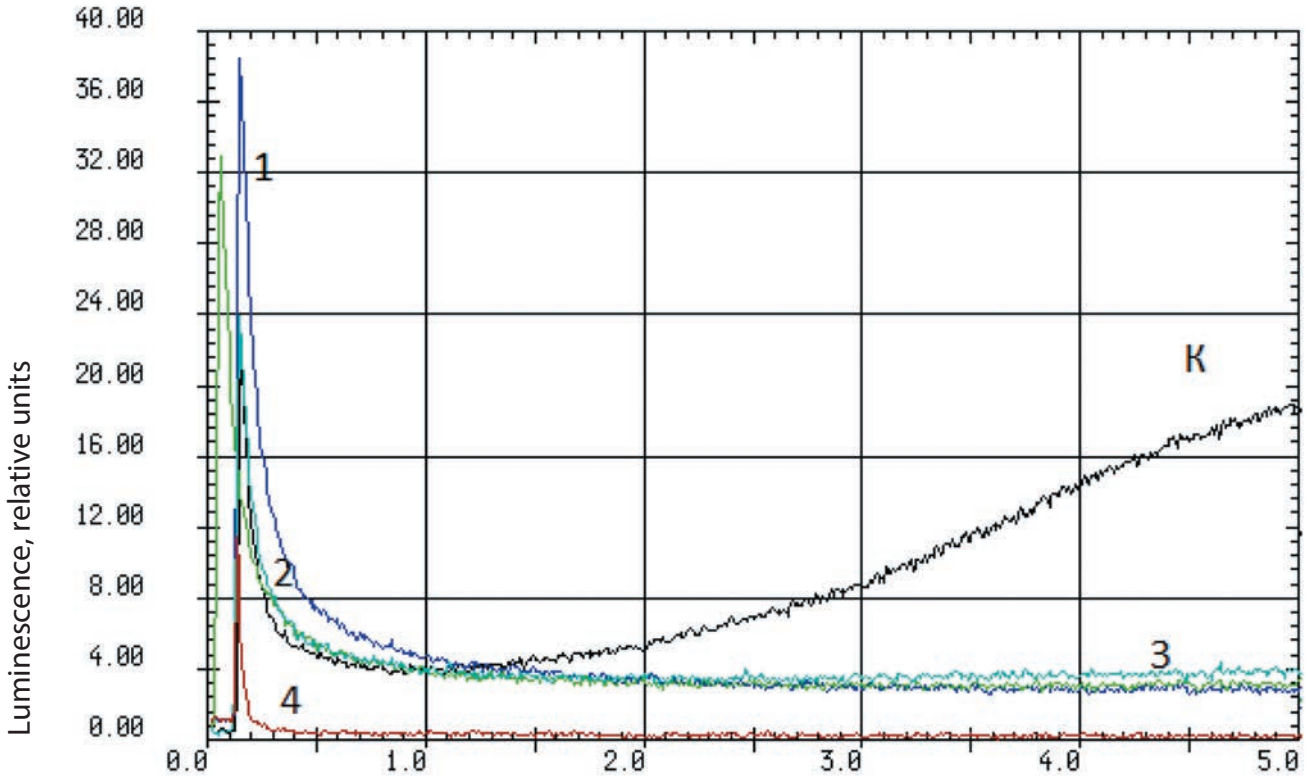

FIG. 3. *Influence of BCP with liquid extract of Humulus lupulus multiple fruits and liquid extract of Humulus lupulus multiple fruits on the FRO processes in the model system of the end lipid peroxidation. Designations: K – control, 1 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C), 2 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C), 3 – water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C), 4 – liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10).*

Table 2

INFLUENCE OF BCP WITH LIQUID EXTRACT OF HUMULUS LUPULUS MULTIPLE FRUITS AND LIQUID EXTRACT OF HUMULUS LUPULUS MULTIPLE FRUITS ON THE LIGHT SUM OF CHL IN THE MODEL SYSTEM WHERE THE END LIPID PEROXIDATION PROCESSES TAKE PLACE

№	Sample of the studied products	Light sum		Spontaneous luminescence, abs.	Flash abs.	Maximal luminescence	
		abs.	% in relation to control			abs.	% in relation to control
1.	Control	45.32	100	0.53	22.77	18.96	100
2.	Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (4.0 g; t = 40°C)	21.56	47.6	1.13	38.48	10.60	55.9
3.	Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (2.0 g; t = 40°C)	20.28	44.7	18.84	32.89	7.22	38.1
4.	Water solution of BCP with liquid extract of Humulus lupulus multiple fruits (0,8 g; t = 40°C)	20.48	45.1	0.48	23.94	6.96	36.7
5.	Liquid extract of Humulus lupulus multiple fruits freshly prepared (50% ethyl alcohol; bismaceration; 1:10)	1.86	4.1	1.16	11.39	0.68	3.6

extract of Humulus lupulus multiple fruits at concentration of 0.8 g.

The model system luminescence level was suppressed to almost zero when adding freshly prepared liquid extract of Humulus lupulus multiple fruits, which is the basis for obtaining BCP.

CONCLUSION

The process flow scheme of production of BCP with liquid extract of Humulus lupulus multiple fruits was developed.

Studies have shown that in both model systems, the studied samples acted in the same direction: they reduced formation of free radicals and showed a direct antioxidant effect.

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