DETERMINATION OF MICROBIOLOGICAL PURITY OF TABLETS FOR THETREATMENT AND PREVENTION OF TYPE II DIABETES MELLITUS

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Introduction. Diabetes mellitus refers to a group of metabolic diseases characterized by hyperglycaemia, which occurs as a result of a defect in secretion or action of insulin. Chronic hyperglycaemia in diabetes mellitus (DM) leads to a damage, dysfunction and insufficiency of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Consistent hyperglycaemia activates the formation of free radicals, reduces the activity of antioxidant protection agents: superoxide dismutase, catalase, glutathione peroxidase, vitamins C and E. Therefore, special attention deserve anti-diabetic drugs which, along with the hypoglycaemic effect, have antioxidant properties, the ability to maintain or improve the secretory function of β-cells [1].

The optimal compositions have been established and the technologies of medicinal products under the conventional names "Glycverin" with Voglibose and quercetin and "Thioquerin " on the basis of solid dispersion of thioctic acid were developed by previous studies [2,3]. As a result of the study of specific pharmacological activity, it has been experimentally proved that the above drugs have a pronounced hypoglycaemic effect and increase the bioavailability of their components, which suggests its relevance in the treatment of type II diabetes. In order to increase biological availability, the method of solid dispersion has been utilized, which has significant advantages, among which: the use of drugs in solution or finely dispersed state provides a high dissolution rate, reduced irritating action on the gastric mucosa, a high therapeutic effect [4]. However, during the technological process of obtaining non-sterile dosage forms, the preparation may be contaminated with microorganisms, which may reduce its stability during storage, which negatively affects its safety profile.

Today, SPU clearly regulates the maximum level of contamination by microorganisms for each dosage form [5].

The purpose of the study was to determine the microbiological purity of the finished medicinal products in the form of tablets "Glycverin" and "Thioquerin".

Materials and methods

The objects of the study were tablets under the conventional names "Glycverin" and "Thioquerin". Studies on the microbiological purity of the tablets were conducted in accordance with the requirements of the SPU, 2nd ed., p.2.6.12 and 2.6.13, category 3A (5.1.4, N). For the study used: soybean casein broth (HIMedia Laboratories Pvt.Ltd India » expiration date of the medium until XI 2019, Manufactured in India). For *Candida albicans* used Saburo-Dextrose Agar (Indian Production, «HIMedia Laboratories Pvt.Ltd India » expiration date of the environment until XI 2019). The environments were prepared according to the manufacturer's requirements (amount of powder per litre, pH, conditions of autoclaving, etc.). Each series used in the experiment was tested on growth quality in accordance with the reference documents. For tests on microbiological purity the following media were obtained on the basis of soy-casein agar: Chistovich's medium, blood agar [6].

Results and discussion

Before conducting research on microbiological purity it was mandatory to test the compliance of the growth qualities of the nutrient media used. For this purpose, the nutrient media were inoculated with a small amount of an appropriate test strain of the microorganism (10 - 10^2 colony forming units per ml of medium - CFU / ml) (Table.1). Saburo dextrose agar was cropped with yeast-like fungi of the genus Candida . Soy-casein agar - *with Pseudomonas aeruginosa* and *Bacillus subtilis*, Chistovich medium - with *Staphylococcus aureus*, Endo - *Escherichia coli*. Nutrient broths (soy-casein and thioglycol) were kept in a thermostat at 35 ^{o} C for three days [7].

The obtained data (Table 1) indicate that all microorganisms were consistent with the taxonomic designation of the strain, the morphology of the colonies when cultured on the media and cell morphology in microscopy were typical. Nutrient broths (soy-casein and thioglycolic medium) met the requirements for sterility - there was no growth of microorganisms, the environment was transparent.

When testing on microbiological purity, the direct sowing method was used for liquid nutrient media. To do this, under sterile conditions, soy-casein broth, thioglycolic medium and Saburo liquid medium were poured of 10 ml into test tubes. In each of the test tubes, 1 g, 0.1 g and 0.001 g of the test sample of the drug were added. Crops on a soy-casein broth were incubated for 14 days, on thioglycolic medium - in a thermostate at a temperature of 35 $^{\circ}$ C., cultures on a Saburo substrate at a temperature of 25 $^{\circ}$ C. Neutralization of the antibacterial properties of the samples under study was carried out with an inactivator containing polysorbate-80 (30 g / l) and lecithin (3 g / l). The results of the study are given in Table 2.

As it is shown in Table 2, after 14 days of incubation, at cultivation on a Saburo, soy-casein broth and thioglycolic medium the growth of fungi was absent.

In a deep sowing study, which was to add 0.1 g of preparation to agar and surface sowing of 0.1 g to agar, the amount of viable cells of microorganisms and fungi has been determined. The study of surface and deep sowing of drugs on Saburo dishes has shown no growth of fungi. Growth was noted when cultivated on soy-casein agar

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Table 1 - Growth Properties of Nutrient media

Test strains	Nutrient media	Terms of cultivation		Conclusion
		T, ° C	Duration of cultivation	
S. aureusATCC 6538	Chistovich	35 ° C	24-72 hours	Morphology of colonies and cells is typical
Escherichia coli ATCC 25922	Endo	35 ° C	24-72 hours	Morphology of colonies and cells is typical
Bacillus subtilis ATCC 6633	Soy-casein agar	35 ° C	24-72 hours	Morphology of colonies and cells is typical
P. aeruginosa ATCC 9027	Soy-casein agar	35 ° C	24-72 hours	Morphology of colonies and cells is typical
Candida albicans ATCC 885/653	Saburo-dextrose agar	25 ° C	24-120 hours	Morphology of colonies and cells is typical
x	Thioglycol medium for sterility monitoring	35 ° C	24-72 hours	Growth of microorganisms is absent
x	Soy-casein broth	35 ° C	24-72 hours	Growth of microorganisms is absent

Note: x - microorganisms were not cropped

Table 2 – Tests of preparations for microbiological purity (combinations of samples that have sprouted)

Samples	Number of medicinal product in test-tube (ml or g)	Terms of cultivation		
		Soy-casein broth 14 days at 35 ° C, growth of microorganisms	Thioglycol medium for 14 days at 35 ° C, growth of microorganisms	Liquid Saburo medium for 14 days at 25 ° C, growth of microorganisms
Glycverin	1.0	is absent	is absent	is absent
	0.1	is absent	is absent	is absent
	0.01	is absent	is absent	is absent
Thioquerin	1.0	is absent	is absent	is absent
	0.1	is absent	is absent	is absent
	0.01	is absent	is absent	is absent

Table 3 – Studies on microbiological purity by direct sowing on dishes

Samples	Weight of sample, g	Number of microorganisms per tenths logarithm of growth rate when cultivated on solid nutrient media x10			
		Deep cropping method g of the drug in agar		Surface cropping method g preparation on agar	
		Soy-casein agar 35 ° 3-5 days	Saburo dextrose agar 25 ° C. 4-7 days	Soy-casein agar 35 ° 3-5 days	Saburo dextrose agar 25 ° C. 4-7 days
Glycverin	0.1	12 ± 0.3	Growth of fungi is absent	14 ± 0.5	Growth of fungi is absent
	0.01	1,2 ± 0,1	Growth of fungi is absent	1.4 ± 0.3	Growth of fungi is absent
Thioquerin	0.1	16 ± 0.4	Growth of fungi is absent	18 ± 0.5	Growth of fungi is absent
	0.01	1,6 ± 0,2	Growth of fungi is absent	1.8 ± 0.1	Growth of fungi is absent

The data obtained in Table 3 indicate that the number of microorganisms does not exceed 10^3 CFU / ml, which meets the requirements of the State Pharmacopoeia of Ukraine.

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Conclusions

1. The study of microbiological purity of tablets "Glycverin" and "Thioquerin" has been conducted. The results indicate absence of viable fungal cells. It has been established that the amount of viable cells of microorganisms does not exceed 10^3 CFU / ml in 1 g of the preparation, which meets the requirements of the SPU for internal use preparations. Strains *S. aureus, P. aeruginosa* and representatives of *Enterobacteriaceae sp.* family have not been detected
2. Investigated samples of drugs meet the requirements of the Pharmacopoeia of Ukraine on the indicators of microbiological purity for oral preparations. The obtained results allow asserting the safety and expediency of further development of medicinal products for use in the treatment of type II diabetes mellitus.

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Keywords: Diabetes mellitus, solid dispersion, microbiological purity, thioctic acid, quercetin

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