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A STUDY ON THE CUMULATIVE EFFECT OF THE SUPPOSITORIES COMBINED ACTION "FENZIN -OB"

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ABSTRACT

The combined composition of suppositories " Fensin -ob " for the results of the study of the specific action and therapeutic activity showed that the drug relieves aseptic edema, has an anti-inflammatory and wound - healing effect with a desinsibilizing effect. A study to study the cumulative properties of Fensulkala suppositories of the new combination "Fensin-OB" was carried out by the method of subchronic toxicity according to the method of Lima et al. The experiments were performed on 1 0 rats. (guinea pigs) of both sexes weighing 20 0 ± 15.0 g. After determining the cumulative properties of the suppositories "Fensin-about", the animals were decapitated for pathomorphological examination of internal organs, with the calculation of the integral indicator - the mass coefficient (MC). The analysis of this indicator makes it possible to detect target organs with a pathologically related effect. The calculation of MC is made according to the formula: MC = Organ mass (g) / body weight (g) × 100. The comparison drug was the suppositories " Fensulkal" at a dose of 150 mg / kg and "Diclofenac" at a dose of 150 mg / kg (manufacturer: De ntafill Plyus LLC , Uzbekistan. Experimental studies have shown that with the rectal administration of the new composition, the suppository "Fensin - about" at a dose of 2000-5000 mg / kg in animals does not cause any negative reactions, as well as pathological changes. In addition , the peripheral blood was examined: the content of hemoglobin, g / l, the number of erythrocytes, 10^{12}/ l and leukocytes 10^{9}/ l . ... Cytograms showed a neutrophilic reaction, but at the same time there was a tendency to an increase in the number of macrophages and polyblasts, which confirms the strengthening of the reparative process. At the end of the study, the cumulation coefficient was calculated using the formula. The experiments indicate that the cumulation coefficient: CC> 1, which indicates the absence of the cumulative effect of the test drug .

KEYWORDS: Fensulkal, Cetirizine, sea buckthorn oil, specific activity, cumulative effect**.**

Domestic non-steroidal anti-inflammatory drug Fensulkal, (RUz IAP patent No. 02245 / 04.10.02). was approved for use in the form of a 0.5% ointment in ophthalmic almological practice . Fensulkal was used in the form of tablets (0.1 g of active substance) and ointments (0.5% and 3%).

3% Fensulkal ointment in accordance with the order of the Ministry of Health of the Republic of Uzbekistan No. 481 is approved for the treatment of gynecological inflammatory processes (cervical erosion, vulvitis, colpitis, myoendometritis, as part of complex therapy - adnexitis and salpingo-oophoritis. The effectiveness of the drug Fensulkal in clinical trials was evaluated in comparison with a steroid anti-inflammatory drug 0.5% hydrocortisone ophthalmic ointment. Experiments on various animals have shown that fensulcal has high anti-inflammatory activity and low toxicity. In terms of the breadth of therapeutic action, it significantly exceeds butadione. The drug is approved for widespread use as an anti-inflammatory agent in the form of an ointment in treatment In the future, vaginal suppositories containing 0.1 g of fensulkal were developed, the obtained experimental preclinical and clinical data made it possible to recommend a new drug - suppositories "Fensulkal" for treatment gynecological diseases as an anti-inflammatory and antimicrobial drug (vulvitis, colpitis, etc.). The approval of the Pharmacological Committee was received and the VFS was approved for vaginal suppositories "Fensulkal" (VFS 42 Uz. - 1292-2008), as well as permission for use in medicine. Currently, the Fensulkala substance has been registered - the quality standard of the medicinal product FS 42 Uz-0185-2020, registration number DV / M 03588/10/20 October 20, 2020.

The purpose of this study is to study acute toxicity and cumulative action, as well as the specific action: anti-inflammatory, wound-healing activity of a new combined dosage form in the form of vaginal suppositories based on fensulcal, cetirizine hydrochloride and sea buckthorn oil.

MATERIALS AND RESEARCH METHODS

Drugs

Vaginal suppositories "Fensulkala" 1.6 g each with a fensulkala content of 0.1 g (reference drug) . Vaginal suppositories - "Fensin-about" 1.6 g each with a fensulcal content of 0.1 g, cetirizine dydrochloride - 0.005 g, sea buckthorn oil - 0.07 (5% of the total mass) , developed and obtained experimental laboratory samples at the department Pharmaceutical Chemistry of the Tashkent Pharmaceutical Institute.

The general effect and acute toxicity of the new composition of vaginal suppositories were carried out according to the Sanotsky method. To determine the parameters of acute toxicity, the method of Lichfield and Wilcoxon was used.[1] The study of the acute toxicity of the drug was carried out on white outbred mice . Vaginal suppositories "Fensin -ob" were injected into the rectum at doses of 2000, 2500, 3000, 4000 and 5000 mg / kg. The last two doses are fractional in 1 hour.

The animals were monitored hourly during the first day of the experiment in laboratory conditions, while survival during the experiment, general condition, possible convulsions and death were used as indicators of the functional state of the animals. Then, every day, for 2 weeks in a vivarium, the animals of all groups were monitored for the general condition and activity, behavioral features, and other indicators.

At the end of the experiment, the average lethal dose (LD_{50}) was calculated and the toxicity class was determined.[3]

The study of the cumulative properties of the suppositories "Fensin-about" was carried out by the method of subchronic toxicity. The cumulative effect of vaginal suppositories was carried out according to the method of Lima et al., Which makes it possible to assess not only cumulation, but also addiction. Experiments were set at 1 0 rats of both sexes weighing 20 0 ± 15,0g.

After determining the cumulative properties of the suppositories "Fensin-about", the animals were decapitated for pathomorphological examination of internal organs, with the calculation of the integral indicator - the mass coefficient (MC). The analysis of this indicator makes it possible to detect target organs with a pathologically related effect. The organs removed during dissection are weighed wet to avoid drying out, the paired organs are weighed together. The calculation of MC is made according to the formula: MC = Organ weight (g) / body weight (g) × 100%.

Statistical processing of the data obtained was carried out using the computer programs Microsoft Office Excel and Staticrica 6.0. The parameters commonly used in toxicology were calculated. The quantitative results of changes in the relative mass of internal organs, in comparison with animals of the control groups, are presented as the mean ± statistical (M ± m).

In the second series of experiments, the specific anti-inflammatory activity of the new composition of suppositories "Fensin-ob" was studied on the model of experimental colpitis, corresponding to vaginitis, and the reparative activity on the model of alterative inflammation of skin wounds.

Experimental Vaginitis Model

Antiinflammatory study conducted on camping on 25 guinea pigs weighing 300-350 g for 5 males in each experimental group. The model of inflammation of experimental colpitis in animals was caused by rectal administration of formalin, 0.15 ml of 25% formalin, into the vagina of guinea pigs.

The dose was calculated according to the guidelines for drug development.[2] 1600mg per person: 70kg = (mg / kg) x 39 (human coefficient): 6 (guinea pig coefficient) mg / kg.

The comparison drug was the suppositories "Fensulkal" at a dose of 150 mg / kg, and "Diclofenac" at a dose of 150 mg / kg. Manufacturer : LLC Dentafill Plyus, Uzbekistan.

Changes in the skin of the vagina were systematically observed under the conditions of an experimental study on days 3, 7, 14 and 21 after rectal formalin administration. The severity of the developing disease was judged by the general condition (general appearance of the condition of the coat, discharge from the anus and vagina, the nature of bowel movements, weight dynamics) and behavior (physical activity, aggressiveness, appetite). Simultaneously, the rectal temperature of the guinea pigs was recorded using an electronic thermometer. In addition , peripheral blood was examined: hemoglobin content, g / l, erythrocyte count, 10^{12} / l and leukocyte $^{10}9$ / l.

Alterative Inflammation Model (Skin Wound Model).

Model skin wounds carried on camping on 25 rats (male) weighing 200 - 215 g of 5 animals in each experimental group. In rats anesthetized with sodium etaminal (dose 50 mg / kg, i.p.), a skin wound of standard diameter and depth, 1.0 x 1.0 cm in size and 0.5 mm in depth, was applied to the previously depilated surface of the back skin under aseptic conditions . The wounds were left open.[2,3]

The observation period was 1, 3, 7, 14 and 21 days after the start of the experiment. The rate of wound healing was assessed:

1. by the presence of a wound exudate and its cellular composition;
2. by the state of the adjacent tissues;
3. by changing its area, mm ^{2} .

RESEARCH RESULTS

Studies of the general action and acute toxicity of the suppository "Fensin-about" were carried out on healthy laboratory animals, white outbred mice, males weighing 18-22 g . As mentioned above , "Fensin - about" was administered in doses of 2000, 2500, 3000, 4000 and 5000 mg / kg.

Observation of the condition of the animals after the introduction of the drug was carried out for 2 days in laboratory conditions and 14 days in a vivarium.

Experimental studies have shown that with the rectal administration of the new composition, the suppository "Fensin - about" at a dose of 2000-5000 mg / kg in animals does not cause any negative reactions, as well as pathological changes.

Analysis of the general condition of the animals showed that in the mice that received the new composition of the suppository "Fensin-about", there were no significant differences in weight. All animals showed satisfactory appetite. During the observation period, the death of animals was not observed.

Consequently, the results of studying the acute toxicity of the new composition of the suppositories "Fensin-about" showed that they belong to the VI class of relatively harmless substances. The LD $_{50}$ in mice after rectal administration was greater than ≥5000 mg / kg.

The toxicological study also included a study of the cumulative effect of the suppository "Fensin - about" according to the method of Lima et al., Which makes it possible to assess not only cumulation, but also addiction. The studied suppositories "Fensin - about" were administered rectally to rats according to the following scheme. (Table 2)

Table 2: Study of the cumulative effect of "Fensin-about" suppositories by the method of subchronic toxicity (M ± m ; n = 10).

Introduction days	The number of animals in group / death toll	Share of LD $_{50}$	LD $_{50}$ or 5000mg / kg
1-4	10/0	0.1	500
5-8	10/0	0.15	750
9-12	10/0	0.22	1100
13-16	10/0	0.34	1700
17-20	10/0	0.50	2500
21-24	10/0	0.75	3750
24-28	10/0	1.12	5600

During EXPERIMENTAL coagulant animal death is not observed to, however cumulation factor could not be determined, therefore, suppositories and "Fenzin ob" Lacking w t cumulative effect.

Macroscopic examination of animals slaughtered under etaminal-sodium anesthesia revealed the correct location of the internal organs, the absence of free fluid in the pleural and abdominal cavities. The tissues of the lungs, liver, stomach and intestines are also of a characteristic color, without signs of edema, hemorrhage and ulceration. The pancreas, kidneys and adrenal glands were unchanged.

As can be seen from the data in Table 3 , in terms of the mass of the internal organs of rats when using Fensin-about suppositories according to the above scheme, no differences were found between the control and experimental groups of animals for 28 days.

Table 3: Average indices of measurements of the mass of internal organs mass coefficient of rats with oral subchronic introduction of candles "Fensin-about" (M ±□m; n = 10).

Index	the control		candles "Fensin-about"	
	Organ mass	Mass coefficient	Organ mass	Mass Coefficient
Body mass	188.5 ± 13.5	-	180.0 ± 12.0	-
A heart	0.73 0 ± 0.04	0.387 ± 0.03	0.93 0 ± 0.05	0 5 1 7 ± 0,03
Lungs	1.40 ± 0.10	0 743 ± 0,04	1.65 ± 0.05	0.917 ± 0.04
Thymus	0.210 ± 0.011	0, 111 ± 0,012	0.300 ± 0.019	0, 167 ± 0,015
Lymph nodes	0.021 ± 0.0011	0.011 ± 0.0010	0.025 ± 0.0014	0.0 14 ± 0.0011
Liver	6.55 ± 0.42	3.48 ± 0.20	6.930 ± 0.31	3, 85 ± 0,18
Spleen	0.99 0 ± 0.06	0.525 ± 0.08	0.520 ± 0.05	0 289 ± 0,04
Kidney	0.62 0 ± 0.04	0 329 ± 0,11	0.670 ± 0.04	0 372 ± 0,05

Adrenal glands	0.022 ± 0.002	0.012 ± 0.001	0.032 ± 0.003	0.018 ± 0.001
Stomach	1.450 ± 0.09	0.769 ± 0.05	1.300 ± 0.07	0 722 ± 0,05
Ovaries	1.36 ± 0.04	0 722 ± 0,05	1.25 ± 0.10	0, 694 ± 0,005

In the second series of experiments, the specific anti-inflammatory activity of the studied drugs and their effect on the treatment of experimental vaginitis were studied.

Against the background of the model of experimental vaginitis induced a 25% formalin solution treatment was carried out in five groups : the first group of animals was administered suppositories n latsebo, this group was a control,a torus th groups ie animals injected suppository and "Fenzin-on" at a dose of 150 mg / kg , third and fourth group experiment cial vaginitis treated comparators "Fensulkal " in the form of suppositories (0.1%) in the dose - 150 mg / kg and candles "diclofenac " in a similar dose.

At the time of introduction of formalin into the vagina, all animals showed excitement, expressed in increased motor activity and increased respiration. The most striking symptoms during this period include vaginal dehiscence, the mucous membrane of which is hyperemic and edematous. On days 14-21 of the experiment, the general condition of the animals was restored to the initial one. The duration of the course of the process of experimental colpitis was observed up to 14-21 days in the experiment.

In the experimental group when applying the novel composition of vaginal suppository "Fenzin ob" and preparation s of comparison, these changes are manifested in the form of light. Symptoms of local inflammation on the 3rd day of the experiment were more pronounced. However, after 5-7 days of treatment, the general condition of the treated animals improved. The guinea pigs displayed their usual activity.

On study day 7, the experimental animals were observed decrease in body weight of animals in control group 2 6 %, and in the test groups when administered compositions pessaries "Fenzin ob" at 10 % and the reference drug at Fensulkala and Diclofenac on 22.3 % and 24.4%, respectively. On the 21st day, we observed in the control group and the group treated with the comparison drug the return of the body weight of the animals to the initial values, and in the group treated with the composition of suppositories "Fensin-about" - on the 14th day. It should be noted that the death of 2 out of 5 control animals on days 6-7, which was accompanied by a sharp drop in body weight. In the experimental groups, there was no death of animals (Table 4 , Fig. 1).

Table 4:Influence of the new composition of vaginal suppositories "Fensin-about" on the weight of guinea pigs with experimental colpitis (M ± m ; n = 5).

Experience condition, dose, mg / kg	**Animal weight, kg / days of research**				
	Exodus	**3**	**7**	**fourteen**	**21**
Intact animals	322 ± 16.3	330 ± 12.4	344 ± 12.5	365 ± 14.5	400 ± 15.0
The control (untreated animals)	300 ± 16.3	264 ± 12.4	254 ± 12.5	285 ± 14.5	300 ± 15.0
New composition supp. Fensin-about	318 ± 13.5	307 ± 13.0	3 10 ± 1 3.8	320 ± 13.9	340 ± 14.8
0.1% supp. Fensulkala	301 ± 13.5	282 ± 13.0	267 ± 3.8	281 ± 13.9	320 ± 14.8
Diclofenac candles	305 ± 1 3 , 0	2 70 ± 1 1 , 0	26 0 ± 3, 1	28 0 ± 13 4	3 10 ± 1 3 , 0

* P≤0.05 relative to the control group.

Fig.1 Influence of the new composition, suppository "Fensin-about" on the weight of guinea pigs with experimental colpitis.

The manifestation of the severity of local inflammation and general intoxication was an increase in rectal temperature by 0.6-0.8 ^{0}C on the 3rd-7th day. In groups of animals treated with a new composition of vaginal suppositories "Fensin-about " and a comparison drug for suppositories Fensulkala and Diclofenac, the temperature reaction was smoothed and short-lived. The maximum n uk it does not exceed 0.3 ^{0}C .

Fig. 2 Influence of the new composition of vaginal suppositories "Fensin-about" on the rectal temperature of guinea pigs with experimental colpitis.

Significant changes were observed on the part of peripheral blood parameters. In the first three days after the reproduction of the pathology, the number of leukocytes increased by 30%, the maximum leukocytosis was observed on days 7-14 (65-100%).

As the inflammatory process increased, all animals showed signs of erythropoiesis disturbance. The changes in the peripheral blood of the treated animals had some peculiarities; leukocytosis, as a rule, was less pronounced.

The hemoglobin level decreased to a lesser extent than in the control group of animals, and by the 7th day reached the initial values. The concentration of erythrocytes did not decrease significantly (Table 5) .

Table No. 5: Influence of the new composition of vaginal suppositories "Fensin-about" on the morphological composition of the peripheral blood of guinea pigs with experimental colpitis with experimental colpitis (M ± m ; n = 5

Indicators	Days of exploration				
	Intact	The control	Soup composition. Fensin-about	0.1% soup. Fensulkala	Diklo candles Fenac
In 3 days					
Hemoglobin, g / dl	1 0 5 ±□0.4	8.8 ±□0.3	1 0 6 ±□0.2	9.3 ±□0.2	8 , 9 ±□0 5
Erythrocytes, 10 ^{12} / l	5.9 ±□0.5	4.6 ±□0.2	5.8 ±□0.3	4.3 ±□0.3	4, 0 ±□0, 4
Leukocytes, 10 ^{9} / l	9.2 ±□1.0	13.5 ±□1.1	10 , 0 ±□1.2	9.8 ±□1.1	11 , 2 ±□1, 5
In 7 days					
Hemoglobin, g / dl	11.0 ±□0.4	8.2 ±□0.3	11.3 ±□0.2	9.3 ±□0.2	9 0 ±□0, 1
Erythrocytes, 10 ^{12} / l	6.0 ±□0.5	4.0 ±□0.2	7.0 ±□0.3	4.8 ±□0.3	4.8 ±□0.3
Leukocytes, 10 ^{9} / l	9.0 ±□1.0	19.2 ±□1.1	9 , 25 ±□1.2	8.2 ±□1.1	8, 0 ±□1.1
After 14 days					
Hemoglobin, g / dl	11.0 ±□0.4	8.8 ±□0.3	1 2 , 0 ±□0.2	10.1 ±□0.2	9 , 5 ±□0.2
Erythrocytes, 10 ^{12} / l	6.0 ±□0.5	4.3 ±□0.2	7.4 ±□0.3	5.2 ±□0.3	5, 0 ±□0.3
Leukocytes, 10 ^{9} / l	10.0 ±□1.0	15.25 ±□1.1	11 , 0 ±□1.2	10.0 ±□1.1	9 , 0 ±□0 , 8
After 21 days					
Hemoglobin, g / dl	10.5 ±□0.4	9.6 ±□0.3	11.0 ±□0.2	10.3 ±□0.2	10, 0 ±□0, 1
Erythrocytes, 10 ^{12} / l	5.8 ±□0.5	5.0 ±□0.2	6.8 ±□0.3	5.7 ±□0.3	5, 3 ±□0, 2
Leukocytes, 10 ^{9} / l	10.5 ±□1.0	12.25 ±□1.1	9 , 0 ±□1.2	10.8 ±□1.1	10.8 ±□1.1

* P <0.05 in relation to control

Based on the results of the pharmacological study, it can be concluded that the drug of the new composition of suppositories "Fensin-about" has an anti-inflammatory and reparative effect, characteristic of its constituent

components, having a beneficial effect on the course of experimental colpitis.

In the second series of experimental studies, the alterative effect of the new composition of suppositories "Fensin-about" on a model of a skin wound was also studied.

A cytological study of prints from experimental wounds on day 1 according to M.P. Pokrovskaya in the control group of animals showed a decrease in the protective cellular reaction, a degenerative nature of the changes. Suppuration of wounds was observed in 40% of the animals. Cytograms on day 7 showed a neutrophilic reaction, but there was a tendency to an increase in the number of macrophages and polyblasts, which confirms the strengthening of the reparative process.

In animals, a new composition of suppositories "Fensin-about" at a dose of 150 mg / kg and a suppository of Fensulkala and Diclofenac at a dose of 150 mg / kg during the first 4-6 days, 50% of the animals also observed suppuration; the rest of the animal wounds remained dry, did not bleed, did not fester. No edematous reaction was observed in any case. The resulting crust was dense and dark.

Later, by 14-18 days, the epidermis was completely restored with the formation of derevates (hair follicles and glandular elements).

Thus, under the influence of a new composition of suppositories Fensin-about at a dose of 150 mg / kg of suppositories Fensulkala and Diclofenac at a similar dose, reparative processes in a skin defect in rats proceed faster than in untreated animals, although the percentage of infected wounds did not differ from the control.

In the course of treatment, dynamic monitoring of the course of the wound process was carried out with the control of the timing of cleansing and healing of wounds. By this time, the area of the wound surface in relation to the outcome decreased in the control by 38% under the influence of the new composition, suppository Fensulkala "Fensin-about" by 64%, and under the influence of the reference drug 1% of suppositories Fensulkala - by 60% (Fig. 3) ... Rice.

Fig. 3: Changes in the area of the wound surface of rats treated with a new composition of suppositories Fensulkala and Dikdofenak in comparison with 1% suppository Fensin-about.

Comparing the drugs to increase the healing rate on the 7th day of treatment, they can be arranged in the following order: control - 24%, 1% Fensulkala suppositories - 37%, the new Fensulkala suppository composition - 46% (Table 8).

Table 8: The results of treatment of experimental cutaneous wounds of the new composition of suppositories Fensin-about suppositories in comparison with 1% suppository Fensulkala (M ±□m ; n = 5)

A drug Dose, mg / kg	Terms, days		**Speed wound healing on day 7,%
	Cleansing wounds	Healing wounds	
The control	14 ±□1.2	29 ±□2.0	24
composition of candles Fensulkala-150	9 ±□1.0 *	21 ±□2.0 *	46
1% candles Fensulkala-100	10 ±□1.0 *	21 ±□2.0 *	37
Diclofenac suppositories	10 ±□1.0 *	21 ±□2.0 *	37

* P <□0.01 in relation to control
** in relation to the initial area of the wound surface

The results obtained on the study of the effect of the new composition of vaginal suppositories Fensin-about on cutaneous wounds in an experiment on rats made it possible to draw the following conclusions:

1. The new suppository composition Fensin-about at a dose of 150mg / kg has a wound healing and healing effect, which reduces the time for cleansing and healing wounds by 4-8 days.

2. Comparative analysis of the wound healing effect of the new composition, the Fensin-about suppository with the reference drug 1% Fensulkala suppositories, revealed the advantage of the former.
3. The experiments indicate that the cumulation coefficient: CC> 1, which indicates the absence of the cumulative effect of the tested drug of the new composition, suppository " Fensin-about ".

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