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Optimal concentration selection of active pharmaceutical ingredients in the cream-mask intended for androgenic alopecia treatment according to experimental results on paramecium caudatum biological model

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Abstract
The article presents the results of the biological study about influence of the active pharmaceutical ingredients (API) concentration on the cream-mask antioxidant properties. Those properties were revealed in prolonging the duration of the Paramecium caudatum motor activity in the environment of toxic substances. The obtained data allowed to select the API optimal concentration in the cream-mask, namely 10% of the Sophora japonica tincture and 5% of the Serenoa repens dry extract.

Keywords: Androgenic alopecia, cream-mask, Sophora japonica tincture, Serenoa repens extract, concentration of active pharmaceutical ingredients, antioxidant action

Introduction
Problems of the skin and its appendages significantly affect the psycho-emotional status and quality of life. Therefore, effective treatment of dermato-cosmetic diseases is an important task of modern medicine. Alopecia (excessive hair loss) is one of the most commonly encountered dermatologic pathology. Alopecia is a multi-etiologic disease that, depending on the trigger factors and external manifestations, is divided into different types: androgenic alopecia (AGA), telogen and anagen effluvium, alopecia areata etc. [1]

AGA is one of the most common forms of baldness that occurs in men (Male pattern hair loss) and women (Female pattern hair loss) with hereditary predisposition [2-4]. A key role in the AGA development is played by the interrelationship between male sex hormones testosterone (TS) and dihydro-testosterone (DHT), specific androgen receptors in the hair follicle (HF) cells and 5-α-reductase enzyme [3, 5]. Modern scientific researches confirm the involvement of new mechanisms in AGA development, such as micro-inflammation, prostaglandin misbalance, oxidative stress [6].

For complex treatment of AGA patients, the following dietary supplements and drugs are used [5-9]:

- Synthetic peripheral vasodilators: minoxidil, aminexil;
- 5-α-reductase enzyme inhibitors (the enzyme converts TS into the most potent androgen DHT): finasteride, dutasteride, β-sitosterol;
- Androgen receptor blockers: spironolactone, cyproterone acetate, fluoride, phytosterols;
- Non-specific irritants that improve the circulation in HF: Capsicum annum tincture, 20% solution of turpentine in Castor oil, fresh-water sponge (Spongilla lacustris L.) powder, nettle juice etc;
- Venotonics and capillary protectors of natural and synthetic origin: escin, escusan, quercetin, routine, xanthinol nicotinate, pentoxifylline, troxerutin etc.
- Antioxidants of plant origin: flavonoids, ginkgolides, hydroxycinnamic acids, phytosterols, carotenoids, tocopherols etc.

For AGA therapy drugs containing a minoxidil solution for the cutaneous application and finasteride for oral administration are most often prescribed [5, 6]. However, these medicines are characterized with side effects and contraindications. In particular, minoxidil negatively affects the activity of the cardiovascular system,

causes the withdrawal syndrome (exacerbates hair loss after treatment cessation). Finasteride weakens the erectile function and contraindicated in fertile women due to fetus feminization [3, 10]. Taking into account the problems, it is important to create effective and safe medicinal cosmetic remedies with plant API, which would affect the main links of the AGA pathological process.

On the basis of theoretical justification and experimental studies, it was developed the emulsion base composition of the cream-mask [11]. Serenoa repens dry extract (SRDE) and Sophora japonica tincture were chosen as API. SRDE contains a group of free and conjugated with fatty acids or sugars phytosterols. These biological active substances reveal anti-androgenic action through 5-α-reductase inhibition and blocking androgen receptors, and they also possess antioxidant and anti-inflammatory effect [12, 13]. Sophora japonica tincture contains a high concentration of flavonoids, which have antioxidant and capillary protective properties [14]. One of the AGA pathogenesis mechanisms is revealed in the activation of oxidation processes. Therefore, the antioxidant effect of API on the HF structure is one of the key directions in AGA treatment [6].

Justification of the API concentration usually is carried out in relation to the data of experimental microbiological or pharmacological studies. Selecting the API concentration on the basis of microbiological studies is possible when they show expressed antimicrobial properties that are necessary for the therapeutic action demonstration. Such studies are relatively non-prolonged, not labor-intensive and provide an opportunity to obtain reliable digital information [15]. In the case of pharmacological activity, which is not related to antimicrobial effect, the selection of API concentration is carried out on animals. However, such studies require the involvement of a large number of animals, and are time-consuming, costly and contradictory to the modern standards of bioethic. Therefore, the use of a biological model on Paramecium caudatum infusorians is a promising research direction to choose the API concentration. Paramecium caudatum combine morphological features of a single cell, but respond to the external environment as independent organisms. Paramecium caudatum infusorians are easily cultivated, so when studying their growth, reproduction and behavior, a large amount of digital information can be quickly and easily obtained [16, 17].

The aim of research: choosing the cream-mask API concentration based on the experimental results of antioxidant properties using the Paramecium caudatum biological model.

Materials and Methods

Objects of research

Samples of the cream-mask with different API concentration:

- 5 samples of the cream-mask containing different concentration of Sophora japonica tincture, namely 5%, 7.5%, 10%, 12.5%, 15%.
- 8 samples of the cream-mask containing different concentration of SRDE from 1% to 8% with 1% increments.

Biological object

Paramecium caudatum infusorians, which were placed into the Lozina-Lozynsky nutrient environment (medium) at 20-26 ºC temperature and 6.2-7.8 pH. Live yeast Rhadotorula gracilis with the addition of wheat flour was used to feed the Paramecium [16].

Method

To investigate the antioxidant properties of the studied samples it was evaluated their influence on the Paramecium life duration in the environment with the addition of toxic substances. The following toxicants were used: 1% hydrogen peroxide solution (which *in vitro* splits to peroxide radicals and damages mainly the lipid part of the membrane); 14% ethanol (which damages the protein structures of the biomembrane) [16].

To conduct the study, 1% solutions (mixtures) of the studied samples with purified water were prepared and their pH was determined. To ensure the normal functioning of the Paramecium the pH should be in the range from 6.2 to 7.8.

In the control group 2 drops of medium were applied to the microscope slide: thirst drop was a culture medium (intact microorganisms), the second drop was with an added toxicant solution of the corresponding volume. In the experimental samples to the second drop, together with toxicant, it was added a drop of the 1% prepared solution (mixture). The duration of Paramecium motor activity up to the microorganism movement termination was evaluated with a microscope.

Results and discussion

The study results of the influence of Sophora japonica tincture concentration in the cream-mask on the duration of the Paramecium physiological activity in the environment of toxic substances are shown in Table 1 and Fig. 1.

Table 1: Influence of the experimental samples with Sophora japonica tincture on the duration of the Paramecium caudatum physiological activity in the environment of toxic substances (n = 5, P = 95%)

Cream-mask experimental samples	Duration of the Paramecium caudatum motor activity			
	1% hydrogen peroxide solution		14% ethanol	
	Time, s	Time, min	Time, s	Time, min
Control samples	307	5.12	132	2.20
	315	5.25	135	2.25
	306	5.10	129	2.15
	312	5.20	132	2.20
	307	5.12	135	2.25
		5.16 ± 0.06		2.21 ± 0.03
Samples with 5% of Sophora tincture	331	5.52	231	3.85
	337	5.62	229	3.82
	330	5.50	230	3.84
	329	5.48	234	3.90
	335	5.58	233	3.88
		5.54 ± 0.05		3.86 ± 0.03

7.5%	345	5.75	231	4.15
	343	5.72	234	4.12
	348	5.80	240	4.10
	349	5.82	220	4.18
	343	5.72	224	4.20
		5.76 ± 0.04		4.15 ± 0.03
10%	373	6.22	255	4.25
	376	6.26	259	4.32
	371	6.18	258	4.30
	372	6.20	257	4.28
	371	6.19	255	4.25
		6.21 ± 0.02		4.28 ± 0.02
12.5%	367	6.12	224	4.71
	369	6.15	228	4.75
	366	6.10	222	4.76
	365	6.09	220	4.70
	368	6.14	217	4.68
		6.12 ± 0.02		4.72 ± 0.03
15%	351	5.85	252	4.20
	353	5.88	255	4.25
	354	5.90	249	4.15
	348	5.80	251	4.18
	349	5.82	253	4.22
		5.85 ± 0.03		4.20 ± 0.03

In the control group the duration of infusorians' motor activity in a toxicant environment of 1% hydrogen peroxide solution was 5.16 min and in 14% ethanol was 2.2 min correspondently. When to the medium it was applied the cream-mask samples with Sophora japonica tincture different concentrations, the Paramecium lifespan lengthened in comparison to the control. In samples with hydrogen peroxide, the time of microorganisms' activity gradually elongated with an increase in the tincture concentration from 5% to 10%, and ranged from 5.54 min to 6.21 min respectively. With the application of the 12.5% API the protective action progress stopped and was characterized by Paramecium activity in the range of 6.12 min. With the introduction of the 15% tincture the antioxidant activity decreased in comparison with the 10% API and lasted 5.85 min.

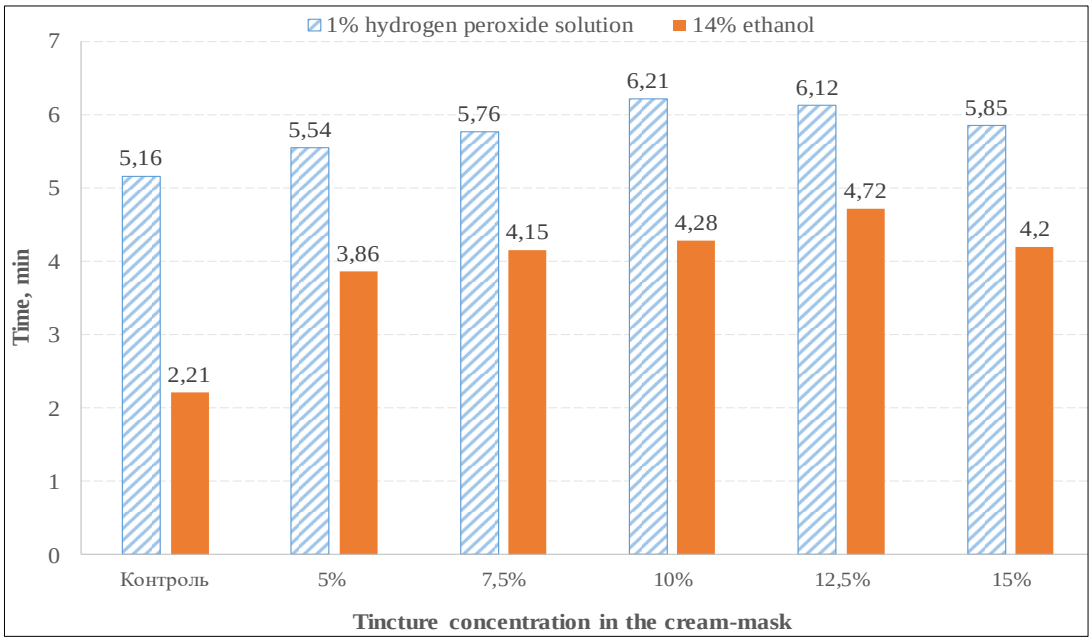

Fig 1: Influence of Sophora japonica tincture concentration in the cream-mask on the duration of the Paramecium caudatum physiological activity in the environment of toxic substances.

In the environment of the toxicant 14% ethanol it was also observed the increase of the Paramecium caudatum motor activity in the studied samples with Sophora japonica tincture compared to the control samples. Time of motor activity intensively increased with the introduction of 5% API and was about 3.9 min in contrast to the control – 2.21 min. The protective effect gradually elongated with an increase of the API concentration up to 12.5% and showed a time delay of up to 4.7 min. However, the introduction of the 15% tincture led to a decrease in the cream-mask antioxidant properties, since the time of microorganisms' motor activity (4.2 min) was lower than in the sample with the 10% API (4.28 min). Reducing the protective action when increasing the API concentration can be explained by the fact that the tincture contains ethyl alcohol, which adversely affects infusorians. Accordingly, taking into account the results of Sophora

japonica tincture concentration influence on the prolongation of the Paramecium caudatum motor activity in the media of both toxicants, it was selected 10% of the API.

The experimental results presented in Table. 2 and on Fig. 2 showed that with an increase in SRDE concentration from 1% to 5% in samples with the toxicant hydrogen peroxide the Paramecium motor activity gradually increased. The control sample had a result of 5.16 min, but in the sample with 5% SRDE the Paramecium lifetime continued to 5.7 min. With the subsequent increase of the API concentration antioxidant action practically did not change and at 8% API content the Paramecium lifetime was 5.63 min.

Table 2: Influence of the experimental samples with SRDE on the duration of the Paramecium caudatum physiological activity in the environment of toxic substances (n = 5, P = 95%)

Cream-mask experimental samples	Duration of the Paramecium caudatum motor activity			
	1% hydrogen peroxide solution		14% ethanol	
	Time, s	Time, min	Time, s	Time, min
Samples with 1% SRDE	312	5.2	153	2.55
	315	5.25	151	2.52
	311	5.18	155	2.58
	310	5.16	154	2.56
	313	5.22	152	2.54
		5.20 ± 0.03		2.55 ± 0.02
2%	322	5.36	161	2.68
	323	5.38	162	2.7
	324	5.40	163	2.72
	320	5.32	159	2.65
	321	5.35	160	2.66
		5.36 ± 0.02		2.68 ± 0.02
3%	329	5.48	173	2.88
	330	5.50	174	2.90
	331	5.52	175	2.92
	328	5.46	171	2.85
	327	5.45	172	2.86
		5.48 ± 0.02		2.88 ± 0.02
4%	335	5.58	183	3.05
	337	5.62	180	3.00
	338	5.64	182	3.04
	334	5.56	178	2.96
	336	5.60	179	2.98
		5.60 ± 0.02		3.01 ± 0.03
5%	341	5.68	189	3.15
	343	5.72	190	3.16
	344	5.74	192	3.20
	340	5.66	189	3.15
	342	5.70	188	3.14
		5.70 ± 0.02		3.16 ± 0.02
6%	340	5.66	192	3.20
	341	5.68	195	3.25
	342	5.70	191	3.18
	337	5.62	192	3.20
	339	5.65	196	3.26
		5.66 ± 0.02		3.22 ± 0.03
7%	338	5.63	197	3.28
	337	5.62	195	3.25
	339	5.65	198	3.30
	339	5.65	192	3.20
	336	5.60	193	3.22
		5.63 ± 0.02		3.25 ± 0.03
8%	339	5.65	199	3.32
	336	5.60	198	3.30
	338	5.64	197	3.28
	338	5.64	195	3.25
	337	5.62	201	3.35
		5.63 ± 0.02		3.30 ± 0.03

In the samples with the toxicant 14% ethanol, there was a significant increase in the Paramecium motor activity compared to control. Time of infusorians' motor activity intensively lengthened with an increase in SRDE concentration from 1% to 5%. So the control sample showed a result of 2.21 min, 1% SRDE – 2.55 min, and 5% SRDE – 3.16 min. SRDE concentration increase up to 6% did not significantly affect the cream-mask antioxidant effect, since the lifetime of the microorganisms was 3.22. The next increase in SRDE content up to 7% and 8% did not significantly affect the infusorians' lifetime, as the values were 3.25 min and 3.3 min respectively. Taking into account the results of the SRDE concentration influence on the Paramecium motor activity prolongation in the environments of both toxicants, it was chosen 5% of the API.

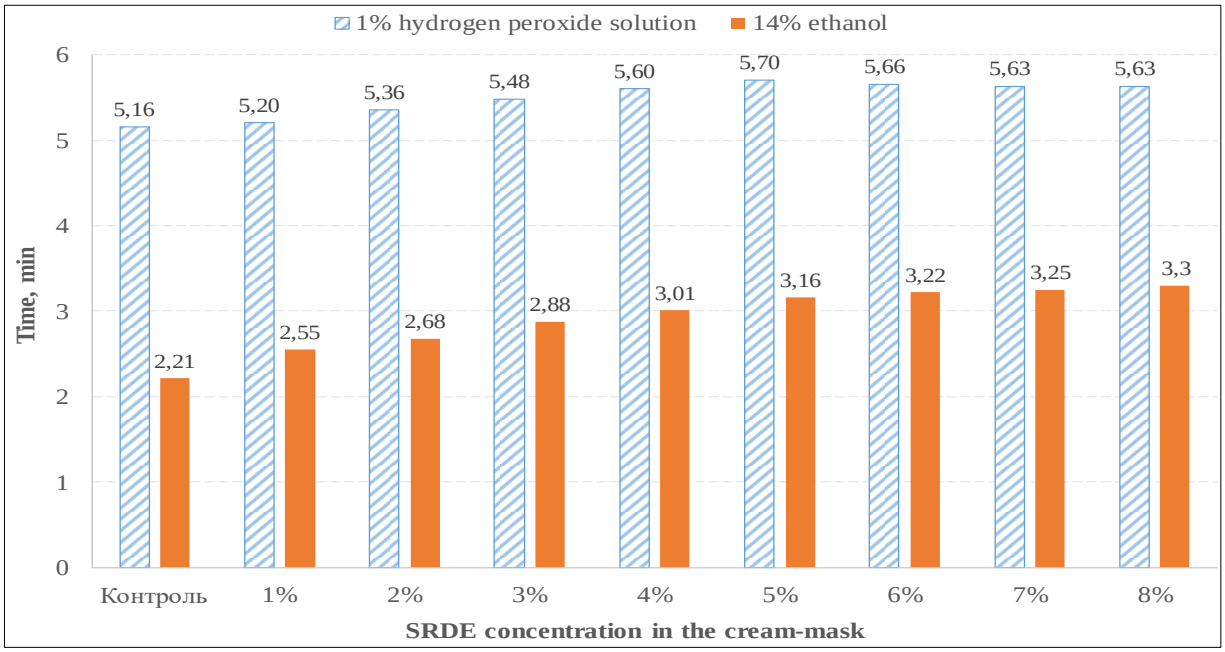

Fig 2: Influence of SRDE concentration in the cream-mask on the duration of the Paramecium caudatum physiological activity in the environment of toxic substances.

Conclusion

The biological study was conducted on the Paramecium caudatum model to investigate the effect of the API concentration on the cream-mask antioxidant properties in the environment of toxic substances. It was proven that the API concentration in the cream-mask, namely 10% of Sophora japonica tincture and 5% of Serenoa repens dry extract, was optimal with regard to prolongation of the Paramecium caudatum motor activity.

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