

ЕКСПЕРИМЕНТАЛЬНА ТА КЛІНІЧНА ФАРМАКОЛОГІЯ

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MICROBIOLOGICAL STUDIES OF THE OINTMENT AND RECTAL SUPPOSITORIES WITH LIQUORICE ROOT EXTRACT

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While developing, preparing, packing, storing and using medicines the measures should be taken to ensure their microbiological purity taking into account the requirements of the general pharmacopoeial article "Microbiological purity of medicines". In order to prevent microbial contamination of products it is necessary to provide them with the effective preservative action due to the introduction of antimicrobial preservatives or due to the antimicrobial preservative action of active substances and appropriate conditions of production. The aim of the work is to study the effectiveness of the preservative action of the ointment and rectal suppositories with liquorice root extract in accordance with the general pharmacopoeial article "Efficiency of antimicrobial preservatives". The criterion for evaluating the efficiency of the preservative action of the samples investigated is reduction of the number of viable cells of microorganisms in the medicines for a certain period of time after their contamination. During the experiment it has been found that the antimicrobial preservative efficiency of the test samples correspond to the requirements of criterion "A" in accordance with the State Pharmacopoeia of Ukraine, section 5.1.3. The efficiency of the preservative action of the samples at the level of the samples with the additional content of nipagin and nipazol (in the ratio of 2:1) allows not to include additional antimicrobial preservatives in their composition.

The current problem of practical medicine is treatment of infectious diseases, which common characteristic feature is abnormality of the immune system. The depth and focus of disturbances in the immune system varies depending on nosology and severity of the disease, the etiological agent, genetic predisposition, age, gender, etc. [1, 7, 9].

Along with causal therapy in the adjuvant treatment of the pathologies mentioned medicines with the immunomodulatory activity are often used [10, 11, 12].

Although the range of the specified medicines today at the pharmaceutical market of Ukraine is small, for the last time professionals in the pharmaceutical industry have been developed and obtained complex medicines not only for correction of the immunity parameters, but also with the antiviral, anti-inflammatory and antibacterial action [3, 5]. The nomenclature of dosage forms of these medicines has been also expanded. Medicines for intravenous, oral, external (ointments, suppositories) application are currently available at the market [1, 3].

In terms of the abovementioned, rectal suppositories and the ointment with liquorice root extract have been developed at the Department of Drug's Technology of the National University of Pharmacy. In the previ-

ous experiments *in vitro* the antiviral activity of these medicines in relation to adenovirus of type 3, coronavirus, herpes virus and vesicular stomatitis virus was determined [5, 6].

The study of the specific activity of the medicines developed has determined their distinct immunostimulatory and immunocorrective properties in immature rats with the normal immune status and in conditions of immunodeficiency caused by hydrocortisone acetate. On the basis of studying acute toxicity it has been found that in accordance with the common classification of K.K. Sidorov the medicines developed belong to the class of low toxic substances.

It is known that when developing, preparing, packing, storing and using medicines the measures should be taken to ensure their microbiological purity taking into account the requirements of the general pharmacopoeial article "Microbiological purity of medicines" [4, 8].

In order to prevent microbial contamination of medicines it is necessary to provide them with the effective preservative action due to the introduction of antimicrobial preservatives to their composition or due to the antimicrobial preservative action of active substances and appropriate conditions of production [13, 14].

Table 1

Efficiency of the antimicrobial preservative action of the ointment with liquorice root extract

Exposition	Requirements of the SPhU		Logarithm of the number of microorganisms		
	Logarithm of reduction	Logarithm of reduction	<i>Staphylococcus aureus</i> ATCC 6538	<i>Pseudomonas aeruginosa</i> ATCC 9027	<i>Candida albicans</i> ATCC 885/653
Microbial load	10 ⁶	10 ⁶	5.30	5.39	5.39
Primary inoculation	-	-	0.62	0.61	NI
2 days	2.00	-	2.05	2.07	NI
7 days	3.00	-	3.62	4.09	NI
14 days	-	-	NI	NI	NI
28 days	NR	NR	NI	NI	NI

Notes: n = 5; P = 95%; * NR – the number of viable cells of microorganisms or fungi did not rise; * NI – viable cells of microorganisms or fungi were not isolated.

The aim of our research was to study the efficiency of the preservative action of the ointment and rectal suppositories with liquorice root extract in accordance with by the methods and criteria described in the general pharmacopoeial article “Effectiveness of antimicrobial preservatives”.

Materials and Methods

Microbiological studies of the samples of rectal suppositories and the ointment with Liquorice root extract were carried out at the laboratory of Biochemistry of Microorganisms and Nutrient Media of the State Institution “Institute of Microbiology and Immunology named after I.I. Mechnikov of the National Academy of Medical Sciences of Ukraine”.

To determine the efficiency of the preservative action of the medicines developed the biological method described in the State Pharmacopoeia of Ukraine (SPhU) 1.0, section 5.1.3. [2] and such test strains of microorganisms as *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 885/653 were used.

While testing the monocultures of these microorganisms were used. In preparation for the research the freshly grown initial culture of each test microorganism was plated on the surface of a dense nutrient medium No. 1 (SPhU, section 2.6.13) in the case of growing bacteria or a dense nutrient medium No. 2 without adding antibiotics (SPhU, section 2.6.13) in the case of fungi cultivation. Bacterial cultures were incubated at 35°C for 18-24 hours, *C. albicans* – at the temperature of 20°C to 25°C for 48 hours.

For preparation of suspensions with bacteria culture and culture of *C. albicans* the microbial mass was washed from the surface of the culture medium with sterile 0.9% sodium chloride solution and transferred to a suitable container. The inoculum was used immediately after preparation.

The test of efficiency of the preservative action was performed as follows: the samples of the medicines studied were contaminated by monoculture of one of the test microorganisms providing the microbial load of 10⁷ CFU/ml. The inoculum volume was less than 1% of the sample volume.

In order to obtain a homogeneous distribution of microorganisms the contaminated samples were stirred thoroughly. The inoculated samples were stored for 28 days at the temperature of 20°C to 25°C protected from light.

From each test specimen the samples were taken immediately after contamination in 2, 7, 14 and 28 days, and they were inoculated on dense nutrient media for determining the number of viable cells of microorganisms (bacteria and fungi).

The samples of the medicines under research with the additional content of synthetic antimicrobial preservatives – nipagin and nipazol (in the ratio of 2:1) were used as reference medicines.

Results and Discussion

The criterion for evaluating the efficiency of the preservative action of the samples studied was reduction of the number of viable microbial cells in medicines for a certain period of time after contamination.

According to the requirements of the SPhU in medicinal products for topical application the logarithm of reduction of the number of viable bacteria colonies in 2 days must be at least 2, in 7 days – at least 3, and then the number of viable bacterial cells should not increase. The logarithm of reduction of the number of viable cells of fungi within 14 days must be at least 2. These figures correspond to criterion “A”.

Criterion “A” corresponds to the efficiency of the preservative action that is recommended and its compliance indicates reliable protection of the medicine from microbial contamination. If criterion “A” cannot be achieved, the medicine must meet criteria “B”.

According to criterion “B” in medicines for topical use the logarithm of the number of viable bacterial cells in 14 days must be at least 3, then the number of viable bacterial cells should not increase. The logarithm of reduction of the number of viable cells of fungi within 14 days must be at least 1 and with no further increase.

After contamination by microorganisms the medicines at regular intervals are inoculated on agar to determine the number of viable cells. The absence of growth on agar or delayed increase in the number of viable colonies in 14 days of incubation indicate the fact that the medicines meet requirements of the SPhU.

Table 2

Efficiency of the antimicrobial preservative action of rectal suppositories with liquorice root extract

Exposition	Requirements of the SPhU		Logarithm of the number of microorganisms		
	Logarithm of reduction	Logarithm of reduction	<i>Staphylococcus aureus</i> ATCC 6538	<i>Pseudomonas aeruginosa</i> ATCC 9027	<i>Candida albicans</i> ATCC 885/653
Microbial load	10 ⁶	10 ⁶	5.30	5.39	5.39
Primary inoculation	-	-	0.62	0.61	0.69
2 days	2.00	-	2.98	2.07	2.05
7 days	3.00	-	NI	3.62	4.09
14 days	-	2.00	NI	NI	NI
28 days	NR	NR	NI	NI	NI

Notes: n = 5; P = 95%; * NR – the number of viable cells of microorganisms or fungi did not rise; * NI – viable cells of microorganisms or fungi were not isolated.

The presence of viable cells of microorganisms and fungi in 28 days of the research indicates that the medicines do not correspond to criteria “A” or “B” and do not meet the requirements of the SPhU. Table 1 shows the results of the study of the efficiency of the antimicrobial preservative action of the ointment with liquorice root extract.

As data presented in Table 1 show, a rapid destruction of microorganisms is observed in the medicine under research after contamination. Viable cells of fungi were not isolated in both the primary inoculation and in the following ones.

In 2 days of cultivation the logarithm of the number of viable cells of microorganisms was more than 2 and was 2.05 for *Staphylococcus aureus* and 2.07 for *Pseudomonas aeruginosa*. In 7 days after contamination the logarithm of the number of viable cells of microorganisms for *Staphylococcus aureus* was 3.62, for *Pseudomonas aeruginosa* – 4.09. On the 14-th and 28-th days of incubation microorganisms were not registered.

Regarding the efficiency of the antimicrobial preservative action of rectal suppositories with liquorice root extract (Tab. 2), in 7 days of cultivation the logarithm of the number of viable cells of *Candida albicans* was 4.09.

The cells of fungi were not isolated after 14 and 28 days of cultivation.

In 2 days after cultivation the logarithm of the number of viable cells of microorganisms was more than 2 and was 2.98 for *Staphylococcus aureus* and 2.07 for *Pseudomonas aeruginosa*. In 7 days of contamination the cells of *Staphylococcus aureus* were not isolated, for *Pseudomonas aeruginosa* the figure was 3.62. On

the 14-th and 28-th days of incubation microorganisms were not registered.

Thus, by efficiency of the antimicrobial preservative action the samples of rectal suppositories and the ointment with liquorice root extract conform to criterion “A” set by the SPhU, section 5.1.3 for medicinal products for topical use.

In addition, the studies have also shown that the efficiency of the preservative action of the samples under research without any preservatives was at the level of the samples with the additional content of nipagin and nipazol (in the ratio of 2:1).

So, we can conclude that the active ingredients and the proper conditions of production of the medicinal products investigated provide the necessary efficiency of the antimicrobial preservative action, which is similar to those of the reference medicines, and it allows not to include additional antimicrobial preservatives in their composition.

CONCLUSIONS

1. The experimental research on the study of efficiency of the preservative action of the test samples of rectal suppositories and the ointment with liquorice root extract has been conducted.

2. During the experiment it has been found that the antimicrobial preservative efficiency of the test samples correspond to the requirements of criterion “A” in accordance with the State Pharmacopoeia of Ukraine, section 5.1.3.

3. The efficiency of the preservative action of the samples at the level of the samples with the additional content of nipagin and nipazol (in the ratio of 2:1) allows not to include additional antimicrobial preservatives in their composition.

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МІКРОБІОЛОГІЧНІ ДОСЛІДЖЕННЯ МАЗІ ТА РЕКТАЛЬНИХ СУПОЗИТОРІЇВ З ЕКСТРАКТОМ СОЛОДКОВОГО КОРЕНЯ

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Ключові слова: мікробіологічні дослідження; мазь; супозиторії; екстракт солодкового кореня

У процесі розробки, приготування, пакування, зберігання та застосування лікарських засобів мають бути вжиті заходи щодо їх забезпечення мікробіологічною чистотою з урахуванням вимог загальної фармакопейної статті «Мікробіологічна чистота лікарських засобів». З метою запобігання мікробному забрудненню препаратів необхідно забезпечувати їх ефективною консервуючою дією за рахунок введення антимікробних консервантів або за рахунок антимікробної консервуючої активності діючих речовин і належних умов виробництва. Метою роботи є вивчення ефективності консервуючої дії мазі та ректальних супозиторіїв з екстрактом солодкового кореня відповідно до вимог загальної фармакопейної статті «Ефективність антимікробних консервантів». Критерієм оцінки ефективності консервуючої дії досліджуваних зразків слугувало зниження числа життєздатних клітин мікроорганізмів у препаратах за визначений період часу після їх контамінації. У ході проведення експерименту було встановлено, що за ефективністю антимікробної консервуючої дії досліджувані зразки препаратів відповідають вимогам критерію «А» відповідно до ДФУ п. 5.1.3. Ефективність консервуючої дії зразків на рівні зі зразками із додатковим вмістом ніпагіну та ніпазолу (у співвідношенні 2:1) дозволяє не включати до їх складу додаткові антимікробні консерванти.

МИКРОБИОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ МАЗИ И РЕКТАЛЬНЫХ СУППОЗИТОРИЕВ С ЭКСТРАКТОМ СОЛОДКОВОГО КОРНЯ

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Ключевые слова: микробиологические исследования; мазь; суппозитории; экстракт солодкового корня

В процессе разработки, приготовления, упаковки, хранения и применения лекарственных средств должны быть приняты меры по их обеспечению микробиологической чистотой с учетом требований общей фармакопейной статьи «Микробиологическая чистота лекарственных средств». С целью предотвращения микробного загрязнения препаратов необходимо обеспечивать их эффективным консервирующим действием за счет введения антимикробных консервантов или за счет антимикробной консервирующей активности действующих веществ и надлежащих условий производства. Целью работы является изучение эффективности консервирующего действия мази и ректальных суппозиториях с экстрактом солодкового корня в соответствии с требованиями общей фармакопейной статьи «Эффективность антимикробных консервантов». Критерием оценки эффективности консервирующего действия исследуемых образцов было снижение числа жизнеспособных клеток микроорганизмов в препаратах за определенный период времени после их контаминации. В ходе проведения эксперимента было установлено, что по эффективности антимикробного консервирующего действия исследуемые образцы препаратов отвечают требованиям критерия «А» в соответствии с ГФУ п. 5.1.3. Эффективность консервирующего действия образцов на уровне с образцами с дополнительным содержанием нипагина и нипазола (в соотношении 2:1) позволяет не включать в их состав дополнительные антимикробные консерванты.