

A close-up photograph of a green plant stem with a coiled tendril, set against a dark green background. The tendril is tightly coiled into a spiral and then extends upwards and to the right.

advanced biobank research & pathophysiology

Volume 1 (1)
February 2018

**THE LEVEL OF ANTIMICROBIAL ACTIVITY OF PRESERVATIVES
HEPATOPROTECTIVE AGENT "OLEOSIL"***Vishnevskaya L.I.¹, Naboka O.I.¹, Polovko N.P.¹, Kryzhna S.I.²**1. National Pharmaceutical University,**2. Kharkiv Academy of Postgraduate Education, Kharkiv, Ukraine***Abstract**

A promising object for the treatment and prevention of diseases of the hepatobiliary system is a new combined remedy based on the vegetable components "Oleosil" (created in NFAU) containing wild carrot seeds, chamomile flowers, corn stalks, thistle oil. The raw materials are rich in phenolic compounds (flavonoids, hydroxycinnamic acids, coumarins), vitamins, minerals. When developing the composition of syrup, one of the main tasks is to minimize the inherent disadvantage of this dosage form - instability when stored and used after opening the package. The optimal concentrations of preservative (among nipagin, nipazole, ethanol, potassium sorbate, benzoic acid) and the state of its antimicrobial activity for hepatoprotecton "Oleosil" have been determined. Used the main strains of bacteria for oral medicines.

It has been established that in developing the composition of syrup one of the main tasks is to minimize instability in storage and use after opening the package, since its constituents are an enabling environment for the reproduction of microorganisms. To determine the effectiveness of the antimicrobial action of preservatives, 6 samples of syrup with different concentrations of preservatives have been studied. The best indicators of preservative in potassium sorbate in the concentration of 0.1% were established.

* **Received Date:** 24 January 2018; **Accepted Date:** 28 January 2018; **Published Date:** 1 February 2018

Introduction.

The prevalence of the pathology of the hepatobiliary system in Ukraine, as in the world as a whole, has no tendency to decrease, and complications are often extremely dangerous and mortally threatening the patient's life. According to the State Statistical Service, hepatobiliary diseases occupy 3rd place in the structure of the morbidity of the population of Ukraine [5]. Therefore, the problem of improving the quality of treatment and prevention of diseases of the hepatobiliary system, first of all, hepatitis – is one of the most urgent in the pharmacy. Drugs that detect hepatoprotective, choleric, anti-inflammatory and applied in diseases of the hepatobiliary system contain medicinal plants, which according to established views are multifaceted and highly safe [7, 9].

A promising object is a new combined plant on the basis of vegetable components Oleosil (created in NFAU) containing wild carrot seeds, chamomile flowers, columns with corn receptacles, thistle oil [1, 3]. The raw materials are rich in phenolic compounds (flavonoids, hydroxycinnamic acids, coumarins), vitamins, minerals [4]. Important is the fact that all components are spread in Ukraine and are available for use. When developing the composition of syrup, one of the main tasks is to minimize the inherent disadvantage of this dosage form – instability when stored and used after opening the package. The auxiliary substances of this dosage form are an enabling environment for the reproduction of microorganisms and microbial contamination during use. Solutions of sweeteners are able to provide a high degree of microbiological purity, but the addition of phytocomposites to syrups leads to a dilution of the concentration of sugars and a decrease in their antimicrobial activity, so in each case it is necessary to conduct research to ensure their microbiological purity [9]. Therefore, the purpose of this work was to determine the optimal concentration of preservative (nipagin, nipazole, ethanol, potassium sorbate, benzoic acid) and its antimicrobial activity for hepatoprotective agent "Oleosil".

Materials and methods.

For conducting research on the effectiveness of the antimicrobial action of preservatives, 6 experimental samples of syrup were manufactured at various concentrations. In studies, the method used to evaluate the effectiveness of antimicrobial preservatives, given in DFU 1.4 (paragraph 5.1.3, pp. 169-171); DFU 2.0 (T. 1, p. 5.1.3) [2]. The principle of the method is that samples of the finished dosage form with different preservatives

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contained in the primary packaging add a certain amount of test microorganisms and store these samples at a certain temperature (20 to 25 °C) in a place protected from light. Immediately after inoculation and at certain intervals (oral use means 14 and 28 days), from inoculated samples, samples are taken (usually 1 ml / g) and determine the number of viable microorganisms [2, 8]. All studies were performed in aseptic conditions using a laminar box (BI2B4 "Esco", Indonesia).

As test microorganisms, *S. aureus* ATCC 6538, *Ps. aeruginosa* ATCC 9027, *C. albicans* ATCC 885-653, *As. brasiliensis* ATCC 16404, as well as *E. coli* ATCC 25922 (for oral medicines).

The initial culture of each of these test microorganisms was transplanted onto the surface of a thick soy-casein nutrient medium in the cultivation of bacteria (*S. aureus*, *Ps. Aeruginosa*, *E. coli*). When cultivating fungi (*C. albicans*, *As. Brasiliensis*), the nutritious Saburo-dextrose medium was transplanted without adding antibiotics.

The statistical analysis of the results was carried out using standard software packages of Exel (2007), and Statistica, v. 6.0 (StatSoft Inc., USA) on a Pentium III PC. Differences between the sample were considered statistically significant at ($p < 0.05$) [6].

Results.

To conduct research on the effectiveness of antimicrobial activity of preservatives, we have manufactured 6 experimental samples of syrup at different concentrations: nipagin-nipazol (3: 1) in 0.1%; nipagin-nipazole (3: 1) in 0.2%; potassium sorbate at 0.1%; potassium sorbate in 0.2%; sodium benzoate at 0.1%; Sodium benzoate at 0.2%.

The results of research on the growth properties of nutrient media show that all microorganism cultures correspond to the taxonomic designation of the strain, while the morphology of the colonies in cultivating on nutrient media and morphology of cells in microscopy are typical.

The results of the research on the antimicrobial efficacy of preservatives in the samples of syrup studied are given in Table. 1, 2.

Table 1

**Results of antimicrobial efficacy of preservative
nipagine: nipazole (3: 1) in the samples under study syrup**

| Sample / preservative | Test-culture | Microbial load after inoculation, lg CFU / ml | Lg decrease initial microbial loading (requirements of DFU / sample) | |
|--------------------------|------------------------------------|--|--|---------|
| | | | 14 days | 28 days |
| № 1 (0,1 %) | <i>S. aureus</i> ATCC 6538 | 5,9 | 3,0/4,45 | MNN/MN |
| № 2 (0,2 %) | | 5,7 | 3,0/MN | MNN/MN |
| № 1 (0,1 %) | <i>Ps. aeruginosa</i> ATCC 9027 | 5,8 | 3,0/3,97 | MNN/MN |
| № 2 (0,2 %) | | 5,9 | 3,0/MN | MNN/MN |
| № 1 (0,1 %) | <i>E. coli</i> ATCC 25922 | 5,8 | 3,0/MN | MNN/MN |
| № 2 (0,2 %) | | 5,7 | 3,0/MN | MNN/MN |
| № 1 (0,1 %) | <i>C. albicans</i> ATCC 885-653 | 5,9 | 1,0/3,55 | MNN/MN |
| № 2 (0,2 %) | | 5,7 | 1,0/MN | MNN/MN |
| № 1 (0,1 %) | <i>As. brasiliensis</i> ATCC 16404 | 5,7 | 1,0/1,83 | MNN/MN |
| № 2 (0,2 %) | | 5,6 | 1,0 /MN | MNN/MN |

Note. MN – microorganisms are not detected; MNN – there is no increase in the number of microorganisms.

The results are shown in the table 1 shows that after 14 days of storage of inoculated samples with preservative (nipagin: nipazol – 3: 1) at concentrations of 0.1 and 0.2%, the logarithm of reducing the number of viable microorganisms of bacteria was more than 3.0 for all cultures of microorganisms *S. aureus*, *ps. aeruginosa*, *E. coli*. At the 28th day of storage of samples, these microorganisms were not detected. For cell mushrooms *C. albicans* and *As. brasiliensis* on day 14, the logarithm of decreasing the number of viable cells

was more than 1.0, and at the 28th day no fungal cells were detected. That is, according to the results of research on samples of preserved syrups, nipagine: nipazole (3: 1) at concentrations of 0.1 and 0.2%, it is evident that they meet the requirements of the SPF for medicinal preparations for oral application in the indicator "antimicrobial efficacy of preservatives".

The results obtained are shown in Table. 2 indicate that after 14 days of storage of inoculated samples of syrups with preservative potassium sorbate at a concentration of 0.1 and 0.2%, the logarithm of reducing the number of viable cells of microorganisms was more than 3.0 and was equal to *S. aureus* 3.54 (0.1%) for *Ps. aeruginosa* - 3.92, for *E. coli* cells bacteria have not been detected. In samples of syrups with a concentration of preservative 0.2% of viable bacterial cells was not detected. At day 28, microorganisms were not detected from inoculated specimens.

After 14 days storage of inoculated samples of syrups with preservative of potassium sorbate 0.1% logarithms for reducing the number of viable cell mushrooms *C. albicans* and *As. brasiliensis* was 3.70 and 2.30 respectively, and with a concentration of preservative 0.2% no viable fungal cells were detected. Mushroom cells *C. albicans* and *As. The brasiliensis* in the studied samples of syrups did not appear on the 28th day of storage.

Consequently, the study of samples of syrup with preservative potassium sorbate at a concentration of 0.1 and 0.2% showed that they fully meet the requirements of the PFU to the effectiveness of antimicrobial preservatives for oral use.

Table 2

Results of antimicrobial efficacy of preservative
potassium sorbate in the samples under study syrup

| Sample / preservative | Test-culture | Microbial load after inoculation, lg CFU / ml | Lg decrease initial microbial loading (requirements of DFU / sample) | |
|--------------------------|------------------------------------|--|--|---------|
| | | | 14 days | 28 days |
| № 1 (0,1 %) | <i>S. aureus</i> ATCC 6538 | 5,6 | 3,0 / 3,54 | MNN/MN |
| № 2 (0,2 %) | | 5,8 | 3,0 / MN | MNN/MN |
| № 1 (0,1 %) | <i>Ps. aeruginosa</i> ATCC 9027 | 5,9 | 3,0 / 3,92 | MNN/MN |
| № 2 (0,2 %) | | 5,7 | 3,0 / MN | MNN/MN |
| № 1 (0,1 %) | <i>E. coli</i> ATCC 25922 | 5,6 | 3,0 / MN | MNN/MN |
| № 2 (0,2 %) | | 5,7 | 3,0 / MN | MNN/MN |
| № 1 (0,1 %) | <i>C. albicans</i> ATCC 885-653 | 5,9 | 1,0 / 3,70 | MNN/MN |
| № 2 (0,2 %) | | 5,8 | 1,0 / MN | MNN/MN |
| № 1 (0,1 %) | <i>As. brasiliensis</i> ATCC 16404 | 5,7 | 1,0 / 2,30 | MNN/MN |
| № 2 (0,2 %) | | 5,9 | 1,0 / MN | MNN/MN |

Note. MN – microorganisms are not detected; MNN – there is no increase in the number of microorganisms.

The results of studies on the antimicrobial efficacy of sodium benzoate preservative in the samples of syrup studied are given in Table. 3. The results shown in Table. 3, indicate that after 14 days of storage of inoculated samples of syrups with preservative sodium benzoate at a concentration of 0.1%, the logarithm of reducing the number of viable cells of microorganisms was more than 3.0 and was for *S. aureus* 3.36, for *Ps. aeruginosa* – 3.84, bacteria were not detected for *E. coli* cells. In samples of syrups with a concentration of preservative sodium benzoate 0.2% of viable cells of bacteria were not detected. At day 28, microorganisms were not detected from inoculated specimens.

Regarding the test cultures of fungi, after 14 days of storage, the logarithm of reducing the number of viable cells was *C. albicans* 3.44 (0.1%), for *As. brasiliensis* - 1.76 (0.1%) and 1.85 (0.2%). Mushroom cells were not detected from specimens after 28 days of storage. That is, samples of a syrup with preservative of sodium benzoate at a concentration of 0.1 and 0.2% meet the requirements of the SPF on the indicator of "effectiveness of antimicrobial preservative".

Table 3

Results of antimicrobial efficacy of preservative
Sodium benzoate in the samples under study syrup

| Sample / preservative | Test-culture | Microbial load after inoculation, lg CFU / ml | Lg decrease initial microbial loading (requirements of DFU / sample) | |
|--------------------------|--------------------------------|--|--|---------|
| | | | 14 days | 28 days |
| № 1 (0,1 %) | S. aureus ATCC 6538 | 5,8 | 3,0/3,36 | MNN/MN |
| № 2 (0,2 %) | | 5,7 | 3,0/ MN | MNN/MN |
| № 1 (0,1 %) | Ps. aeruginosa ATCC 9027 | 5,6 | 3,0/3,84 | MNN/MN |
| № 2 (0,2 %) | | 5,8 | 3,0/ MN | MNN/MN |
| № 1 (0,1 %) | E. coli ATCC 25922 | 5,7 | 3,0/ MN | MNN/MN |
| № 2 (0,2 %) | | 5,9 | 3,0/ MN | MNN/MN |
| № 1 (0,1 %) | C. albicans ATCC 885-653 | 5,8 | 1,0/3,44 | MNN/MN |
| № 2 (0,2 %) | | 5,7 | 1,0/ MN | MNN/MN |
| № 1 (0,1 %) | As. brasiliensis ATCC 16404 | 5,9 | 1,0/1,76 | MNN/MN |
| № 2 (0,2 %) | | 5,7 | 1,0 /1,85 | MNN/MN |

Note. MN – microorganisms are not detected; MNN – there is no increase in the number of microorganisms.

Consequently, when developing the composition of syrup, one of the main tasks is to minimize instability when stored and used after opening the package, since its components are an enabling environment for the reproduction of microorganisms. To determine the effectiveness of the antimicrobial action of preservatives, 6 samples of syrup with different concentrations of preservatives have been studied. The best indicators of preservative in potassium sorbate in the concentration of 0.1% were established.

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