DETERMINATION OF MICROBIOLOGICAL PURITY OF RECTAL SUPPOSITORIES WITH DIOSMINE AND HESPERIDINE

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Introduction

The use of conservative methods of treatment, in terms of increasing the number of patients with anorectal diseases, is becoming increasingly important in modern realities. Proctological pathologies are becoming more characteristic for people of working age, and can affect not only the physical and mental health of an individual, but also the future reproductive state of the nation as a whole [5]. To prevent the development of malignant pathologies, it is necessary to timely use a comprehensive treatment of diseases of the anorectal area in the early stages detection of the symptoms. To that end, it is advisable to use dosage forms that will effect on several factors in the pathogenesis, while showing resorptive and local effects. As an example, of such a dosage form (DF) are rectal suppositories, which according to a complex of structural-mechanical and biopharmaceutical factors is the optimal DF for the release of active substances on the place of the pathological process. Due to the possibility of introducing a combination of substances with different pharmacological effects, as a result of the treatment with this form is a fast and multifaceted effect of the drug.

As the main active substances we have chosen the complex of micronized fraction of diosmin and hesperidin [4]. According to a preliminary study of a literature sources, oral administration of these substances leads to reduces permeability of venous capillaries and distensibility of veins, toning up a vascular wall, what are important in the treatment of diseases of the anorectal area. We have been proposing the introduction of a local anesthetic – benzocaine for alleviate the pain symptom. Due to the fast local anesthetic effect and minimal side effects of allergic type, this substance is an alternative choice for the treatment of pain in acute forms of proctological diseases [3].

An important stage in the formulation of rectal suppositories it isn`t only the choice of active substances, but also quality control of ready-made DF.

The SPhU of Ukraine regulates the quality indicator "microbiological purity" for suppositories as a critical stage of control, which means the necessity for a full range of studies to identify possible of microbial contamination in the DF [2].

The aim of the work was to study of indicator "microbiological purity" in rectal suppositories, which contain as main substances the complex of micronized fraction of diosmin and hesperidin.

Material & Methods

We have selected 3 samples of rectal suppositories with diosmin and hesperidin for microbiological studies. In order to substantiate the stability during storage, we have proposed to use samples with different durations from the date of manufacture (1- just made; 2- 2 weeks storage; 3- 6 months storage).

We have used the following mediums in conducting research to study the growth properties of nutrient medium: soybean casein digest broth, soybean casein digest agar («HIMedia LaboratorlesPvt. LtdIndia» expiration date to XI 2020, India). We have used a sabouraud dextrose agar for Candida albicans («HIMedia LaboratorlesPvt. LtdIndia» expiration date to XI 2020, India). The mediums had been prepared for according to technological requirements of the manufacturer (autoclaving conditions, the amount of substance per liter, pH of the medium, etc.). The growth quality of each series of samples was checked in accordance with regulatory documentations [1].

The testing of suppository samples for microbiological purity was carried out using with the Chistovich medium, blood agar-based soybean casein digest agar and Endo medium.

As a preliminary preparation for the study of microbiological purity, tests were performing for compliance with the growth qualities of nutrient mediums. The nutrient mediums were inoculated with a small amount of the appropriate test strain of the microorganism (10-10^2 colony forming units per ml of medium - CFU/ml). Candida albicans was seeded on sabouraud dextrose agar. Pseudomonas aeruginosa and Bacillus subtilis were seeded on the soybean casein digest agar. Staphylococcus aureus was seeded on the Chistovich medium. Escherichia coli was seeded on the Endo medium. The nutrient mediums (soybean casein and thyoglycollate) were kept in a thermostat for three days at a temperature of 35ºC.

The definitions of microscopic study were carried out a microscopic method using a Konus Academi Microscope of Italian production with a DLT-Cam Basic 2MP camera.

The test of microbiological purity was conducted with methods direct seeded on liquid nutrient mediums. The soybean casein digest broth, thyoglycollate medium and liquid Sabouraud medium have been sterile poured in the test tubes of each volume of 10 ml. The test simples have been made in each test tube on 1.0, 0.1 and 0.001. The cultures was incubating during 14 days on soybean casein digest broth and thyoglycollate medium at a temperature of 35ºC, culture on liquid Sabouraud medium was incubating at a temperature of 25ºC. The definitions of neutralization of antibacterial properties of samples were made with inactivator (composition: polysorate-80 – 30.0 g/l; lecithin – 3.0 g/l).

The definitions of quantity of viable cells of microorganisms and fungus for methods of deep seeding have been conducting with adding sample in quantity 0.1 in agar medium.

Results & discussion

DOI: 10.5281/zenodo.3885040

The results of the study of growth properties of nutrient mediums are responded in table 1, where is interpreted, that all cultures of microorganisms met to the taxonomic designation of the strain. The morphology of the colonies within cultivation on the medium and the morphology of the cells at microscopy studied were typical.

The nutrient mediums (soybean casein digest broth and thyoglycollate medium) are corresponded to requirements for sterility. The microorganisms wasn`t growing and mediums were transparent.

Table 1.The study of growth properties of nutrient mediums, which have been used for testing suppositories samples

Indicator microorganisms	**Culture medium**	**Culture conditions**		**Conclusion**
		Temperature	***Culture duration***	
Staphylococcus aureus *ATCC 6538*	Chistovich medium	35ºC,	24-72 hours	The morphology of the colonies and cells is typical
Escherichia coli *ATCC 25922*	Endo medium	35ºC,	24-72 hours	
Bacillus subtilis *ATCC 6633*	Soybean casein digest agar	35ºC,	24-72 hours	
Pseudomonas aeruginosa *ATCC 9027*	Soybean casein digest agar	35ºC,	24-72 hours	
Candida albicans *ATCC 885/653*	Saburo	25ºC,	24-120 hours	
x	Thyoglycollate medium for sterility test	35ºC,	24-72 hours	
x	Soybean casein digest broth	35ºC,	24-72 hours	

Note: x – microorganisms are not seeded.

The next step was to study microbiological purity of suppositories. The results are interpreted on the table 2.

Table 2. The study of microbiological purity of suppositories

Sample	**The quantity of DF in the test tube** *(g)*	**The conditions of cultivation**		
		Soybean casein digest broth *14 days at 35ºC*	***Thyoglycollate medium*** *14 days at 35ºC*	***The liquid Saburo medium*** *14 days at 25ºC*
1	1,0	MNG	MNG	FNG
	0,1	MNG	MNG	
	0,01	**microorganisms have growth**	**microorganisms have growth**	
2	1,0	MNG	MNG	
	0,1	MNG	MNG	
	0,01	**microorganisms have growth**	**microorganisms have growth**	
3	1,0	MNG	MNG	
	0,1	MNG	MNG	
	0,01	**microorganisms have growth**	**microorganisms have growth**	

Note: MNG – microorganisms haven't growth; FNG – fungus haven't growth.

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The obtained results have shown that after 14 days of incubation of suppositories samples on the Saburo medium - the fungus haven't growth. The results of researches samples with soybean casein digest broth and thyoglycollate medium in the quantity 1.0 and 0.1 haven`t shown the growth of microorganisms. Registration of growth of the microorganisms has been shown in research of samples in the quantity of 0.01.

The microscopic study of samples has shown gram-positive vegetative spores. Confirmation was obtained by sieving into differential nutrient mediums. The results are interpreted on the table 3.

Table 3. Identification of microorganisms that germinated on soybean casein digest broth and thyoglycollate medium

Sample	The quantity of sample, g	The growth of microorganisms on nutrient mediums				
		Chistovich medium	***Endo medium***	***Blood agar***	***Saburo***	***Nutrient medium***
1	0,01	x	x	Dry gray colonies, with jagged edges, not shiny, hemolysis	x	Dry gray colonies, with jagged edges, not shiny
	0,01					
2	0,01					
	0,01					
3	0,01					
	0,01					

Note: x – microorganisms are not seeded.

As can be seen from table 3 microorganisms that have been detected during the studies by morphology of colonies and biological properties belong to the genus Bacillus sp.

Studies of suppositories samples on the presence of representatives of intestinal microorganisms and pathogenic staphylococci on differential mediums (Chistovich and Endo) have been showed a negative result.

The next step was to study microbiological purity by the method of direct seeding on cups. The results are interpreted on the table 4.

Table 4. Research on the microbiological purity of the suppositories samples by the method of direct seeding on cups

Sample	The quantity of sample, g	The number of microorganisms for the decimal logarithm of the degree of growth while cultivating on solid nutrient media			
		Deep seeding method		***Surface seeding method***	
		Soybean casein digest agar 35°C; 3-5 days	Sabouraud dextrose agar 25°C; 4-7 days	Soybean casein digest agar 35°C; 3-5 days	Sabouraud dextrose agar 25°C; 4-7 days
1	0,1	9±0,3	FNG	10±0,2	FNG
	0,01	MNG		1,0±0,1	
2	0,1	16±0,4		12±0,3	
	0,01	MNG		1,2±0,1	
3	0,1	14,2±0,5		13,4±0,2	
	0,01	1,4±0,1		1,3±0,1	

Note: MNG – microorganisms haven't growth; FNG – fungus haven't growth

According to the research results, the growth of fungus has been absented in all suppositories samples. The quantity of microorganisms which was growth on 0.1 g samples haven`t been more than 10^3 CFU/ml that meets the requirements of SPhU [2].

Conclusion

1) Viable fungal cells weren`t detected in the suppositories samples;
2) The quantity of microorganisms was lower by 10^3 CFU/ml that meets the requirements of SPhU;
3) Microorganisms that have been detected during the studies belong to the genus Bacillus sp.

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4) The strains of S. aureus, P. aeruginosa and representatives of the genus Enterobacteriacea sp. haven`t been detected.
5) Suppositories samples that have been determinations on indicator of microbiological purity, meets the requirements of SPhU.

Determination of microbiological purity of rectal suppositories with diosmine and hesperidine
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Introduction. The use of conservative methods of treatment, in terms of increasing the number of patients with anorectal diseases, is becoming increasingly important in modern realities. Proctological pathologies are becoming more characteristic for people of working age, and can affect not only the physical and mental health of an individual, but also the future reproductive state of the nation as a whole. , It is advisable to use dosage forms that will effect on several factors in the pathogenesis, while showing resorptive and local effects. As an example, of such a dosage form (DF) are rectal suppositories, which according to a complex of structural-mechanical and biopharmaceutical factors is the optimal DF for the release of active substances on the place of the pathological process. An important stage in the formulation of rectal suppositories it isn`t only the choice of active substances, but also quality control of ready-made DF. The SPhU of Ukraine regulates the quality indicator "microbiological purity" for suppositories as a critical stage of control, which means the necessity for a full range of studies to identify possible of microbial contamination in the DF.**Material & Methods.** We have selected 3 samples of rectal suppositories with diosmin and hesperidin for microbiological studies. In order to substantiate the stability during storage, we have proposed to use samples with different durations from the date of manufacture (1- just made; 2- 2 weeks storage; 3- 6 months storage). The testing of suppository samples for microbiological purity was carried out using with the Chistovich medium, blood agar-based soybean casein digest agar and Endo medium. As a preliminary preparation for the study of microbiological purity, tests were performing for compliance with the growth qualities of nutrient mediums. The definitions of microscopic study were carried out a microscopic method using a Konus Academi Microscope of Italian production with a DLT-Cam Basic 2MP camera. The test of microbiological purity was conducted with methods direct seeded on liquid nutrient mediums. The definitions of quantity of viable cells of microorganisms and fungus for methods of deep seeding have been conducting with adding sample in quantity 0.1 in agar medium. **Results & discussion.** The results of the study of growth properties of nutrient mediums was shown, that all cultures of microorganisms met to the taxonomic designation of the strain. The obtained results have shown that after 14 days of incubation of suppositories samples on the Saburo medium - the fungus haven't growth. The results of researches samples with soybean casein digest broth and thyoglycollate medium in the quantity 1.0 and 0.1 haven`t shown the growth of microorganisms. Registration of growth of the microorganisms has been shown in research of samples in the quantity of 0.01. The microorganisms that have been detected during the studies by morphology of colonies and biological properties belong to the genus Bacillus sp. According to the research results by the method of direct seeding on cups the growth of fungus has been absented in all suppositories samples. **Conclusion.** Taking into account the obtained data we was indicated that viable fungal cells weren`t detected in the suppositories samples. The quantity of microorganisms was lower by 10^3 CFU/ml that meets the requirements of SPhU. Microorganisms that have been detected during the studies belong to the genus Bacillus sp. The strains of S. aureus, P. aeruginosa and representatives of the genus Enterobacteriacea sp. haven`t been detected. Suppositories samples that have been determinations on indicator of microbiological purity, meets the requirements of SPhU.

Keywords: microbiological purity, rectal suppositories, diosmine, hesperidine

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DOI: 10.5281/zenodo.3885040