STUDY OF ANTIBACTERIAL PROPERTIES OF THE EMULGEL WITH SCUTELLARIA BAICALENSIS EXTRACT

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Introduction

One of the important problems of medicine for many years remains the search for effective treatments for infected wound processes. Wound healing is a complex biological process, in the complex therapy of which a relevant direction is the development of wound healing drugs with a wide range of pharmacological action that affect different parts of the wound process. Modern medicine offers a large number of methods for the treatment of wound processes of various aetiology, mainly due to pathogenic microflora and the development of inflammatory response.

Many antimicrobials have been developed and proposed, but the phenomenon of microorganisms' resistance to drugs, reduction of general and local immunological activity require improvement of existing and finding new treatments and drugs capable of providing complex antibacterial, anti-inflammatory and reparative action.

Semisolids remain an indispensable dosage form in the treatment of wound injuries, the interest in which has increased recently due to the new tend of including phytopreparations, providing soft action and low toxicity against the background of high efficiency, and the complex of biologically active substances has a versatile and complementary effect. This makes it possible to use them for a long time with a minimal number of side effects [2].

Particular attention is paid to the properties of the Scutellaria baicalensis among the plants. The main constituents of Scutellaria root are baikaleine, baikalin, vogonin, acetophenone, neobaicaleine, palmitic acid, oleic acid, benzoic acid, beta-sitosterol and others. All these chemical compounds provide the plant anti-allergic, antibacterial, antimicrobial, diuretic, sedative, antioxidant, anticancer, antiviral, anticonvulsant, vasoconstrictor, antitoxic, haemostatic and antiparasitic properties. Considering the variety of compounds and the action inherent in the raw material, we have developed an emulgel based on the dry extract of Scutellaria root and rhizomes. The purpose of our work is to study the antimicrobial activity of the created emulgel and to choose a preservative.

Materials and methods

For the study of microbiological purity, three samples of emulgel with extract content of 1%, 2% and 2.5% were taken.

The following test strains were used to evaluate the activity of the drug samples: *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633, Proteus vulgaris ATCC 4636, Candida albicans ATCC 885/653.*

Preparation of the microbial suspension was performed using a Densi-La-Meter device (manufactured by PLIVA-Lachema, Czech Republic; wavelength 540 nm). The suspension was prepared in accordance with the instructions provided with the instrument and information letter on innovations in the health care system No. 163-2006 "Standardization of microbial suspensions preparation ", Kyiv. The synchronization of the cultures was performed using a low temperature (4 ° C). The microbial load was 10^7 microbial cells per 1 ml of medium and was calculated according to the McFarland standard. In operation took 18-24 days culture of microorganisms. The study used the Muller- Hinton agar (HIMedia Laboratorles Pvt.Ltd India » medium shelf life up to XII 2020, Manufactured in India). For Candida albicans Sabouraud -dextrose agar (Manufactured in India by HI Media Laboratories Pvt. Ltd India) was used. Medium shelf life up to XII 2020).
Determination of antibacterial activity of the study drug was carried out by the method of diffusion into agar (method of "wells") on two layers of agarised nutrient medium on Petri dishes. The bottom layer is a floor with 10 ml of agar on which horizontally installed 3-6 cylinders made of stainless steel. Around them, the top layer of agar medium was filled with the appropriate daily culture standard of the test strain (14 ml of agar + 1 ml of microbial mixture). After solidification of the agar, the cylinders were removed with sterile tweezers and the test sample of the preparation (0.25 - 0.3 ml) was introduced into the hole.

The following criteria were used to evaluate the antibacterial properties:
- the absence of zones of growth retardation around the hole indicates the insensitivity of the microorganism to the studied sample;
- the diameter of the growth retardation zone 10-15 mm indicates a weak sensitivity of the microorganism to the sample;
- the diameter of the growth retardation zone of 15-25 mm indicates the sensitivity of the microorganism to the test sample;
- the diameter of the growth retardation zone more than 25 mm is evaluated as high sensitivity of the microorganism to the test specimen;

Cultivation media were used according to the type of microorganisms in accordance with existing methodological developments and recommendations.

Antimicrobial and anticancer effects of new drugs were determined by the standard method of two-fold serial dilutions in nutrient broth (macromethod). Testing was carried out in a volume of 1 ml of each dilution with a final concentration of the tested microorganism 5×10^5 CFU / ml. After incubation for 24 hours or 48-72 hours for *C. albicans* cultures. The test tubes with crops were observed in ray light to determine the growth of the microorganism. During the

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experiments, additional controls were performed on the growth of culture in an environment without the test substances, in the solvent; control of the purity of the microbial suspension (by seeding on non-selective media) and sterility of the medium.

Microbiological purity tests were performed by direct cropping on liquid nutrient media. The soybean-casein broth, thioglycol medium and Sabouraud liquid medium were sterile poured into tubes of 10.0 ml. In each of the tubes introduced 0.1 g of the test drug. Crops were incubated for 14 days on soybean-casein broth and thioglycol medium in a thermostat at 35 ° C, crops on Sabouraud liquid medium at 25° C. Neutralization of antibacterial properties of the samples was carried out by inactivator, which includes polysorbate-80 (30 g / l). and lecithin (3 g / l).

The most common substances that are authorized for use in pharmacy in Ukraine were used to select a preservative. Parabens derivatives (Nipagin, Nipasol, germaben) and glycol (euxyl) were selected for the research. The preservatives concentration was selected according to the literature data.

The effectiveness of preservatives' action was evaluated by the logarithm of reducing the number of viable microorganisms [3, 4].

Statistical processing of the obtained results was carried out using «Statgraphics» software in accordance with the requirements of the SPU [5-7].

Results and discussion

As a result of the study, it was found that the samples of the emulsifier based on the dry extract of Scutellaria root (1%, 2% and 2.5%) possess antibacterial action against the test strains [8, 9] .The diameters of growth retardation zones were 20-23 mm relative to *Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633, 18-20* mm - to *Escherichia coli ATCC 25922*, to *Proteus vulgaris ATCC 4636 and Pseudomonas aeruginosa ATCC 27853 - the diameter was 16* - 19 mm, relative to the fungus *Candida albicans ATCC 653/885* the zone was 16-17 mm. In comparison drug, the solution of chlorophyllin the diameter of the growth retardation zone was smaller, and with respect to Proteus vulgaris ATCC 4636 and Pseudomonas aeruginosa ATCC 27853 antibacterial properties were not registered. The data are presented in table 1.

Table1 Antibacterial properties of the test samples

samples	Diameters of growth inhibition zones in mm (M ± m) n = 6, (p≤0.05)					
	S. aureus ATCC 25923	*E. coli ATCC 25922*	*Ex. vulgaris ATCC 4636*	*P. aeruginosa ATCC 27853*	*B.subtilis ATCC 6633*	*C. albicans ATCC 653/885*
1%	20.2 ± 1.2	18.6 ± 0.4	16.3 ± 1.1	17.2 ± 0.2	21.2 ± 1.1	16.2 ± 0.8
2%	21.6 ± 1.5	19.7 ± 1.6	17.4 ± 1.4	18.5 ± 1.1	22.2 ± 0.7	16.3 ± 1.1
2.5%	20.6 ± 0.9	18.4 ± 1.3	17.1 ± 0.8	18.2 ± 0.4	21.3 ± 1.3	16.2 ± 0.82
Control (Chlorophyllipt solution)	22 ± 1.1	16.7 ± 0.3	growth	growth	16.4 ± 1.2	12.2 ± 1.6

It was also found (Table 2) that the samples had antibacterial activity against clinical strains. The diameters of growth inhibition zones were 15–20 mm relative to *Pseudomonas aeruginosa 46*, *Proteus vulgaris* 18, *Staphylococcus aureus 25, Escherichia coli 7.*

Table 2 Antibacterial properties in relation to clinical strains

Samples	Diameter and growth inhibition zone in mm number of repetitions n = 6, (p≤0,05)			
	P. aeruginosa 46	*Pr. vulgaris 18*	*S. aureus 25*	*E. coli 7*
1%	17.1 ± 0.4	15.2 ± 0.1	17.4 ± 0.3	17.3 ± 0.41
2%	18.3 ± 0.8	17.3 ± 0.5	20.1 ± 0.28	19.2 ± 0.25
2.5%	17.1 ± 0.3	16.2 ± 0.35	19, 2 ± 0.25	18.2 ± 0.21
Control (Chlorophyllipt solution)	growth	growth	16.2 ± 0.18	growth

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As a result of the screening, the samples were found to have moderate antimicrobial activity against the tested reference strains of gram-positive and gram-negative microorganisms, as well as to clinical isolates of the microorganisms.

The results of the studies show that the sample of emulgel with 2% content of Scutellaria extract exceeds the sample with 1% content of the extract by antimicrobial activity. Increasing the amount of extract to 2.5% is not rational, since it does not increase the activity. In this regard, a sample with a dry extract content of 2% was selected for further studies.

For microbiological purity studies of freshly made emulgel, the growth characteristics of nutrient media used in the experiment have been investigated. The data are presented in table 3.

Table 3 - Growth properties of nutrient media

Test strains	Nutrient media	Cultivation conditions		Conclusion
		Temperature	Duration of cultivation	
S. aureus ATCC 6538	Chistovich	35 º C,	24-72 hours	Colony and cell morphology is typical
E. coli ATCC 25922	Endo	35 º C,	24-72 hours	Colony and cell morphology is typical
B. subtilis ATCC 6633	Soybean casein agar	35 º C,	24-72 hours	Colony and cell morphology is typical
P. aeruginosa ATCC 9027	Soybean casein agar	35 º C,	24-72 hours	Colony and cell morphology is typical
C. albicans ATCC 885/653	Sabouraud - dextrose agar	25 º C,	24-120 hours	Colony and cell morphology is typical
x	Thioglycol medium for sterility control	35 º C,	24-72 hours	The growth of microorganisms is absent
x	Soybean casein broth	35 º C,	24-72 hours	The growth of microorganisms is absent

Note: x - microorganisms were not cropped

The data in table 3 indicate that all cultures of microorganisms corresponded to the taxonomic designation of the strain, and the morphology of colonies when cultured on media and the morphology of cells under microscopy were typical. Nutrient broths (soybean-casein and thioglycol medium) met sterility requirements - microbial growth was absent, medium was transparent.

Another point of study for microbiological purity was the testing of specimens on selective nutrient media.

Studies have shown that after 14 days of incubation during cultivation on Sabouraud medium, growth of fungi was absent. On soybean-casein broth and thioglycol medium, the growth of microorganisms was absent when tested for samples of the drug in the amount of 0.1 g.

In the study of microbiological purity by direct sowing on the dishes it has been found that the growth of fungi was absent in the study of all samples. The number of microorganisms that grew on 0.1 g samples of the preparation did not exceed 10^3 CFU / ml, which meets the requirements of the State Pharmacopoeia of Ukraine.

Microscopy showed the presence of a gram-positive vegetative spore bacillus in the 2.0% emulgel samples based on the dry extract of Scutellaria. Confirmation was obtained by cropping on differential nutrient media. Obtained data showed that in morphology of the colonies and some biological properties the isolated microorganisms found in the study belong to the genus *Bacillus sp*. On differential media (Chistovich medium and Endo medium) for the isolation of intestinal group and pathogenic staphylococci the growth among other microorganisms was not observed.

The next step was to investigate the effectiveness of the antimicrobial preservatives (Nipagin + Nipasol, germaben and euxyl). The results are shown in table.4.

The data of the table indicate that when using Nipagin and Nipasol at a concentration of (0,15 / 0,5) after 7 days of cultivation, the logarithm for reducing the number of viable fungal cells was 4.05 for *C. albicans;* on days 14 and 28, viable *C. albicans* cells were not isolated. After 2 days of cultivation, the logarithm of the number of colonies of microorganisms was 1.22 for *S. aureus* and 1.05 for *P. aeruginosa* . At day 7, the logarithm of viable cell count for *S. aureus* was 3.37, for *P. aeruginosa* it was 3.09. On the 14th and 28th days of incubation, colonies of *S. aureus* and *Ps. aeruginosa were* not observed.

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Table 4 The activity of antimicrobial preservatives in the samples tested

Preservative	Concentration, %	Microbial load 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						
Nipagin/ Nipasol	0,15 / 0,5	5,76	1,22	3,37	NF	NF
Euxyl	0,1	5,44	2,95	NF	NF	NF
Germaben	0,1	5,76	2,11	3,59	NF	NF
Pseudomonas aeruginosa ATCC 9027						
Nipagin/ Nipasol	0,15 / 0,5	5,74	1,05	3,09	NF	NF
Euxyl	0,1	5,74	2,51	4,44	NF	NF
Germaben	0,1	5,76	2,08	3,37	NF	NF
Candida albicans ATCC 885-653						
Nipagin/ Nipasol	0,15 / 0,5	5,74	2,2	4,05	NF	NF
Euxyl	0,1	5,74	1,07	3,31	NF	NF
Germaben	0,1	5,72	2,55	4,03	NF	NF

Notes: NF - viable cells of test microorganisms were not found.

In the study of the effectiveness of euxyl in the amount of 0.1% after 7 days of cultivation, the logarithm of reducing the number of viable fungal cells was 3.31 for *C. albicans.* On days 14 and 28, viable *C. albicans* cells were not isolated. After 2 days of cultivation, the logarithm of the number of colonies of microorganisms was 2.95 for *S. aureus* and 2.51 for *P. aeruginosa* . On the 7th day of the study viable cells of *S. aureus were* not observed, and the logarithm of the number of colonies of microorganisms *P. aeruginosa* was 4.44. On 14th and 28th days of incubation, colonies of *S. aureus* and *P. aeruginosa were* not recorded.

A study of the effectiveness of germaben in the amount of 0.1% showed that after 7 days of cultivation, the logarithm of reducing the number of viable fungal cells was 4.03 for *C. albicans*. On days 14 and 28, viable *C. albicans* cells were not isolated. After 2 days of cultivation, the logarithm of the number of colonies of microorganisms was 2.11 for *S. aureus* and 2.08 for *P. aeruginosa* . At day 7, the logarithm of viable cell count for *S. aureus* was 3.59, for *Ps.aeruginosa* it was 3.37. On the 14th and 28th day of incubation, colonies of *S. aureus* and *P. aeruginosa were* not observed. At the 14th and 28th day of incubation, no microorganisms were isolated.

Based on the studies and according to the literature, euxyl has been chosen as a preservative, as it has the widest spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, yeast and fungi in the pH range of 3.0-12.0 and is a safer and economically reasonable option.

Thus the obtained sample of the emulgel meets the criterion "A", according to the requirements of the SPU.

Conclusions

1. According to the results of the study of the antimicrobial activity of samples of emulgel with Scutellaria baikalensis extract, it has been found that the optimal content of dry extract in the developed drug is 2%.
2. The microbiological purity of the freshly made specimen was investigated and antibacterial activity against a wide range of microorganisms of different taxonomic groups has been proven.
3. Studies on the preserving ability of antimicrobial preservatives (Nipagin + Nipasol, germaben and euxyl) have established the effectiveness of all selected substances. All samples met the requirements of SPU (criterion "A"). As the most promising one Euxyl preservative has been selected, which is the safest and most economically reasonable with a wider pH range.

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Study of antibacterial properties of the emulgel with *Scutellaria baicalensis* extract

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Introduction. One of the important problems of medicine for many years remains the search for effective treatments for infected wound processes. Semisolids remain an indispensable dosage form in the treatment of wound injuries, the interest in which has increased recently due to the new tend of including phytopreparations, providing soft action and low toxicity against the background of high efficiency, and the complex of biologically active substances has a versatile and complementary effect. This makes it possible to use them for a long time with a minimal number of side effects. Particular attention is paid to the properties of the Scutellaria baicalensis among the plants. Considering the variety of compounds and the action inherent in the raw material, we have developed an emulgel based on the dry extract of Scutellaria root and rhizomes. The purpose of our work is to study the antimicrobial activity of the created emulgel and to choose a preservative.

Materials and methods. For the study of microbiological purity, three samples of emulgel with extract content of 1%, 2% and 2.5% were taken. The following test strains were used to evaluate the activity of the drug samples: *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633, Proteus vulgaris ATCC 4636, Candida albicans ATCC 885/653.* Determination of antibacterial activity of the study drug was carried out by the method of diffusion into agar (method of "wells") on two layers of agarised nutrient medium on Petri dishes. Cultivation media were used according to the type of microorganisms in accordance with existing methodological developments and recommendations. Antimicrobial and anticancer effects of new drugs were determined by the standard method of two-fold serial dilutions in nutrient broth (macromethod). Microbiological purity tests were performed by direct cropping on liquid nutrient media. The soybean-casein broth, thioglycol medium and Sabouraud liquid medium were sterile poured into tubes of 10.0 ml. The most common substances that are authorized for use in pharmacy in Ukraine were used to select a preservative. Parabens derivatives (Nipagin, Nipasol, germaben) and glycol (euxyl) were selected for the research. **Results and discussion.** As a result of the study, it was found that the samples of the emulsifier based on the dry extract of Scutellaria root (1%, 2% and 2.5%) possess antibacterial action against the test strains. The diameters of growth retardation zones were 20-23 mm relative to *Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633, 18-20* mm - to *Escherichia coli ATCC 25922,* to *Proteus vulgaris ATCC 4636 and Pseudomonas aeruginosa ATCC 27853 - the diameter was 16* - 19 mm, relative to the fungus *Candida albicans ATCC 653/885* the zone was 16-17 mm. In comparison drug, the solution of chlorophyllin the diameter of the growth retardation zone was smaller, and with respect to Proteus vulgaris ATCC 4636 and Pseudomonas aeruginosa ATCC 27853 antibacterial properties were not registered. It was also found that the samples had antibacterial activity against clinical strains. The diameters of growth inhibition zones were 15–20 mm relative to *Pseudomonas aeruginosa 46*, *Proteus vulgaris* 18, *Staphylococcus aureus 25, Escherichia coli 7.* In the study of microbiological purity by direct sowing on the dishes it has been found that the growth of fungi was absent in the study of all samples. The number of microorganisms that grew on 0.1 g samples of the preparation did not exceed 10^3 CFU / ml, which meets the requirements of the State Pharmacopoeia of Ukraine. Microscopy showed the presence of a gram-positive vegetative spore bacillus in the 2.0% emulgel samples based on the dry extract of Scutellaria. Confirmation was obtained by cropping on differential nutrient media. Obtained data showed that in morphology of the colonies and some biological properties the isolated microorganisms

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Keywords: antibacterial; emulgel; *Scutellaria baicalensis*; extract

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