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Therapeutic efficacy and tolerability profiles of Proteflazid®, suppository and drops among the patients with herpesvirus infection in the exacerbation phase

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Rationale. In obstetric-gynecological and urological practice, diseases caused by herpes simplex virus (HSV) are one of the major healthcare problems. According
to the WHO, about 90% of the world's population are carriers of one or more types of herpes viruses [1].

On the territory of Ukraine, no compulsory registration of the diseases caused by HSV is provided. Officially recorded incidence of genital herpes on the territory of Ukraine is averagely 15 cases per 100,000 population per year [2]. However, literature data indicate that 10% of women and 15% of men are regularly affected with genital herpes, and the number of carriers is not accountable [2].

In the Russian Federation, compulsory registration of genital herpes was introduced in 1993. The incidence rate of genital herpes in Russia within the period of 1994-2001 amounted to 19.0 cases per 100 thousand of people. At the same time, women between the ages of 18 and 39 are the risk group, in which the incidence of genital herpes is 135.7 cases per 100,000 population of this category [3].

In Western Europe, seroepidemiological studies have shown a significant difference between the prevalence of seropositivity and the actual incidence of genital herpes, which exceeds 80 cases per 100 thousand of population, and in the USA this value approaches 200 cases per 100 thousand inhabitants [3].

The common strategy of the management of various forms of herpetic infection is regulated by the standards of dermatovenerological care provided in accordance with the Order of the Ministry of Health of Ukraine No. 312 dated 08.05.2009 and the Order of the Ministry of Health of Ukraine No. 286 dated 07.06.2004. According to the Orders, the treatment program used for the therapy of herpesvirus infection includes the administration of the drug Acyclovir in standard dosages. However, despite the available treatment strategy, the relapses of the disease are common, especially during pregnancy.

To prevent the recurrence of the disease, various remedies for the immune system stimulation are currently applied, however, a stable clinical effect cannot be achieved in all patients. One of the drugs with proven immunotropic, antiviral and anti-recurrence properties for this purpose is Proteflazid® in the form of suppositories [4].

Proteflazid® is an active antiviral drug, the active substance of which (flavonoids) inhibits the synthesis of DNA and RNA-viruses in the infected cells due to inhibition of the activity of virus-specific RNA and DNA-polymerases, thymidine kinase [5] and reverse transcriptase [6]. The drug promotes the synthesis of endogenous alpha- and gamma-interferons to the level of physiological activity (without the development of refractoriness), which increases the non-specific resistance to viral and bacterial infections [7].

Clinical studies of Proteflazid® suppositories have established that they restore the protective function of the mucous membrane of the vagina and cervix due to the normalization of local immunity factors (sIgA, lysozyme, and complement C3) [8, 9, 10].

It was found that the active substance of the drug possesses specific antiviral activity and inhibits the reproduction of HPV in experimental models of oncogenic HPV strains *in vitro*. In the cytological studies, the suppression of proliferative and destructive effect of HPV on cells under the influence of the drug has been demonstrated [11].

In case of genital herpes, the application of Proteflazid® suppositories prevents the formation of new rash elements, reduces the risk of dissemination and visceral complications, and accelerates healing of the lesions. In case of vaginosis, vaginitis, and inflammatory diseases of the cervix, the drug helps to restore the local immunity and eliminate the pathogen faster and more effective. Proteflazid® in suppositories shows antioxidant properties inhibiting the course of free-radical processes, thereby preventing the accumulation of products of lipid peroxidation, and enhancing the antioxidant status of the cells [12]. The drug is a modulator of apoptosis, enhancing the effect of apoptosis-inducing factors [13], namely by activating caspase-9, which facilitates more rapid elimination of the virus-infected cells [14] and primary prevention of chronic disease associated with latent viral infections [15].

During phase I of the clinical study of Proteflazid® in the form of suppositories, an assessment of the efficacy and tolerability of the drug was carried out in 30 patients with genital herpes in remission. A decrease in the levels of IgG and sIgA and increase in the levels of lysozyme and complement C3 in the cervical mucus of patients were observed in connection with the treatment, indicating the enhancement of both general and local immunological resistance of the body. Good drug tolerability was noted, as well as the absence of serious adverse effects and negative changes in the laboratory indicators [16].

Study objective: to evaluate the efficacy and tolerability of the drug Proteflazid® in the form of suppositories (Ecopharm Ltd. RPC, Ukraine) in patients with exacerbation of the herpetic infection.

Study object and methods. This study presents the phase II of the clinical trial of the drug Proteflazid® in the form of suppositories. By design, the study is classified as an open, randomized controlled trial with parallel groups. Seventy patients aged 18-50 years with a verified diagnosis of genital herpes (HSV-1, HSV-2) in the acute stage, undergoing inpatient treatment in the Department of Rehabilitation of the Reproductive Function of Women of SI "Institute of Pediatrics, Obstetrics and Gynecology of NAMS of Ukraine", Kiev, were enrolled in the study.

Patients were randomized in the main (n = 35) and control (n = 35) groups by simple randomization method. All subjects received Herpevir, tablets 200 mg each (Kievmedpreparat OJSC, Ukraine), 1 tablet 5 times a day for 5 days as a basic therapy. In addition, the patients of the main group were prescribed with the study drug Proteflazid®, suppositories (Ecopharm Ltd. RPC, Ukraine), 1 suppository once a day for 10 days. The patients of the control group were prescribed with the reference drug Proteflazid®, drops (Ecopharm Ltd. RPC, Ukraine), in the form of vaginal tampons with a solution of the drug. To prepare the solution, 3.0 ml (72-75 drops) of the drug were diluted in 20 ml of saline sodium chloride solution. The time of vaginal tampon exposure was 30-40 minutes; the procedure was to be performed 2 times a day for 10 days.

The indicators were monitored before the study beginning, at the end of the treatment course (10 days) and after the treatment completion (after 8 weeks). The treatment efficacy was assessed by the severity of clinical signs of the herpetic infection at the end of the course of treatment (10 days after the treatment began), and also 2 and 8 weeks after the completion of the course of treatment. The evaluation of clinical signs of the herpetic infection was based on the subjective complaints of the subjects (itching, burning, painfulness) using a 4-point scale (from 0 to 3).

When examining the affected area of the mucosa, the nature of the changes in the vagina mucous membranes was described, and the severity of inflammatory changes was also evaluated (in points). The degree of manifestation of the inflammation signs was evaluated according to the following scale: 0 - no signs; 1 - mild; 2 - moderate; 3 - severe. The degree of severity of the morphological elements of rash was also evaluated by the scale: 0 - absence of the sign; 1 - mild severity; 2 - moderate severity; 3 - significant

severity. The drug was considered effective if the severity of clinical signs of the herpetic infection was not more than 1 point (0 – 1 point) by the end of the treatment course (10 days after the start of treatment).

Furthermore, the conclusion about the efficacy was based on the difference in levels of HSV-1, HSV-2 (IgG, IgM) markers, levels of HSV DNA in smears from the epithelium of the vaginal/cervical mucosa and local immunity indicators (sIgA, lysozyme, complement C3). The frequency, duration, and severity of relapses during the follow-up period were also compared. In order to assess the state of the body to reveal the possible adverse effects or reactions, data of physical examination (heart rate, blood pressure, body temperature indicators, auscultation of the heart and lungs, examination of the skin and mucous membranes) were taken into account as well as the indicators of the complete and biochemical analysis of blood and urine.

Results and discussion. At the screening phase, normality of distribution of the subjects in the main and control groups was assessed by a number of indicators. Thus, the "Average age" indicator was 31.1 years and 33.2 years in the main and control groups, respectively. As for the duration of the disease, the subjects with the duration of the disease from 1 to 5 years prevailed – 16 of 35 patients in the main group and 18 of 35 in the control group. The groups were also comparable in terms of the following indicators: "Frequency of the herpetic infection relapses", "Gynecological history data", "Frequency of concomitant diseases" and "Degree of vaginal purity."

Seventy women included in the study received a course of treatment within 10 days. At the end of the course of treatment and 8 weeks after its completion, the detection of HSV DNA in smears from the epithelium of the mucous membrane of the vagina/cervix was repeated, as well as the identification of HSV markers (IgG, IgM).

The HSV DNA at screening was detected in 100% of the subjects of each group, which was the eligibility criterion for the selection of subjects to participate in the study. By the end of the 10-day course of treatment, no chlamydia DNA was determined in any of the cases in each of the groups. Dynamics of HSV DNA viral load in the groups is shown in Fig. 1.

The assessment data of IgG, IgM against HSV in the groups over time are shown in Fig. 2 and 3.

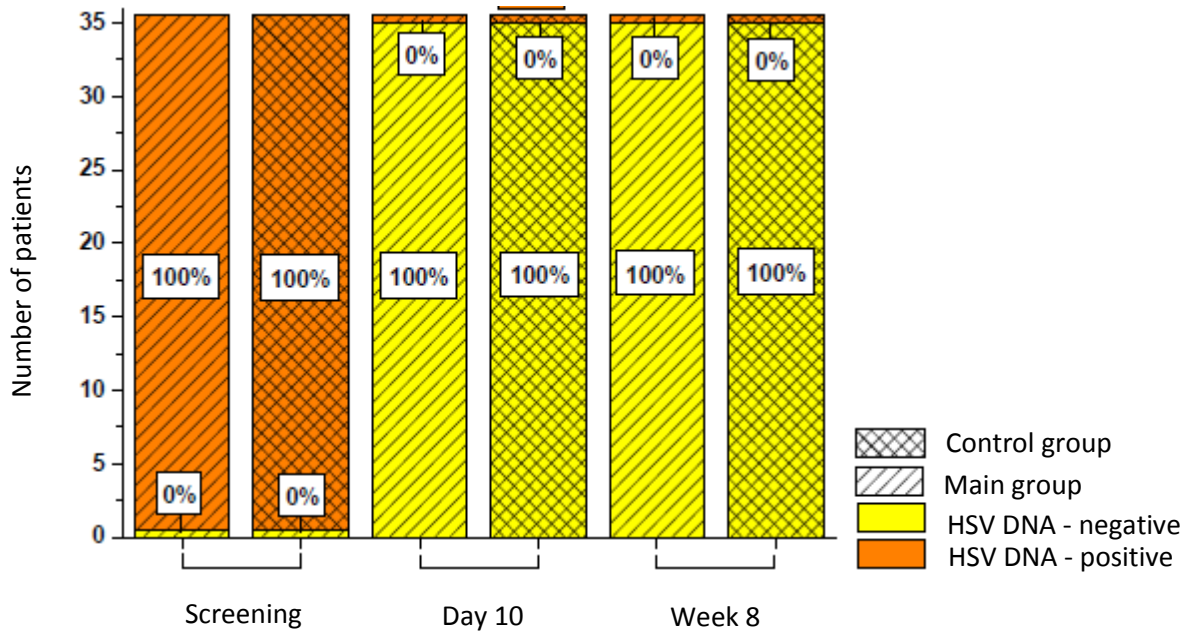

Fig. 1. Detection of HSV DNA over time after administration of the drug Proteflazid®, suppositories (main group, n = 35), and Proteflazid®, drops (control group, n = 35)

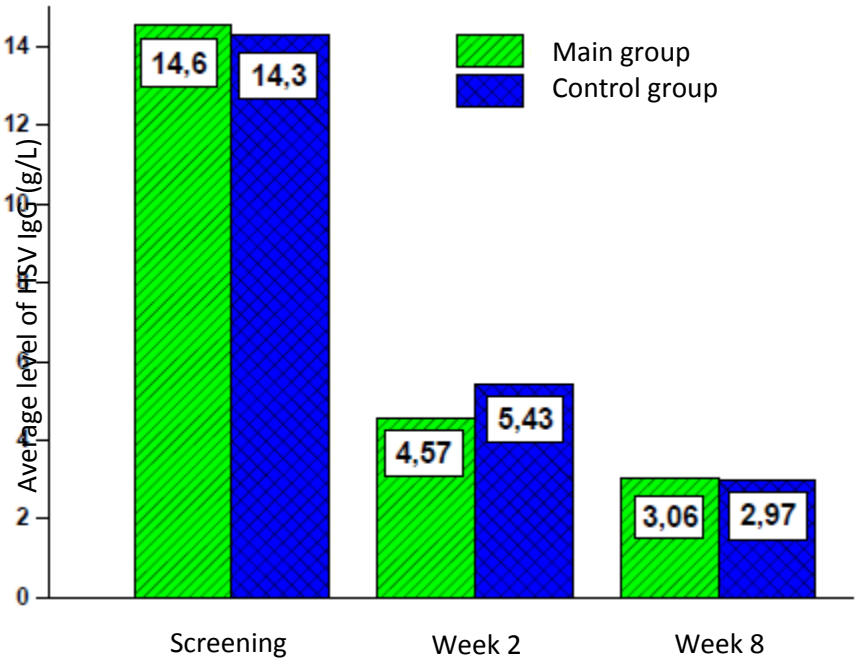

Fig. 2. Dynamics of the "IgG" indicator (g/L) after administration of the drug Proteflazid®, suppositories (main group, n = 35), and Proteflazid®, drops (control group, n = 35)

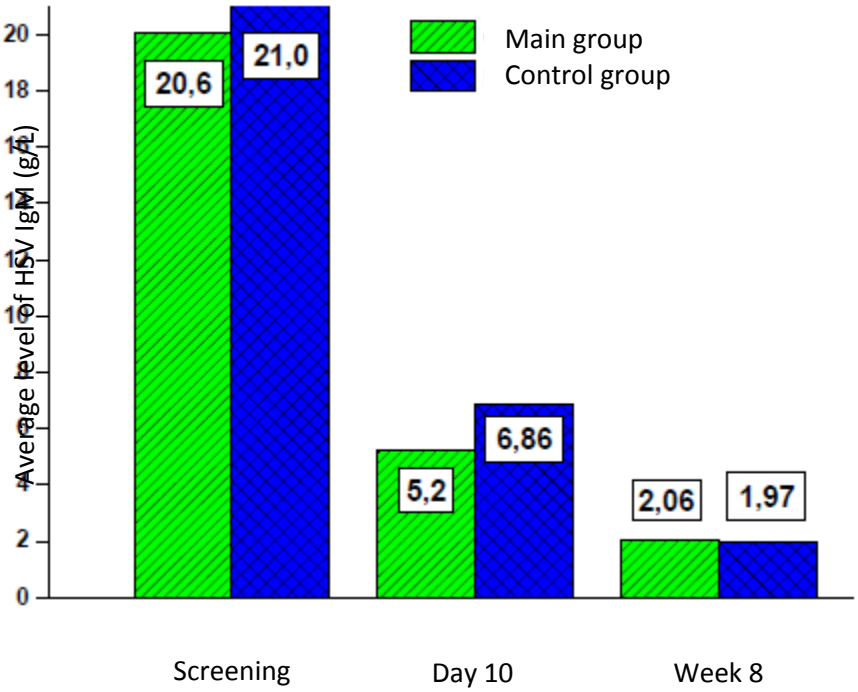

Fig. 3. Dynamics of the "IgM" indicator (g/L) after administration of the drug Proteflazid®, suppositories (main group, n = 35), and Proteflazid®, drops (control group, n = 35)

The above data allow us to note a significant decrease in the level of HSV IgM and HSV IgG serological markers in both groups.

At the end of the course of treatment and 8 weeks after the completion of the course, a reassessment of local immunity indicators (sIgA, lysozyme, complement C3) was performed. Data on the evaluation of the local immunity indicators over time are shown in Fig. 4-6.

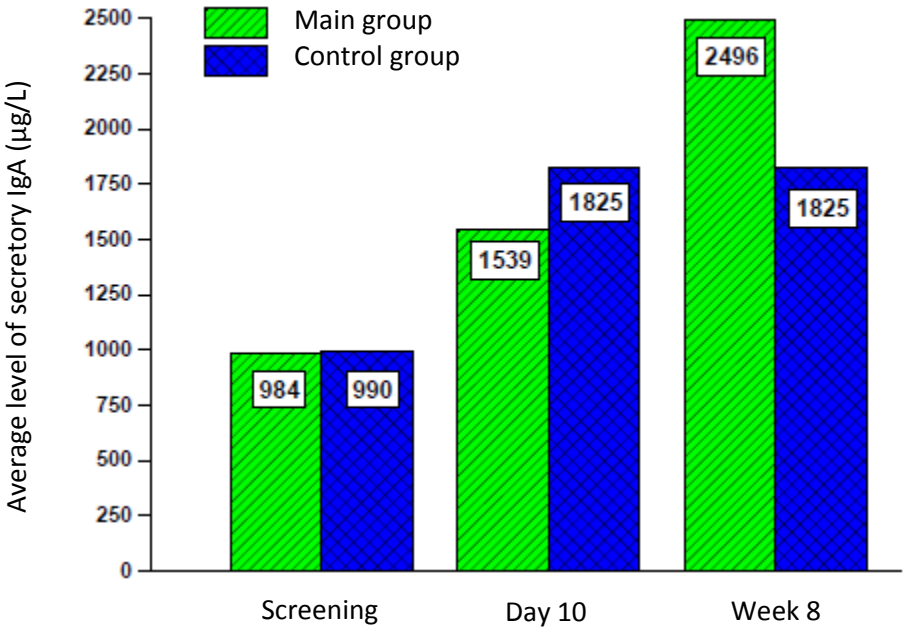

Fig. 4. Dynamics of the "Secretory IgA" indicator (µg/L) after administration of the drug Proteflazid®, suppositories (main group, n = 35), and Proteflazid®, drops (control group, n = 35)

In both groups, a significant increase in the level of sIgA and complement C3 at the 2nd and 8th week was observed in comparison with the status at screening (Fig. 4-5).

The level of complement C3 significantly increased by the end of the course of treatment in both groups and returned to the baseline by the end of the follow-up period (Fig. 6).

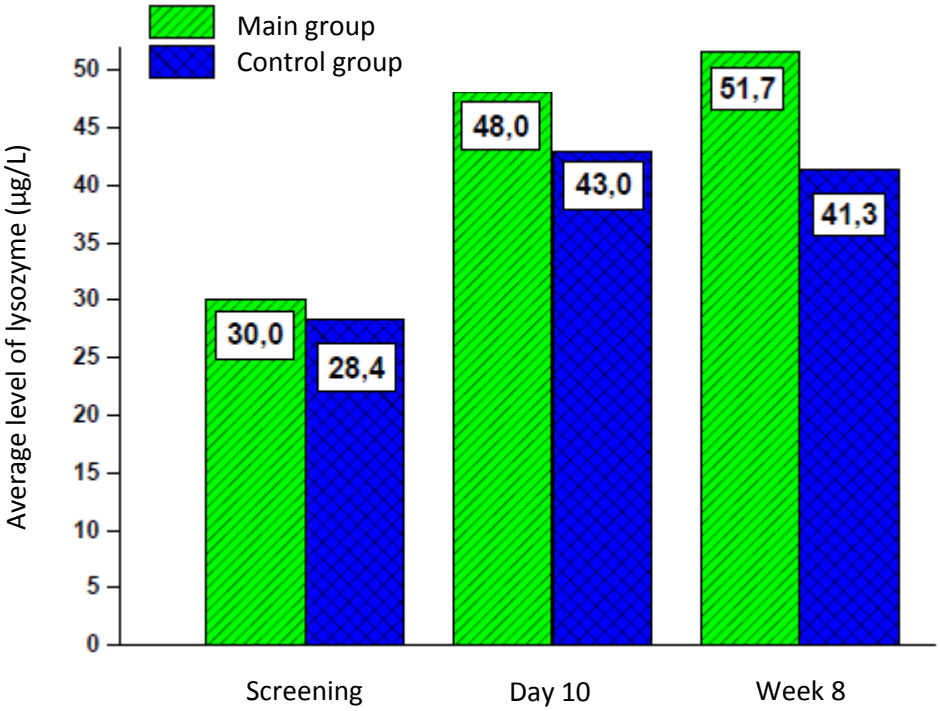

Fig. 5. Dynamics of the "Lysozyme" indicator (µg/L) after administration of the drug Proteflazid®, suppositories (main group, n = 35), and Proteflazid®, drops (control group, n = 35)

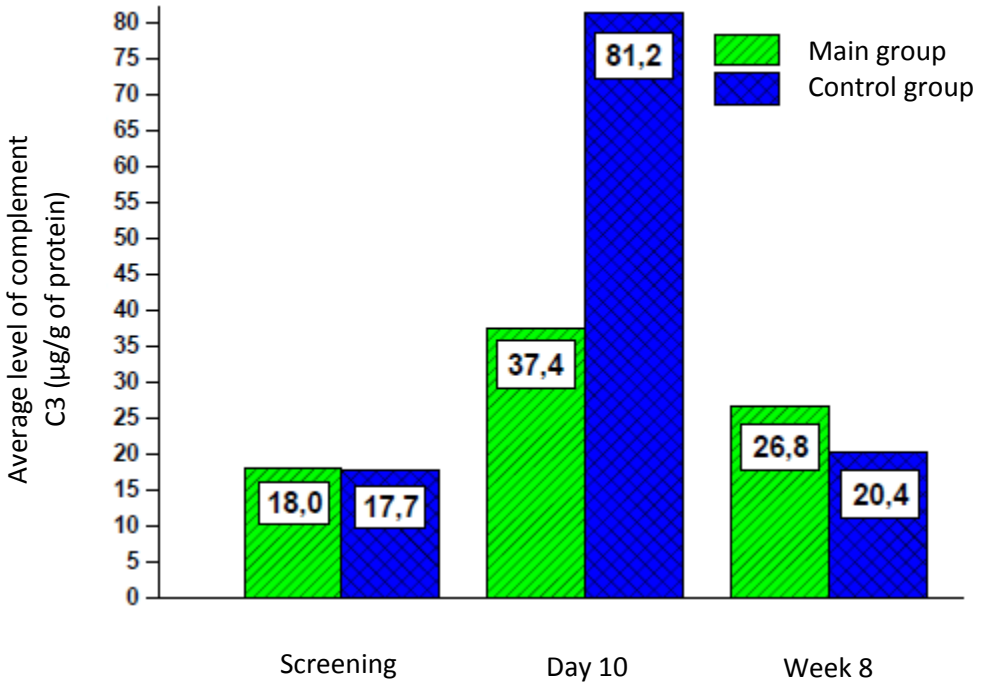

Fig. 6. Dynamics of the "Complement C3" indicator (µg/g of protein) after administration of the drug Proteflazid®, suppositories (main group, n = 35), and Proteflazid®, drops (control group, n = 35)

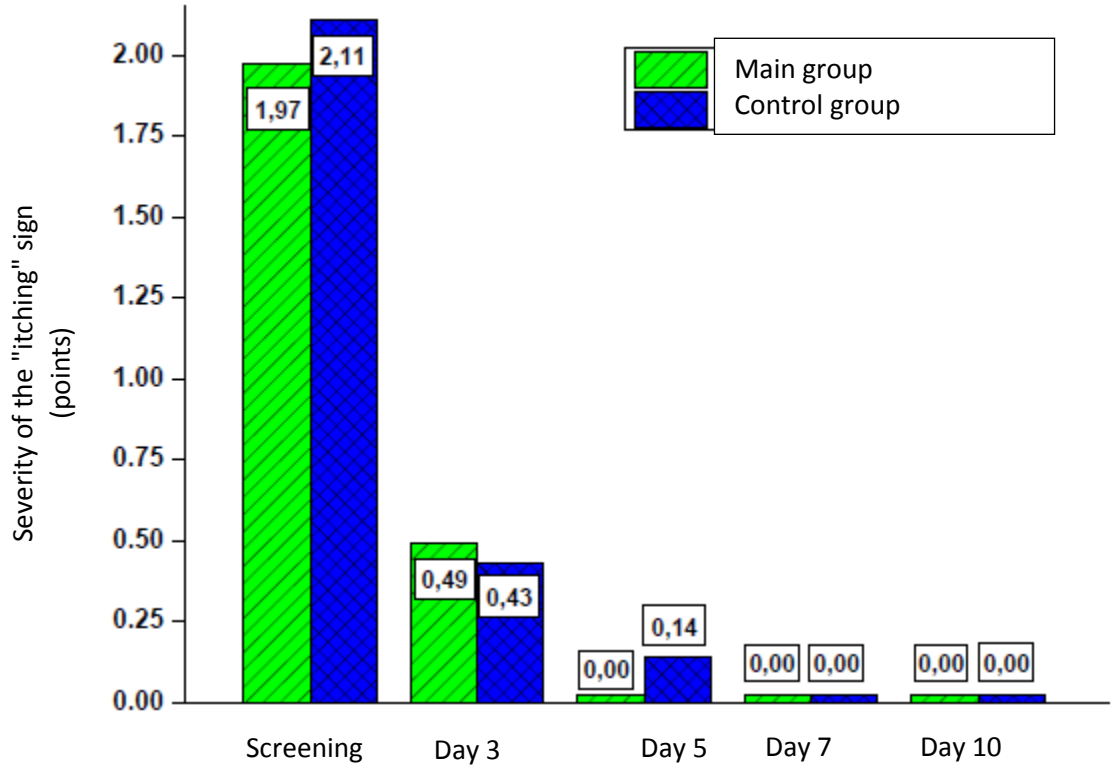

Fig. 7. Dynamics of the severity of the clinical "Itching" sign (according to subjective scale from 0 to 3 points) after administration of the drug Proteflazid®, suppositories (main group, n = 35), and Proteflazid®, drops (control group, n = 35)

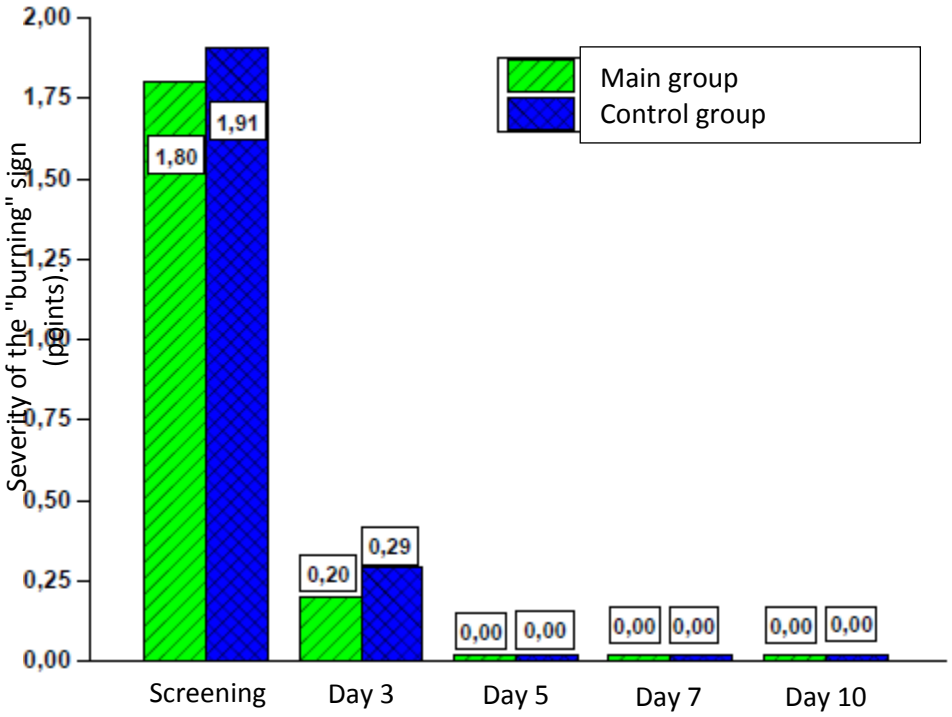

Fig. 8. Dynamics of the severity of the clinical “Burning” sign (according to subjective scale from 0 to 3 points) after administration of the drug Proteflazid®, suppositories (main group, n = 35), and Proteflazid®, drops (control group, n = 35)

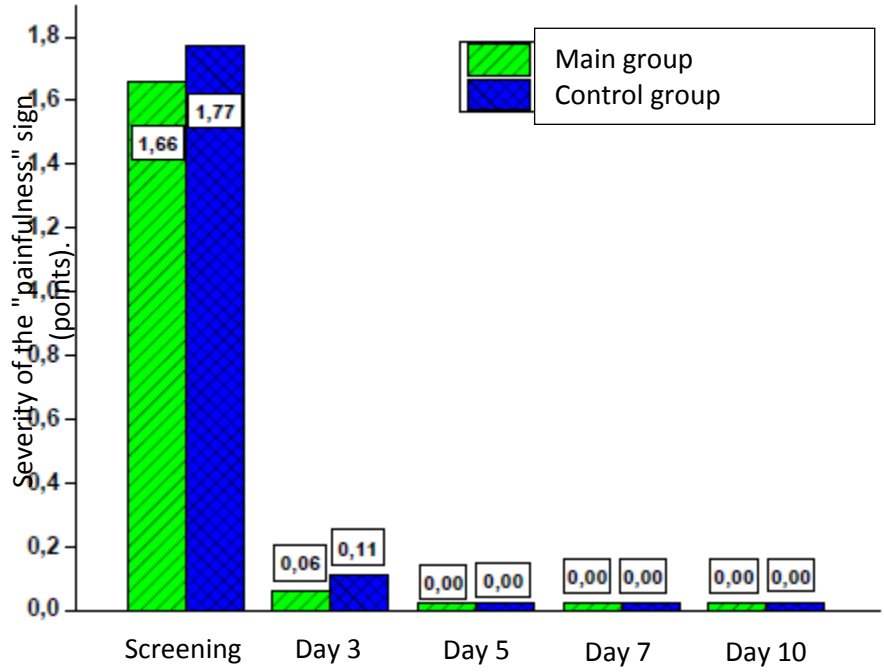

Fig. 9. Dynamics of the severity of the clinical “Painfulness” sign (according to subjective scale from 0 to 3 points) after administration of the drug Proteflazid®, suppositories (main group, n = 35), and Proteflazid®, drops (control group, n = 35)

During each next visit following the screening, the severity of subjective complaints of subjects (itching, burning, painfulness) was evaluated according to the 4-point scale (from 0 to 3). Evaluation data on subjective complaints of patients of the main and control groups over time are shown in Fig. 7-9. The severity of subjective complaints significantly decreased by the third day of treatment and subsequently, there were no complaints in either of the groups. According to the statistical analysis of the patients' subjective complaints, high therapeutic efficacy of the study drug was noted. Moreover, on the basis of statistical analysis of the registered laboratory indicators, a significant decrease in the level of HSV DNA viral load and significant improvement of local immunity indicators were observed in both groups.

Instrumental studies of the vaginal and cervical mucosa showed that the drug did not have any negative irritant effect on the mucosa condition. Throughout the course of treatment and the follow-up period, the detection and evaluation of a possible recurrence of the herpetic infection were carried out. Within the 8-week period, no recurrence of the herpetic infection was registered. A significant improvement was noted in the main and control groups in terms of the colpocervicoscopy indicators in most cases.

Based on the data given in Table 1, it is concluded that the groups had no statistically significant differences in terms of the treatment efficacy. The treatment was found to be equally effective in 35 patients of the main and 35 patients of the control group.

Table 1. Distribution of patients by the efficacy variable categories

Efficacy	**Main group, n=35**		**Control group, n=35**	
	Prevalence	**Proportion, %**	**Prevalence**	**Proportion, %**
The drug is effective	35	100.0	35	100.0
The drug is not effective	0	0	0	0

The conclusion about the non-inferior efficacy of the drug Proteflazid®, suppositories, in comparison with the reference drug Proteflazid®, drops, in the specified category of patients was made based on the concept of confidence intervals (see Table 2).

Table 2. 95% CI limits for the difference between the proportions of positive results

Statistical parameter	**Value**
Probability of type I error, α	0.025
Percentage point of the standard normal distribution for α	1.96
Zone of efficacy (δ), %	-20%
Proportion of positive outcomes for the main group,%	100.0

Size of the main group	35
Proportion of positive outcomes for the control group,%	100.0
Size of the control group	35
Proportion difference, %	0.0
95% confidence interval lower limit	-9.89
95% confidence interval upper limit	9.89

No serious adverse reactions or events were registered during the study. None of the patients had to terminate the participation in the study early due to undesirable events. The compared drugs did not have any negative effect on blood pressure, heart rate, and body temperature. No negative changes in these indicators in the patients of both groups were observed at the end of the clinical study as compared to the baseline before the treatment. Any negative changes in the laboratory indicators were also not found in any of the cases. No case of exacerbation of the existing chronic disease was registered.

There were no anaphylactic reactions, delayed-type reactions, or clinically significant fluctuations of hemodynamic and basic laboratory indicators noted. Based on the presented data, the tolerability assessment of the studied drugs was performed (Table 3).

Table 3. Results of the drug tolerability assessment

Tolerability	**Main group, n=35**		**Control group, n=35**	
	Prevalence	**Proportion, %**	**Prevalence**	**Proportion, %**
Good	35	100.0	35	100.0
Satisfactory	0	0	0	0
Unsatisfactory	0	0	0	0

According to the results of the statistical analysis, it was concluded that there were no statistically significant differences in the most studied parameters of the complete blood analysis, blood biochemistry analysis, and complete urine analysis in both groups in most cases, revealed before and after the course of the treatment.

Conclusions:

1) High efficacy of the study drug Proteflazid®, suppositories (Ecopharm Ltd. RPC, Ukraine), was shown in the treatment of the herpetic infection exacerbation in a comparative clinical study involving 70 patients.

2) The study drug Proteflazid®, suppositories, is as effective as Proteflazid®, drops, in the treatment of the herpetic infection relapses.

3) A reversal of the clinical manifestations of genital herpes was observed in all women participating in the study by the end of the 10-day course of treatment.

4) During the 8-week follow-up period after the end of the course of treatment, no recurrence of the herpetic infection was noted in patients of both studied groups.

5) In both studied groups, a significant increase of the values of local immunity indicators (sIgA, lysozyme, complement C3) was observed as compared to the baseling. In particular, sIgA level increased by the 10^{th} day of treatment, remaining significantly high throughout the 8-week follow-up period (from 984.32 to 2496.19 μg/L); lysozyme level increased by the 10^{th} day of treatment, remaining significantly high during the 8-week follow-up period (from 30.06 to 51.67 μg/L); complement C3 level increased by the 10^{th} day of treatment and returned to the baseline by the end of the 8-week follow-up period (from 17.99 μg/g of protein at screening to 37.47 μg/g of protein on the 10^{th} day and 20.37 μg/g of protein on the 8^{th} week)

6) In both groups of subjects, a significant reduction of HSV DNA viral load was observed in comparison with the baseline. After the completion of the 10-day course of treatment, and after the 2- and 8-week follow-up period, no HSV DNA in smears from the epithelium of the mucous membrane of the vagina and cervix was detected in any of the cases.

7) In both studied groups, a significant decrease in the level of HSV markers (IgG, IgM) was observed compared to the baseline after the 10-day course of treatment, and the 2- and 8-week follow-up period.

8) Both drugs showed good tolerability; no cases of adverse reactions or events were noted. In addition, no cases of negative changes in the data of physical and laboratory examination were registered. The tolerability of the treatment in all cases was interpreted as good.

9) The drug Proteflazid®, suppositories, provided more convenient dosage regimen in contrast to the reference drug Proteflazid®, drops, the use of which required time to prepare the necessary dilution and to make a tampon.

10) Based on the data received and presented, the drug Proteflazid®, suppositories (Ecopharm Ltd. RPC, Ukraine), can be recommended as a highly effective and safe antiviral agent in the treatment of gynecological diseases caused by herpetic infection.

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