CHOICE OF THE PRESERVATIVE IN THE COMPOSITION OF VAGINAL GEL WITH RESVERATROL AND HYALURONIC ACID FOR TREATMENT OF UROGENITAL SYMPTOMS IN THE CLIMAX PERIOD

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Hormonal disorders in women in the climax period are usually accompanied by a series of unpleasant symptoms from the genital and urinary system such as dryness and inflammation of the mucous membranes of the genitals, painful urination, and the like. These affect the quality of daily life of a woman and require treatment. Rational is the use of local semisolid drugs in the form of gels based on plant material of phytoestrogenic action. They have a localized mild effect and exhibit no systemic side effects, unlike synthetic hormonal drugs.

Previous studies have found the optimal composition and technology of vaginal gel with resveratrol, which exhibits phytoestrogenic and anti-inflammatory effects, and hyaluronic acid, which moisturizes and promotes the regeneration of mucous tissues [1,2].

Microbial contamination of a product is possible in the process of production, storage and use, which can shorten the shelf life and adversely affect the safety profile of the drug. Therefore, an important criterion for evaluating the quality of a medicinal product is microbiological purity [3].

To ensure stability and quality of the drug throughout the shelf life, a preservative must be added to the gel. To date, it is relevant to use combined non-paraben preservatives, which have a wide range of antibacterial action and are safe for the human body [4, 5].

The purpose of the study was to determine and compare the antimicrobial effectiveness of preservatives in the composition of the vaginal gel.

Objects and methods of research

The objects of the study were samples of vaginal gel with various antimicrobial preservatives (Table 1), which have no irritant effect on the mucous membranes, and are safe for the human body [4,6]. The preservative content in a sample was selected according to the manufacturer's recommendations.

Table 1 Samples for microbiological research.

Sample number	Type of preservative
1	Cosgard 0.5% (Incl: Benzyl alcohol, Dehydroacetic acid, Aqua). Manufactured by Aroma zone (France).
2	Leucidal 2.0% (Incl: Leuconostoc / Radish Root Ferment Filtrate). Manufactured by Active Micro Technologies US.
3	Euxyl 0.7% (Incl: Ethylhexylglycerin, Phenoxyethanol). Manufactured by Schülke & Mayr GmbH, Germany.
4	without preservative

Preservative selection studies have been conducted using the antimicrobial preservative efficacy evaluation technique described in SPU 2.0 (Vol. 1, Sec.5.1.3, p. 773). The principle of the method is that to the samples of the finished dosage form with various preservatives, which are in the primary packaging, introduce a certain number of test microorganisms and store these samples at a certain temperature (from 20 to 25 ºC) in a dark place. Immediately after inoculation and at specified intervals (2, 7, 14 and 28 days), samples (usually 1.0 g) are taken from the inoculated samples and the number of viable microorganisms is determined [6].

All studies were performed in aseptic conditions, using a laminar box (Biological Safety Cabinet AC2-4E1 " Esco", Indonesia).

Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Candida albicans ATCC 885-653, Aspergillus brasiliensis ATCC 16404 were used as test microorganisms for inoculation of gel samples [6].

We used the nutrient media of HiMedia Laboratories Pvt. Limited (India), namely, soya-casein agar and Saburo-dextrose agar without antibiotics. The nutrient media were prepared according to the manufacturer's requirements. Each series was tested for growth properties [6].

Results and Discussion

Prior to the studies, experiments were performed to determine the growth properties of the nutrient media (the number of colonies grown when cropped with an appropriate number of microorganisms).Nutrient media were inoculated with a small number of test strains of microorganisms (10-10^2 colony forming units per 1ml of medium - CFU / ml).The original culture of each of the test microorganisms (*Staphylococcus aureus* , *Pseudomonas aeruginosa*) was sown on the surface of a thick soybean-casein nutrient medium, while fungi cultivation (*Candida albicans* , *Aspergillus brasiliensis*) was done at a thick Sabouraud-dextrose nutrient medium without addition of antibiotics. The results of the studies are shown in table 2.

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For the preparation of cultures of test microorganisms cropped bacteria on the surface of a dense nutrient soybean-casein medium, in the case of fungi sowing used Sabouraud-dextrose nutrient medium without the addition of antibiotics. *Staphylococcus aureus* and *Pseudomonas aeruginosa* cultures were incubated in TCO-80 thermostat at 30-35 ° C for 18-24 h, *Candida albicans* culture incubated at 20-25 ° C for 2-3 days, *Aspergillus brasiliensis* culture at 20-25 ° C for 7 days.

For the preparation of suspensions of bacterial cultures and cultures of the fungus *Candida albicans, the* microbial mass was washed from the surface of the nutrient medium with a sterile suspending solution containing 9 g / l sodium chloride P, transferred into a sterile tube and brought the content of microorganisms to 10^8 cells per 1ml.In the preparation of *Aspergillus brasiliensis* culture suspension used a sterile suspending solution containing 9 g / l sodium chloride P and 0.5 g / l polysorbate-80 P and brought the content of spores to 10^8 in ml. From each suspension, immediately after its preparation, a sample was taken and the number of colony forming units (CFU) in 1 ml was determined by direct crop on Petri dishes on dense culture media used for the initial cultivation of the test cultures.

The results of the studies are shown in table 2.

Table 2. The effectiveness of the antimicrobial action of preservatives.

Test strains of microorganisms	Nutrient media	Cultivation conditions		Conclusion
		temperature, ºC	cultivation period	
Staphylococcus aureus ATCC 6538	Soy-casein	30-35 ºC	18-24 h	colonies and cells morphologies are typical
Pseudomonas aeruginosa ATCC 9027	Soy-casein	30-35 ºC	18-24 h	colonies and cells morphologies are typical
Candida albicans ATCC 885-653	Sabouraud-dextrose	20-25 ºC	2-3 days	colonies and cells morphologies are typical
Aspergillus brasiliensis ATCC 16404	Sabouraud-dextrose	20-25 ºC	5-7 days	colonies and cells morphologies are typical

The data presented in table 2 demonstrate that all cultures of microorganisms corresponded to the taxonomic designation of the strain, and the morphology of colonies when cultured on nutrient media and the morphology of cells under microscopy are typical.

To determine the antimicrobial activity of the preservatives in the gel composition, each sample of the semisolid dosage form under study was introduced with a suspension containing test microorganisms with a load of 10^8 CFU in 1 ml. In the sample, the microbial load should be from 10^5 CFU / ml to 10^6 CFU / ml.

Non-preservative drug samples were also inoculated with cultures of *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 885-653, *Aspergillus brasiliensis* ATCC 16404 and left for microbial contamination determination .On the 5th day of storage, all samples were contaminated and had a characteristic growth of microorganisms, thus the result showed that this dosage form requires the addition of antimicrobial preservatives.

The criterion for evaluating the effectiveness of antimicrobial preservatives was the logarithm (lg) of reducing the number of viable cells of microorganisms for the appropriate storage period after contamination of samples, which, according to the requirements of SPU, should be at least 2 after 2 days, and not less than 3 after 7 days. Further (after 28 days), the number of viable bacteria cells should not increase. This figure relative to viable fungal cells for 14 days should be at least 2, after 28 days of storage of inoculated gel samples, the number of viable fungal cells should not increase.

After inoculation of the samples with microorganisms (loading 10^5 CFU / ml - 10^6 CFU / ml), they were thoroughly stirred for uniform distribution of microorganisms within the sample .Then, sampling of each sample was done, immediately after seeding and at regular intervals of time (2 days 7, 14 and 28 days) and carried out direct sowing on agar nutrient medium to determine the number of viable microorganisms and calculate the logarithm of the reduction in the number of viable bacteria

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Staphylococcus aureus ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027 and fungi *Candida albicans* ATCC 885-653 and *Aspergillus brasiliensis* ATCC 16404.

The results of the studies are shown in Table 3.

Table 3. Antimicrobial effectiveness of preservatives in the analysed gel samples.

Test cultures of microorganisms	Preservative (Concentration)	Microbial loading after inoculation, lg CFU / ml	Lg of decrease in initial microbial load (SPU requirements / sample)			
			2 days	7 days	14 days	28 days
Staphylococcus aureus ATCC 6538	Cosgard 0.5%	5,66±0,02	2/4,39 ±0,01	3 / ND	ND	NI / ND
	Leucidal 2.0%	5,54±0,01	2/3,32 ±0,01	3 / ND	ND	NI / ND
	Euxyl PE 9010 0.7%	5,74±0,01	2/3,49 ±0,01	3 / ND	ND	NI / ND
Pseudomonas aeruginosa ATCC 9027	Cosgard 0.5%	5,74±0,01	2/2,85 ±0,03	3 / ND	ND	NI / ND
	Leucidal 2.0%	5,66±0,02	2/2,43 ±0,03	3/3,57 ±0,02	ND	NI / ND
	Euxyl PE 9010 0.7%	5,74±0,01	2/2,74 ±0,02	3/4,05 ±0,03	ND	NI / ND
Candida albicans ATCC 885-653	Cosgard 0.5%	5,66±0,02	2,27 ±0,02	3,57 ±0,02	2 / ND	NI / ND
	Leucidal 2.0%	5,54±0,01	1,27 ±0,02	3,12 ±0,01	2/3,97 ±0,02	NI / ND
	Euxyl PE 9010 0.7%	5,74±0,01	1,49 ±0,01	3,97 ±0,01	2/4,05 ±0,03	NI / ND
Aspergillus brasiliensis ATCC 16404	Cosgard 0.5%	5,36±0,02	1,49 ±0,01	3,12 ±0,02	2/HB	NI / ND
	Leucidal 2.0%	5,39±0,01	1,35 ±0,02	2,49 ±0,02	2/3,12 ±0,01	NI / ND
	Euxyl PE 9010 0.7%	5,66±0,01	1,27 ±0,01	3,12 ±0,01	2/3,97 ±0,02	NI / ND

Note: ND - microorganisms are not detected; NI - there is no increase in the number of microorganisms.

According to the results given in table. 3, it can be concluded that the investigated samples of vaginal gel with Cosgard 0.5%, Leucidal 2.0% and EuxylPE 9010 0.7% preservatives fully meet the requirements of SPU (class A criterion) both in relation to cells of *Staphylococcus aureus* ATCC 6538 *and Pseudomonas aeruginosa* ATCC 9027 and fungi *Candida albicans* ATCC 885-653 *and Aspergillus brasiliensis*.

The obtained data, shown in Table. 3 indicate that after 2 days of storage of inoculated gel samples with various preservatives Cosgard 0.5%, Leucidal 2.0% and Euxyl PE 9010 0.7% logarithm of reducing the number of viable microorganisms for culture of *Staphylococcus aureus* ATCC 6538 was 4.39 (Cosgard preservative 0.5%), 3.32 (Leucidal preservative 2.0%) and 3.49 (Euxyl PE 9010 0.7%); for culture of *Pseudomonas aeruginosa* ATCC 9027 logarithm of reduction in the number of viable microorganisms was for samples of gel with the preservative Cosgard 0.5% - 2.85; for Leucidal 2,0% gel samples - 2,43 and for samples with Euxyl PE 9010 0.7% - 2.74.All logarithm values obtained were at least 2.0, which corresponds to the requirements of SPU.

At day 7, viable cells of *Staphylococcus aureus* ATCC 6538 in gel samples of Cosgard 0.5%, Leucidal 2.0% and Euxyl PE 9010 0.7% were not allocated (according to the requirements of the State Pharmacopoeia of Ukraine the logarithm of reduction should be at least 3.0).While the logarithm of reducing the number of viable cells of *Pseudomonas aeruginosa* ATCC 9027 in samples with the preservative Leucidal 2.0% was 3.57, in samples with Euxyl PE 9010 0.7% - 4.05; viable cells of *Pseudomonas aeruginosa* ATCC 9027 bacteria in Cosgard preservative gel samples 0.5% were not allocated .All logarithm values obtained were not less than 3.0, which corresponds to the requirements of SPU.

On the 14th and 28th day of incubation in gel samples with Cosgard 0.5%, Leucidal 2.0%, Euxyl PE 9010 0.7% viable microorganisms of *Staphylococcus aureus* ATCC 6538 *and Pseudomonas aeruginosa* ATCC 9027 were not detected.

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For *Candida albicans* fungus cells ATCC 885-653 for the 14th day the reduction lg of viable cell number in Leucidal preservative 2.0% and Euxyl PE 9010 0.7% gel samples was 3.97 and 4.05, respectively. While in gel samples with Cosgard 0.5% viable cells of the fungus *Candida albicans* ATCC 885-653 were not detected. For the culture of *Aspergillus brasiliensis* ATCC 16404 on the 14th day the decrease lg in the number of viable cells in samples with preservatives Leucidal 2.0% and Euxyl PE 9010 0.7% was 3.12 and 3.97, respectively, and in samples of gel with preservative Cosgard 0.5% viable cells were not detected. All logarithm values obtained were at least 2.0, which corresponds to the requirements of SPU. On the 28th day of storage of inoculated gel samples with all preservatives viable cells of fungi *Candida albicans* ATCC 885-653 and *Aspergillus brasiliensis* ATCC 16404 were not isolated in any of the samples with preservatives Cosgard 0.5%, Leucidal 2.0% and Euxyl PE 9010 0.7%.

During the experiment, it has been found that all the preservatives tested meet the requirements of SPU and can be used in the manufacture of semisolid dosage forms, namely in the composition of vaginal gel with resveratrol and hyaluronic acid .But the best antimicrobial indexes has a sample of a gel with a preservative Cosgard at a concentration of 0.5%.

Conclusions

1) The research on the choice of antimicrobial preservative in the composition of vaginal gel for the treatment of urogenital symptoms, has been conducted, according to which it can be concluded that samples of gels with preservatives Cosgard 0.5%, Leucidal 2.0% and EuxylPE 9010 0.7% meet the criterion "A » According to the requirements of SPU for non-sterile medicines and are promising for further research on the development of composition and optimal technology of semisolid dosage form of vaginal gel.

2) According to the research, the antimicrobial effectiveness of 0.5% Cosgard preservative gel was higher than the 2.0% Leucidal preservative gel samples and 0.7% Euxyl PE 9010, which will contribute to the quality of the developed gel during its storage.

3) On the basis of the conducted researches the expediency of preservative Cosgard at a concentration of 0.5% introduction into the composition of vaginal gel with resveratrol and hyaluronic acid has been established.

4) The conducted studies are promising for further research on the development of composition and optimal technology of semisolid dosage forms.

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Aim. Microbial contamination of a product is possible in the process of production, storage and use, which can shorten the shelf life and adversely affect the safety profile of the drug. Therefore, an important criterion for evaluating the quality of a medicinal product is microbiological purity. The aim of the study was to determine and compare the antimicrobial effectiveness of preservatives in the composition of the vaginal gel with resveratrol and hyaluronic acid. **Materials and methods.** The objects of the study were samples of vaginal gel with various antimicrobial preservatives, which have no irritant effect on the mucous membranes, and are safe for the human body. Preservative selection studies have been conducted using the antimicrobial preservative efficacy evaluation technique described in SPU 2.0 (Vol. 1, Sec.5.1.3, p. 773). The principle of the method is that to the samples of the finished dosage form with various preservatives, which are in the primary packaging, introduce a certain number of test microorganisms and store these samples at a certain temperature (from 20 to 25 ºC) in a dark place. Immediately after inoculation and at specified intervals (2, 7, 14 and 28 days), samples (usually 1.0 g) are taken from the inoculated samples and the number of viable microorganisms is determined. All studies were performed in aseptic conditions, using a laminar box (Biological Safety Cabinet AC2-4E1 " Esco", Indonesia). Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Candida albicans ATCC 885-653, Aspergillus brasiliensis ATCC 16404 were used as test microorganisms for inoculation of gel samples. **Results.** The research on the choice of antimicrobial preservative in the composition of vaginal gel for the treatment of urogenital symptoms, has been conducted, according to which it can be concluded that samples of gels with preservatives Cosgard 0.5%, Leucidal 2.0% and EuxylPE 9010 0.7% meet the criterion "A » According to the requirements of SPU for non-sterile medicines and are promising for further research on the development of composition and optimal technology of semisolid dosage form of vaginal gel. According to the research, the antimicrobial effectiveness of 0.5% Cosgard preservative gel was higher than the 2.0% Leucidal preservative gel samples and 0.7% Euxyl PE 9010, which will contribute to the quality of the developed gel during its storage. On the basis of the conducted researches the expediency of preservative Cosgard at a concentration of 0.5% introduction into the composition of vaginal gel with resveratrol and hyaluronic acid has been established. **Conclusions.** It has been established that the optimal antimicrobial preservative in the composition of vaginal gel is the preservatives Cosgard in concentration 0,5%. The conducted studies are promising for further research on the development of composition and optimal technology of semisolid dosage forms.

Key words. Vaginal gel, antimicrobial preservative, resveratrol, hyaluronic acid.

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