DEVELOPMENT OF COMBINED COMPOSITION PESSARIES FOR GENITAL HERPES TREATMENT

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Among sexually transmitted infections, Genital herpes (GH) is the second most common disease after trichomoniasis. According to the statistics data, only in the USA, every 4–6 people of this country are carriers of this virus [1]. Thereby, in the world the active search for anti-herpetic drugs is being carried out; it led to the creation of the anomalous nucleosides group – Acyclovir and its derivatives [2].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Acyclovir is one of the most frequently prescribed antiviral drugs (AVD), the "gold" standard of GH treatment. Among the existing synthetic AVD, it is the safest, but at the same time its bioavailability is rather low (about 20 %) [3]. It is well-known that the use of Acyclovir combination with other drugs is one of the ways to increase its bioavailability and efficiency. The best results are shown when using drugs with different mechanisms of action.

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author

On the modern Ukrainian pharmaceutical market, anti-herpetic remedies are mainly represented by synthetic mono composition drugs, which together with high therapeutic activity have an impressive range of adverse effects [4].

Anti-herpetic therapy includes both the use of topical drugs (to stop the spread of infection to other tissues), as well as systemic drugs that help to eradicate the viral infection after absorption into the bloodstream [5].

The modern assortment of the combined anti-herpetic drugs is extremely limited, and there are no remedies for GH local treatment. Therefore, development of new combined drugs on the basis of Acyclovir and herbal compounds applied for the genital form of herpetic infection local treatment is expedient to create new combination drugs based on acyclovir and plant components for topical treatment of the genital form of herpetic infection [6].

Among herbal substances, there are some of them with proved antiviral activity, but they are not referred to official drugs in the given pharmacotherapeutic group. Tea tree and Thyme essential oils, having wide spectrum of pharmacological activity (bactericidal, antiviral, immunostimulant and anti-inflammatory) are the most promising among herbal substances [7–9].

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

Scientific researchers from different countries have shown that the combination of essential oils with synthetic substances in a single dosage form increases their anti-
viral and antibacterial activity [10]. Besides, essential oils components can serve as penetrators, due to their ability to accelerate the active substances penetration into the skin by changing the lipids fluidity in the stratum corneum.

This approach, that combines potential therapeutic properties of chemotherapeutic agents and natural substances, is promising in drug technology [11, 12]. In addition, replenishment of Ukrainian pharmaceutical market with new medicinal products will change the market structure in favor of Ukraine.

5. Formulation of goals (tasks) of Article

Therefore, development of the combined remedy containing Acyclovir and Tea tree and Thyme essential oils in the form of pessaries for local GH treatment is relevant for modern pharmacy.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

Materials and methods. Acyclovir substance «Guimica Sintetica, S.A.,» Spain was used for the pessaries composition development.

It is known, when creating drugs for GH local therapy, firstly it is necessary to select a suppository base, since it is a carrier and is in constant contact, both with active substances and with vaginal mucosa. At development of the pessaries containing Acyclovir and the essential oils, in accordance with the medical and biological GH treatment requirements, fatty bases for suppositories (Witepsol, Hard fat, etc.) were used. Well-known hydrophilic bases, namely Macrogols having high osmotic activity, are unacceptable in case of the given disease due to the mentioned above requirements.

To substantiate the suppository base choice, the samples of pessaries containing Acyclovir and Tea tree and Thyme essential oils on fatty bases were prepared under laboratory conditions. Disintegration analysis of the pessaries was carried out according to the SPHU 2.0 requirements. Acyclovir concentration in the solutions was determined by the absorption spectroscopy method at a wavelength of (265 ± 2) nm [13].

As it is shown in Figure 1, about 70–72% of Acyclovir released into the solution in 30 minutes from the pessaries on Hard fat and Suppocire bases; about 98% of Acyclovir released from the pessaries on Witepsol base. Also, Witepsol-based pessaries showed the best disintegration results.

Results and discussion. The results of Acyclovir quantitative determination in the process of its release from the samples of pessaries with hydrophobic bases are shown in Figure 1 [17, 18].

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Therefore, in result of the research, produced in Germany Witepsol base was chosen as a suppository base for pessaries containing Acyclovir and the essential oils.
In the laboratory of experimental chemotherapy of viral infections, for determination of the antiviral activity of Acyclovir, Tea tree and Thyme essential oils, cytotoxic concentration (CC50), effective dose (ED50), maximum tolerated dose (MTD), minimum effective concentration (MEC) and chemotherapeutic index (HTI) of the mentioned substances were analyzed. In result of the study, MTD (the highest dose that does not cause cell death) for the substances was: Tea tree essential oil (1:1000) – 0.1 %, Thyme essential oil (1:400) – 0.25 %, Acyclovir (1:800) – 0.125 %.

Results of the MEC (the minimum amount of a drug that delays the virus-specific cytopathogenic action (CPA) development by 50%). To determine the effective dose (ED50), test-virus in the dose of TCD50 /0.1 ml (infectious titer of CPA in cell culture is 6.0-9.0 lg) was put into Vero cells culture and incubated for 60 minutes at 37° C. After the virus absorption, its residues were removed, the cells were washed with a nutrient medium, and the remedies in different concentrations were put into a supporting medium (RPMI-1640 with 2 % fetal serum) [15]. Results of the study are shown in Table 1.

Besides, a chemotherapeutic index of the substances (IS) was carried out by MTD and MEC correlation. Results of the study are shown in Table 2.

In vitro analysis of the antiviral activity of the pessaries containing Acyclovir and the essential oils for genital herpetic infection therapy was carried out using daily transplantable cell culture, which formed a continuous layer on the substrate. The cells were grown in the bottles (NuncIon, Surface, Denmark) in RPMI-1640 medium with 10 % fetal serum addition at a temperature of 37° C in a thermostat with carbon dioxide (CO2).

In accordance to the results of Herpes virus type 2 infectious titre determination, all the studied samples effectively inhibited the reproduction of the herpes virus (Tables 3, 4).

According to the results of the study, all substances of the pessaries were effective inhibitors of Herpes virus type 2 reproduction in preventive and therapeutic experimental schemes. ED50 for the pessary № 1 - the dilution was 1: 64000, while Herpes virus type 2 reproduction inhibition was 6.0 to 9.0 lg ID50.

Anti-herpetic activity study of the pessaries containing Acyclovir and the essential oils, and the pessaries’ suppository mass for the genital form of herpes viral infection treatment was carried out in vivo on the model of genital herpes in guinea pigs (Marennikova S.S. model) [15]. The results of research are presented in Table 5.

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**Table 1**

| Samples            | Infectious titer in lg ED50 at dilutions of the remedies | MEC               |
|--------------------|----------------------------------------------------------|-------------------|
|                    | 50000 | 100000 | 200000 | 400000 | 800000 | VC    |               |
| Tea tree essential oil | 0.0   | 2.0    | 2.0    | 2.0    | 2.0    | 2.0   | >1:50000   |
| Thyme essential oil   | 1.5   | 1.0    | 1.5    | 1.0    | 2.0    | 2.0   | 32000  |
| Acyclovir            | 0.0   | 0.0    | 0.0    | 0.0    | 2.0    | 2.0   | >1:400000 |

**Table 2**

| Samples            | MTD       | MEC      | Chemotherapeutic index (IS) |
|--------------------|-----------|----------|----------------------------|
| Tea tree essential oil | 0.1 %    | > 0.002 %| 50.0                       |
| Thyme essential oil   | 0.25 %   | 0.0031 % | 80.0                       |
| Acyclovir            | 125 μg/ml| 0.25 μg/ml| 500.0                      |
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Table 3
Antiviral activity of the pessaries against Herpes virus (Preventive administration mode)

| Dilution of the drug (pessaries) | Pessaries containing Acyclovir and the essential oils, their influence on Herpes virus infectious titre in lg ED$_{50}$ |
|----------------------------------|--------------------------------------------------------------------------------------------------|
|                                  | Pessary No. 1 | Pessary No. 2 | Pessary No. 3 | Acyclovir |
| 1:4000                           | 0             | 0             | 0             | 3.0       |
| 1:8000                           | 0.5           | 0             | 0             | 4.0       |
| 1:16000                          | 2.5           | 0             | 1.0           | 4.0       |
| 1:32000                          | 2.5           | 1.5           | 1.5           | 3.0       |
| 1:64000                          | 2.0           | 2.5           | 3.0           | 5.0       |
| Virus control                    | 9.0           | 9.0           | 9.0           | 9.0       |

Table 4
Antiviral activity of the pessaries against Herpes virus (Therapeutic administration mode)

| Dilution of the drug (pessaries) | Pessaries containing Acyclovir and the essential oils, their influence on Herpes virus infectious titre in lg ED$_{50}$ |
|----------------------------------|--------------------------------------------------------------------------------------------------|
|                                  | Pessary No. 1 | Pessary No. 2 | Pessary No. 3 | Acyclovir |
| 1:4000                           | 4.0           | 3.0           | 4.0           | 2.0       |
| 1:8000                           | 4.0           | 3.0           | 3.0           | 3.0       |
| 1:16000                          | 2.0           | 3.0           | 2.0           | 3.0       |
| 1:32000                          | 3.0           | 3.0           | 3.0           | 1.0       |
| 1:64000                          | 1.0           | 4.0           | 3.0           | 3.0       |
| Virus control                    | 9.0           | 9.0           | 9.0           | 9.0       |

Table 5
Efficiency of the pessaries on the model of genital herpes in guinea pigs

| Influence | Duration of the disease (days) | P | OIS, points | TEI % |
|-----------|--------------------------------|---|-------------|-------|
| Herpes virus | 15.0                           |   | 51.0        |       |
| The pessaries containing Acyclovir and the essential oils (therapeutic administration mode) | 9.0 | < 0.05 | 22.0 | 56.0 |
| The suppository mass containing Acyclovir and the essential oils (therapeutic administration mode) | 5.0 | < 0.05 | 12.0 | 76.4 |
| The pessaries containing Acyclovir and the essential oils + the suppository mass (therapeutic administration mode) | 5.0 | < 0.05 | 18.0 | 64.7 |
| The pessaries + HSV (preventive administration mode) | 0 | < 0.05 | 0 | 100.0 |

Note: OIS – overall index of symptoms; TEI – therapeutic effect index; HSV – Herpes simplex virus

The studies have shown that the single use of the pessaries containing Acyclovir and the essential oils within 5 days reduces the severity of symptoms to 22.0 points, which corresponds to therapeutic effect index (therapeutic effect) at 56.0 % and reliably shortens the disease duration.

The use of the suppository mass containing Acyclovir and the essential oils also decreases the symptoms severity to 12.0 points, and therapeutic effect is 76.4 %. The disease duration in animals was 5 days, which is statistically reliable for all parameters and indicates the efficiency of this dosage form of the remedy. Thus, the basis (Witepsol) and the emulsifier (Lecithin) in the suppository mass and pessaries composition cannot significantly affect OIS, but increase the therapeutic action index. At the combined therapeutic scheme using both the pessaries and the suppository mass, the disease duration was 5 days, and the therapeutic effect index was 64.7 %.

In the preventive treatment scheme – administration of the pessaries 24 hours before Herpes virus infection – the disease did not occur during the whole study period (10 days).

In result of the studies it was proved that the developed remedy in the form of pessaries containing Acyclovir and Tea tree and Thyme essential oils inhibits type 2 herpevirus reproduction and is effective preventive drug on the experimental model of genital herpes infection in guinea pigs.

The additional study of antibacterial activity was carried out using the samples of pessaries containing Acyclovir and pessaries containing Acyclovir and Tea tree and Thyme essential oils in ratio (1:1): 2.5: 5.0 and 7.5 %. Earlier studies carried out by the authors showed that Tea tree essential oil shows its antimicrobial activity even at concentration of 2%. To substantiate the essential oils rational concentration (Tea tree and Thyme essential oils), microbiological research was carried out. For the oils antibacterial sensitivity determination, the samples of pessaries with 2.5 %, 5 % and 7.5 % essential oils concentrations in the ratio (1:1) were obtained; then they were tested on the strains of the cultures shown in Table. 6.
The given samples of pessaries containing essential oils show pronounced antimicrobial activity, but the maximum diameters of the microorganisms’ growth retardation zones were observed for the samples of pessaries containing the essential oils concentration 5% in the ratio (1:1). The further essential oils concentration rise does not lead to a significant increase in antimicrobial activity. The optimal concentration of essential oils in pessaries is 5% concentration (1:1 ratio); they can effectively inhibit the growth of such pathogens as Staphylococcus, Pseudomonas, Gonococcus, E. coli, as well as some pathogenic fungi of C. Albicans genus.

During antimicrobial activity studying for № 1 test sample (pessaries containing Acyclovir) and № 2 test sample (pessaries containing Acyclovir and Tea tree and Thyme essential oils) it was found that test sample № 1 does not show antibacterial properties. Test sample № 2 has antibacterial properties since it shows the maximum diameters of the microorganisms growth retardation zones (Table 7).

### Table 6

| Essential oils concentration, the sum, % | St. aureus ATCC 25923 | E. coli ATCC 25922 | Ps.aeruginosa ATCC 27853 | Basillus subtilis ATCC 6633 | Proteus vulgaris ATCC 4636 | Calbicans ATCC 885/653 |
|----------------------------------------|-----------------------|-------------------|--------------------------|---------------------------|---------------------------|-----------------------|
|                                        |                       |                   |                          |                           |                           |                       |
| 2.5                                    | 18.7±1.8              | 16.8±2.3          | growth                   | 12.0 ±1.5                 | 17.2±2.5                  | 14.2±1.6              |
| 5.0                                    | 22.7±1.6              | 18.2±1.2          | growth                   | 13.0 ±1.4                 | 20.2±1.2                  | 14.7±1.4              |
| 7.5                                    | 21.8±1.7              | 18.5±2.3          | growth                   | 13.1 ±1.3                 | 22.2±1.8                  | 14.9±1.7              |

Notes: n=5; P=95 %

### Table 7

| Pessaries | St. aureus ATCC 25923 | E. coli ATCC 25922 | Ps.aeruginosa ATCC 27853 | Basillus subtilis ATCC 6633 | Proteus vulgaris ATCC 4636 | Calbicans ATCC 885/653 |
|-----------|-----------------------|-------------------|--------------------------|---------------------------|---------------------------|-----------------------|
|           |                       |                   |                          |                           |                           |                       |
| Test sample No. 1 Pessaries containing Acyclovir (5 %) | 15.6±1.7          | 14.3±1.7          | growth                   | growth                    | 16.4±1.7                  | 13.4±1.9              |
| Test sample No. 2 Pessaries containing Acyclovir (5 %) and the essential oils (5 %) | 22.9±1.6          | 18.2±1.5          | growth                   | 13.0 ±1.3                 | 20.8±1.9                  | 14.7±1.4              |

Note: n=5; P=95 %

### 7. Conclusion

On the basis of pharmaco-technological, physico-chemical, and pharmacological studies, the composition of combined composition pessaries containing Acyclovir and Tea tree and Thyme essential oils was substantiated. The novelty of the research is protected by the Patent of Ukraine № 107464.

Anti-herpetic activity study for the substances (Acyclovir, Tea tree essential oil, and Thyme essential oil) and pessaries containing Acyclovir and the essential oils have shown that they can effectively inhibit Herpes virus type 2 reproduction.

Results of the pessaries antibacterial activity research point to antibacterial effect of Tea tree and Thyme essential oils in concentration 5%.

It was found that the original pessaries containing Acyclovir and the essential oils are effective preventive and therapeutic remedy, which was confirmed by the experimental model of herpetic infection in guinea pigs.

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ПРЕДИКЦІЯ КОНКУРЕНТОСПРОМОЖНОСТІ ФАРМАЦЕВТИЧНИХ СЕТЕЙ

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У ринковій економіці одним із головних завдань будь-якого підприємства (організації) є перемога в конкурентній боротьбі. Перемога не разова, не випадкова, а як закономірний підсумок ефективного управління цим процесом.

Метою роботи є розробка методичних підходів до моделювання динаміки конкурентоспроможності аптечних мереж в умовах мінливості ринкової ситуації.

Методи. В процесі роботи був використаний метод багатовимірного кореспонденційного аналізу (correspondence analysis).

Результати дослідження. Для прогнозування динаміки конкурентоспроможності аптечних мереж на початковому етапі було проаналізовано 53 потенційних факторів, що впливають на рівень результативного показника. З використанням багатовимірного кореспонденційного аналізу розроблено математичну модель, що дозволяє прогнозувати динаміку конкурентоспроможності аптечних мереж на основі обчислення індексу конкурентоспроможності – числового показника, що приймає позитивні значення в разі підвищення конкурентоспроможності та негативні – при відсутності її позитивної динамики.

Висновки. Запропоновано методичні підходи до прогнозування конкурентоспроможності аптечних мереж, що дозволяють приймати управлінські рішення, спрямовані на протистояння негативним зовнішнім впливам та досягнення лідерства у відповідності з поставленими стратегічними цілями організації.

Ключові слова: прогнозування, динаміка конкурентоспроможності, аптечні мережі