RESEARCH ON THE SELECTION OF EMULGEL PRESERVATIVE WITH THICK EXTRACT OF FEVERFEW

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Introduction

One of the main medical and social problems of our time is diseases that are accompanied by musculoskeletal pain: arthritis, osteoarthritis, rheumatism, etc. Analysis of the domestic market shows that the means of "the first lane" for the treatment of these diseases are nonsteroidal anti-inflammatory drugs.

However, long-term use of these drugs causes the development of local and systemic side effects. Unlike synthetic drugs, herbal medicines have a higher degree of safety and, at the same time, are not inferior in effectiveness. Therefore, the development of herbal preparations for the treatment of diseases of the musculoskeletal system and connective tissues is an urgent task of modern pharmaceutical science.

The leading place for the treatment of diseases of the musculoskeletal system is provided by means of topical application of a mild form of release. The effectiveness of drugs for the local treatment of these diseases is assessed by the severity of analgesia and improving the functional capacity of the joints. Due to the complex mechanism of pain, modern mild drugs in the form of gels are a priority for topical therapy. Gels containing active substances of only natural origin are not available on the pharmaceutical market of Ukraine.

The current trend in pharmaceutical technology is the creation of emulsifiers consisting of an aqueous phase in the form of a gel with the active substance and an oily phase, which ensures the prolongation of the drug and improves its consumer characteristics.

The composition of the emulsifier with a thick extract of feverfew obtained at the Department of Botany of NUPh, was developed and experimentally substantiated for the prevention and treatment of inflammatory diseases of joints and connective tissues at the Department of Factory Technology of Drugs of the National University of Pharmacy. According to the literature, the chemical composition of feverfew is represented by phenolic compounds: hydroxycinnamic acids (chlorogenic, dicafeoiloquinic, chicory, etc.), flavonoids, sesquiterpene lactones (parthenolide, artecanin, chrysanthemum, cyanophemine, cyphemine, etc.)., bornyl acetate, etc.).

Flavonoids are dominated by flavones and flavonols. Flavones are represented by such compounds as apigenin, apigenin-7-glucuronide, luteolin, luteolin-7-glucuronide, chrysoeriol, and among flavonols – 1,6-hydroxycamppherol 3,6-dimethyl ether, 3,6,4-trimethyl ether 6-hydroxyceppherol (tanetin), 3,6-dimethyl ester of quercetamine, quercetagetin 3,3,6-trimethyl ether (jacedin), quercetin, santine and centauredin [1]. It should be noted that among these compounds, there is a large percentage of lipophilic flavonoids, namely methyl esters of flavonols 6-hydroxyceppherol and quercetamine [2].

The composition of the emulgel, in addition to a thick extract of feverfew, included sunflower oil, which serves as the oil phase of the emulsion of the 1st kind of emulgel, emulsifier-gelling agent Sepiplus-400 and purified water.

An important stage in the development of new drugs is the choice of optimal cooking technology [3]. The main technological parameters that affect the quality and stability of emulsion dosage forms are the order of mixing phases, cooking temperature, speed and duration of emulsification. Based on experimental studies, the technological parameters of emulgel production were determined, namely: temperature – 60 ° C, emulsification by mixing oil and water phases with a stirrer for 5 minutes, using a relatively low mixing speed – from 60 to 200 rpm min to prevent air bubbles from entering the emulgel, gradually adding residual water.

However, the drug contains API of plant origin and purified water – both components affect the stability of the product during storage and use due to the risk of biological contamination of the finished dosage form [4, 5]. The presence of microorganisms in non-sterile drugs can cause a reduction or even inactivation of their therapeutic effect, so at the stage of pharmaceutical development should be considered to ensure microbiological purity [6].

Ensuring the required level of microbiological purity of non-sterile drugs, especially those that do not have sufficient antimicrobial activity, is possible through the introduction of antimicrobial preservatives in the drug [7, 8]. The range of preservatives used in domestic medicines is quite wide. Among them, the following are often used in the development of mild preparations for dermal use: methyl parahydroxybenzoate (nipagin), methyl parahydroxybenzoate + propyl parahydroxybenzoate (nipazol), benzyl alcohol, benzalkonium chloride, phenoxyethanol, 2-hydroxyethylbenzole (benzyl), sodium benzoate, potassium sorbate [9-11].

The aim of the study is to investigate the effectiveness of antimicrobial preservatives in the composition of the emulgel with a thick extract of feverfew made on the basis of the gelling agent Sepiplus-400 for the treatment of diseases of the musculoskeletal system.

Materials and methods

The following antimicrobial substances were selected during the development of the emulgel composition with a thick extract of feverfew to select the preservative and its concentration: methyl parahydroxybenzoate (nipagin) + propyl parahydroxybenzoate (nipazol) in a ratio of 1: 3, sodium benzoate, phenoxyethanol, phenethyl alcohol.

Tests on the effectiveness of antimicrobial preservatives were performed according to the method of SPhU (State Pharmacopoeia of Ukraine) 2.3, paragraph 5.1.3 [12]. The studies were performed in aseptic conditions of laminar boxing (biosafety cabinet AC2-4E1 "Esco", Indonesia).

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Soybean-Casein agar and Sabouraud dextrose agar were used as nutrient media, and buffer solution with sodium chloride and peptone pH = 7.0, containing 50 g / lpolysorbate-80, 5 g / l lecithin, 1 g / l histidine hydrochloride were used as a solvent.

Staphylococcus aureus ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404 were used as test cultures of microorganisms, and preparation of their inoculum was carried out according to SPhU 2.3, item 5.1.3.

According to the requirements of the SPhU, the sterility of nutrient media, solvent, growth properties of nutrient media (soy-casein nutrient medium for growing bacteria and Saburo-dextrose medium without the addition of antibiotics for growing mushrooms) check and tests of the suitability of methods for determining total number of cells were conducted. The control in determining the growth qualities of the environment is a standard environment with guaranteed growth properties, which correctly manifests the quantitative and qualitative growth of microorganisms (morphology of colonies) [13]. The results of testing the sterility of nutrient media, solvent and growth properties of nutrient media are shown in table 1.

Table 1. Growth Properties of Nutrient Media

Test-microorganisms	Nutrient media	Cultivation conditions		Conclusion
		temperature, °C	duration, hours	
Staphylococcus aureus ATCC 6538	Soybean-Casein	30-35	18-24	Morphology of colonies and cells is typical
Pseudomonas aeruginosa ATCC 9027	Soybean-Casein	30-35	18-24	Morphology of colonies and cells is typical
Candida albicans ATCC 10231	Sabouraud dextrose	20-25	48-72	Morphology of colonies and cells is typical
Aspergillus brasiliensis ATCC 16404	Sabouraud dextrose	20-25	120-168	Morphology of colonies and cells is typical
–	Sterility control	35	24-72	No microorganisms growth

The data presented in table 1 show that the nutrient media met the growth properties and passed the sterility test according to the requirements of SPhU 2.0, paragraph 2.6.12., and test microorganisms met the taxonomic characteristics – morphology of colonies of the media and cell morphology under microscopy were typical for the corresponding strain.

Checking the suitability of the method for determining the total number of viable cells is based on the comparison of the results of counting the number of test microorganisms obtained in the presence of the test drug and on control inoculations. To do this, suspension of the test strain of one of the species of microorganisms, which contains about 100 colony-forming units (CFU), was put in test tubes with dilution of the drug, prepared for each test microorganism separately. Control of test microorganisms was prepared. The inoculated samples were mixed thoroughly. 1 ml of dilutions of the drug and controls separately for each test strain containing not more than 100 CFU were plated by surface method on dense nutrient media: soybean-casein agar to detect bacteria sabouraud dextrose agar to detect fungi [13]. The results of the tests of the suitability of the method for determining the total number of viable cells are shown in table 2.

Table 2. The results of checking the suitability of the method

Sample name	The average number of CFU in 1 ml of sample			
	S. aureus	***P. aeruginosa***	***C. albicans***	***A. brasiliensis***
Suspension of microorganisms + emulgel with a thick extract of the feverfew	78	64	75	68
Control suspension of microorganisms	89	76	69	65

The results of table 2 were obtained by counting each of the test microorganisms in the presence and absence of the test sample differ by no more than 1.2 times, which meets the criterion of acceptability (not more than twice). Thus, the method of surface plating using a standard solvent is suitable for determining the number of microorganisms in the preparation and can be used in testing the effectiveness of antimicrobial preservatives.

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To test the effectiveness of selected antimicrobial preservatives, each gel sample container was inoculated with freshly prepared suspension with one of the test microorganisms, providing a microbial load of 10^5 CFU to 10^6 CFU in 1 ml of sample, mixed thoroughly to distribute evenly in sample volume and stored at a temperature of 20 °C to 25 °C in a dark place. Immediately after inoculation and at certain intervals (for preparations for dermal use after 2, 7, 14 and 28 days) 1 ml of sample was taken from each sample and the number of viable microorganisms was determined by direct plating. The criterion for evaluating the effectiveness of the preservative in the dosage form is the reduction of the number of viable cells of test microorganisms in the drug after a certain period of time after its contamination. In accordance with the requirements of the SPhU, the logarithm of the reduction in the number of viable bacterial cells after 2 days should be at least two, after 7 days – at least 3, then the number of viable bacterial cells should not increase; the logarithm of the decrease in the number of viable fungal cells after 14 days should be at least two, and the number of viable fungal cells should not increase in the future [12].

Results and discussion

The results of the study of antimicrobial efficacy of preservatives are shown in table 3. The obtained data indicate that the sample of emulsifier without preservative does not meet the requirements of SPhU, because the logarithm of the reduction of viable bacterial microorganisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) is less than 2.0 and 3.0 in 2 days and 7 days respectively. For cells of *Candida albicans* and *Aspergillus brasiliensis*, on the 14th day lg, the reduction in the number of viable cells in samples required by the SPhU should be at least 2.0, and in samples without preservatives – 1.76 for *Candida albicans* and 1.65 for *Aspergillus brasiliensis*, which also do not meet the requirements. Thus, the obtained results prove the need to add to the composition of the developed emulsion antimicrobial preservatives.

Table 3. The results of the effectiveness of antimicrobial preservatives in the samples of the studied emulgel

Test-culture of microorganisms	Preservative (concentration, %)	lg of the number of viable microorganisms immediately after inoculation, lg CFU/ml	lg of the reduction of the number of viable microorganisms, lg CFU/ml (SPhU requirements 2.3 / results obtained)			
			2 days	7 days	14 days	28 days
Staphylococcus aureus ATCC 6538	No preservative	5,28	2 / 0,95	3 / 2,10	–	NI /2,71
	Nipagin + Nipazol (0,2)	5,34	2 / 2,92	3 / 3,76	–	NI / ND
	Nipagin + Nipazol (0,4)	5,36	2 / 3,05	3 / 3,94	–	NI / ND
	Sodium benzoate (0,15)	5,38	2 / 3,37	3 / 3,53	–	NI / ND
	Sodium benzoate (0,25)	5,36	2 / 3,40	3 / 3,99	–	NI / ND
	Phenylethyl alcohol (0,5)	5,42	2 / 3,02	3 / 3,85	–	NI / ND
	Phenylethyl alcohol (1,0)	5,38	2 / 3,75	3 / 3,97	–	NI / ND
	Phenoxyethanol (0,5)	5,52	2 / 2,05	3 / 3,22	–	NI / ND
	Phenoxyethanol (1,0)	5,59	2/2,67	3/3,95	–	NI / ND
Pseudomonas aeruginosa ATCC 9027	No preservative	5,44	2 / 1,15	3 /2,23	–	NI /2,50
	Nipagin + Nipazol (0,2)	5,48	2 / 2,74	3 / 3,70	–	NI / ND
	Nipagin + Nipazol (0,4)	5,44	2 / 2,79	3 / 3,78	–	NI / ND
	Sodium benzoate (0,15)	5,51	2 / 2,94	3 / 4,35	–	NI / ND
	Sodium benzoate (0,25)	5,44	2 / 2,97	3 / 4,50	–	NI /NI
	Phenylethyl alcohol (0,5)	5,52	2 / 3,05	3 / 3,74	–	NI /NI
	Phenylethyl alcohol (1,0)	5,48	2 / 3,17	3 / ND	–	NI / ND
	Phenoxyethanol (0,5)	5,48	2 / 2,76	3 / 3,70	–	NI / ND
	Phenoxyethanol (1,0)	5,52	2/3,05	3/4,22	–	NI / ND
Candida albicans ATCC 10231	No preservative	5,74	–	–	2 / 1,76	NI /1,95
	Nipagin + Nipazol (0,2)	5,59	–	–	2 / 3,15	NI / ND
	Nipagin + Nipazol (0,4)	5,59	–	–	2 / ND	NI / ND
	Sodium benzoate (0,15)	5,57	–	–	2 / 3,50	NI / ND
	Sodium benzoate (0,25)	5,54	–	–	2 / 3,86	NI / ND
	Phenylethyl alcohol (0,5)	5,47	–	–	2 / 4,14	NI / ND
	Phenylethyl alcohol (1,0)	5,58	–	–	2 / ND	NI / ND
	Phenoxyethanol (0,5)	5,54	–	–	2 / 3,24	NI / ND
	Phenoxyethanol (1,0)	5,68	–	–	2 / ND	NI / ND
Aspergillus brasiliensis ATCC 16404	No preservative	5,57	–	–	2 / 1,65	NI /1,9
	Nipagin + Nipazol (0,2)	5,66	–	–	2 / 3,70	NI / ND
	Nipagin + Nipazol (0,4)	5,52	–	–	2 / ND	NI / ND
	Sodium benzoate (0,15)	5,59	–	–	2 / 4,05	NI / ND

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	Sodium benzoate (0,25)	5,59	–	–	2 / ND	NI / ND
	Phenylethyl alcohol (0,5)	5,57	–	–	2/4,28	NI / ND
	Phenylethyl alcohol (1,0)	5,53	–	–	2/ ND	NI / ND
	Phenoxyethanol (0,5)	5,53	–	–	2/ ND	NI / ND
	Phenoxyethanol (1,0)	5,66	–	–	2/ ND	NI / ND

Notes: NI – no increase in the number of microorganisms compared to the number of viable microorganisms at the previous control point; ND – no viable cells of microorganisms were detected in the experiment.

The results given in table 3, indicate that after 2 days of storage of inoculated samples of emulgel with a combination of preservatives nipagin + nipazol lg, the reduction in the number of viable bacteria was more than 2 and at the concentration of 0.2% was 2.92 for *Staphylococcus aureus*, and 2.74 for *Pseudomonas aeruginosa*; at the concentration of 0.4% was 3.05 for *Staphylococcus aureus*, and 2.79 for *Pseudomonas aeruginosa*. After 7 days of storage of inoculated samples of emulgel with a combination of preservatives nipagin + nipazol lg decrease in the number of viable bacteria was more than 3 and at a concentration of 0.2% was 3.76 for *Staphylococcus aureus*, and 3.70 for *Pseudomonas aeruginosa*; at a concentration of 0.4%, it was 3.94 for *Staphylococcus aureus*, and 3.78 for *Pseudomonas aeruginosa*. For cells of the fungus *Candida albicans* on the 14th day lg, the decrease in the number of viable cells in these samples at a concentration of 0.2% was 3.15 (at least 2 required), and was not detected at 0.4%; for the culture of *Aspergillus brasiliensis* on the 14th day at a concentration of 0.2% lg decrease was 3.70,) and was not detected at 0.4%. On the 28th day, no viable bacterial and fungal cells were detected in the emulgel samples with the combination of nipagin + nipazol preservatives of the selected concentrations. Based on the results of the table 3, samples of emulgel with sodium benzoate also showed antimicrobial activity against *Staphylococcus aureus*. Thus, on the 2nd day, the lg decrease in the number of *Staphylococcus aureus* bacteria was 3.37 and 3.42 at a preservative concentration of 0.15% and 0.25%, respectively; on the 7th day a similar trend was observed – lg decrease in the number of bacteria was 3.53 and 3.99 at a concentration of sodium benzoate of 0.15% and 0.25%, respectively; on the 28th day the cells were not detected. For the test microorganism *Pseudomonas aeruginosa* at a concentration of sodium benzoate 0.15% on the 2nd day lg decrease in the number of bacteria was 2.94, at a concentration of 0.25% it was – 2.97, on the 7th day – 4.35 and 4.50, respectively. On the 28th day, there was no increase in the number of microorganisms compared to the previous control point. For *Candida albicans*, in samples with sodium benzoate in concentrations of 0.15% and 0.25% on the 14th day of observation, the lg reduction in cell number was 3.50 and 3.86, respectively, and for *Aspergillus brasiliensis* – 4.05 and no cells were detected. In both cases, on the 28th day of the experiment, the cells of these test microorganisms were not registered.

The following results were observed in samples with preservative phenylethyl alcohol: at concentrations of 0.5% and 1.0%, respectively: lg decrease in the number of *Staphylococcus aureus* cells was 3.02 and 3.75 on the 2nd day and 3.85 and 3.97 on the 7th day; for *Pseudomonas aeruginosa* – 3.05 and 3.17 on the 2nd day and 3.74 and not detected on the 7th day. For *Candida albicans* and *Aspergillus brasiliensis* at a preservative concentration of 0.5% on the 14th day lg, the decrease in cell number was 4.14 and 4.28, and at a concentration of 1.0% – it was not detected. On the 28th day of observations from samples with both concentrations of preservative, cells of all test microorganisms were not registered. Similar trends were observed for emulgel samples with the addition of the preservative phenoxyethanol at concentrations of 0.5% and 1.0%: lg decrease in the number of *Staphylococcus aureus* cells was 2.05 and 2.67 on the 2nd day and 3.22 and 3.95 on the 7th day; for *Pseudomonas aeruginosa*, the numbers were 2.76 and 3.05 on the 2nd day and 3.70 and 4.22 on the 7th day. For *Candida albicans* and *Aspergillus brasiliensis* at a preservative concentration of 0.5% on the 14th day lg, the decrease in cell number was 3.24 and was not detected, and at a concentration of 1.0% – not detected. On the 28th day of observations from samples with both concentrations of preservative, cells of all test microorganisms were not registered. Summarizing the results of table 3, it is possible to note compliance of the received data with the requirements of SPhU to preparations for dermal application in terms of emulgel with selected preservatives.

Conclusions

Tests have shown that the studied preservatives (sodium benzoate, phenoxyethanol, phenylethyl alcohol and the combination of nipagin + nipazol), which are used in the emulgel with a thick extract of the feverfew on the basis of gelling agent Sepiplus-400 for treatment of diseases of the musculoskeletal system, showed high antimicrobial efficacy and met the requirements of SPhU 2.3 criterion A for drugs for dermal use.

Given the requirements of safety and economy, physicochemical properties and the possibility of using as a flavoring, the most acceptable preservative in this development is phenylethyl alcohol, as the most acceptable concentration of the preservative was 1.0%. Samples of emulgel with preservative in the selected concentration were laid on the preservation and determination of quality indicators during the offered storage time.

Research on the selection of emulgel preservative with thick extract of feverfew

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Introduction. The composition of the emulsifier with a thick extract of feverfew obtained at the Department of Botany of NUPh, was developed and experimentally substantiated for the prevention and treatment of inflammatory diseases of joints and connective tissues

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at the Department of Factory Technology of Drugs of the National University of Pharmacy. The drug contains API of plant origin and purified water - both components affect the stability of the product during storage and use due to the risk of biological contamination of the finished dosage form. The presence of microorganisms in non-sterile drugs can cause a reduction or even inactivation of their therapeutic effect, so at the stage of pharmaceutical development should be considered to ensure microbiological purity. **Materials and methods.** Tests on the effectiveness of antimicrobial preservatives were performed according to the method of SPhU (State Pharmacopoeia of Ukraine) 2.3, paragraph 5.1.3. Soybean-Casein agar and Sabouraud dextrose agar were used as nutrient media, and buffer solution with sodium chloride and peptone pH = 7.0, containing 50 g / lpolysorbate-80, 5 g / l lecithin, 1 g / l histidine hydrochloride were used as a solvent. *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404 were used as test cultures of microorganisms. **Results and discussion.** The obtained data indicate that the sample of emulsifier without preservative does not meet the requirements of SPhU, because the logarithm of the reduction of viable bacterial microorganisms (*S. aureus* and *P. aeruginosa*) is less than 2.0 and 3.0 in 2 days and 7 days respectively. For cells of *C. albicans* and *A. brasiliensis*, on the 14th day lg, the reduction in the number of viable cells in samples required by the SPhU should be at least 2.0, and in samples without preservatives - 1.76 for *C. albicans* and 1.65 for *A. brasiliensis*, which also do not meet the requirements. Thus, the obtained results prove the need to add to the composition of the developed emulsion antimicrobial preservatives. According to the research, it is possible to note compliance of the received data with the requirements of SPhU to preparations for dermal application in terms of emulgel with selected preservatives (sodium benzoate, phenoxyethanol, phenylethyl alcohol and the combination of nipagin + nipazol). **Conclusion.** Given the requirements of safety and economy, physicochemical properties and the possibility of using as a flavoring, the most acceptable preservative in this development is phenylethyl alcohol, as the most acceptable concentration of the preservative was 1.0%. Samples of emulgel with preservative in the selected concentration were laid on the preservation and determination of quality indicators during the offered storage time.

Key words: emulgel, antimicrobial preservatives, antimicrobial activity, phenylethyl alcohol.

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