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COMPREHENSIVE STUDY FOR THE DEVELOPMENT OF RECTAL SUPPOSITORIES WITH DIOSMIN AND HESPERIDIN

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The aim. *To conduct a comprehensive study of biphasic-type suppositories that contain diosmin and hesperidin.*
Materials and methods. *Samples of biphasic-type suppositories with a mass of 4.0 were objects of the study. Pharmacological, technological and analytical research methods were used to directly or indirectly analyze the strength and completeness of the drug activity. Thus, in this work we combined the study of the specificity of the pharmacological action of the drug, experimental verification of the quantitative content of API, analysis of the structural properties of suppositories and study of the profile of the release of active pharmaceutical ingredients.*
Results. *Therefore, based on the obtained data, the most effective dose was 75 mg/kg (in terms of a human dose of 300 mg per suppository). The drug in the selected dose showed a significant therapeutic effect, which significantly exceeded that of the test sample at a lower dose and the reference agent. According to the results of technological studies, it was determined that all samples of suppositories had satisfactory structural and mechanical properties. Studies of the histological structure of the mucous membrane of rats proved that there is a positive effect of treatment with suppositories with diosmin and hesperidin due to the improvement of the normal condition of the mucous membrane, the absence of edema and ulcerative defect. Research of the release profile of active pharmaceutical ingredients showed that the best percentage of release is characteristic of sample 2 (99.8 %).*
Conclusions. *Therefore, suppositories with diosmin and hesperidin in therapeutically dose of 75 mg/kg of animal weight can be used for further research and will be of interest in the treatment of hemorrhoids of both acute and chronic forms*
Keywords: *suppositories, diosmin, hesperidin, hemorrhoids, biphasic-type system, pharmacological activity, release profile, technological parameters, quantitative determination*

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1. Introduction

Hemorrhoids is a disease of anorectal area that could cause the decrease of person adaptation in the socio-cultural community of the modern world. A relapse of this pathology occurs in almost 49 % of people who had the acute circulatory disorders in cavernous formations [1]. One of the possible factors in the relapse development is the use of therapy aimed solely at the symptomatic treatment of the disease [2]. Such underestimation of phleboprotective drugs in the treatment regimen contributes to a significant increase in the probability of surgery (hemorrhoidectomy) [3]. Therefore, it was important to find new methods of conservative treatment of hemorrhoids by developing combined drugs, the active substances of which belong to the group of phleboprotectors. Their main advantage is the ability to act precisely at the site of the disease and purposefully reduce the pathological process [4]. Such drugs should be developed in the form of rectal suppositories, since their complex of structural, mechanical and mucoadhesive properties provide the necessary pharmacological effect [5, 6].

Monocomponent media are usually used in the rectal suppositories technology: hydrophobic – Witepsol (W32, H35, E76), Suppocire (AGP, A, AML), solid fat or hydrophilic – PEO alloys with different molecular weight [7]. This technological approach did not allow the introduction of active pharmaceutical ingredients (API) with different solubility properties into the drug, which significantly narrows the spectrum of action and leads to the necessity to select active substances with a similar lipophilicity coefficient. Therefore, we focused on the development of biphasic-type suppositories.

An innovation in this basis will be a combination of hydrophobic components with purified water. In our opinion, a dispersed system of this type will have the following positive aspects:

1) the absence of components that will contribute to the irritation of the anorectal area;

2) a combination of several APIs of hydrophilic and hydrophobic nature is possible;

3) improving the bioavailability of insoluble substances.

This approach would allow the development of a new drug, which can include several active pharmaceutical ingredients, the combination of which was not previously possible. By creating an optimal ratio of hydrophilic-lipophilic balance of the system and the addition of polymeric compounds (e.g., sodium alginate complex with calcium stearate), the indicators of disintegration, distribution and release of API from the drug could be significantly improved [8].

Thus, according to the results of previous studies, we have selected phleboprotective substances diosmin and hesperidin as API [9]. During the Ukrainian pharmaceutical market research, it was found that this combination of active substances is used only in oral dosage forms [10]. Given the technological advantages of suppositories, introduction of the aforementioned substances into the rectal dosage form of biphasic type may be rational. In addition, it could possibly reduce the manifestation of adverse systemic effects typical for tablets with diosmin and hesperidin.

In order to select the optimal dose of API, the adsorption mechanism of active substances from the anorectal area was taken into account. As hepatic filtration during rectal administration of the drug is limited, the conventional dose of 500 mg was irrational. A preliminary literature sources study revealed the presence of a phleboprotective effect in the fraction of diosmin and hesperidin at a dose of 300 and 150 mg [11, 12]. In addition, the optimal ratio of diosmin and hesperidin in the dosage form was established – 9:1. In this ratio, hesperidin potentiated the phleboprotective effect of diosmin [13].

Thus, **the aim of the research** was a comprehensive study of rectal suppositories of biphasic type containing diosmin and hesperidin.

2. Planning (methodology) of research

Development of a methodology for the study of rectal suppositories of the biphasic type required the study of a large number of critical parameters [14]. Therefore, in our work we focused on the complex study of rectal suppositories with diosmin and hesperidin. Pharmacological, technological, and analytical research methods were used to directly or indirectly analyze the strength and completeness of the drug activity. Thus, in this work we combined the study of the specificity of the pharmacological action of the drug, experimental verification of the quantitative content of API, analysis of the structural properties of suppositories and study of the profile of the release of active pharmaceutical ingredients.

The research algorithm that will be presented in this article is shown in Fig. 1.

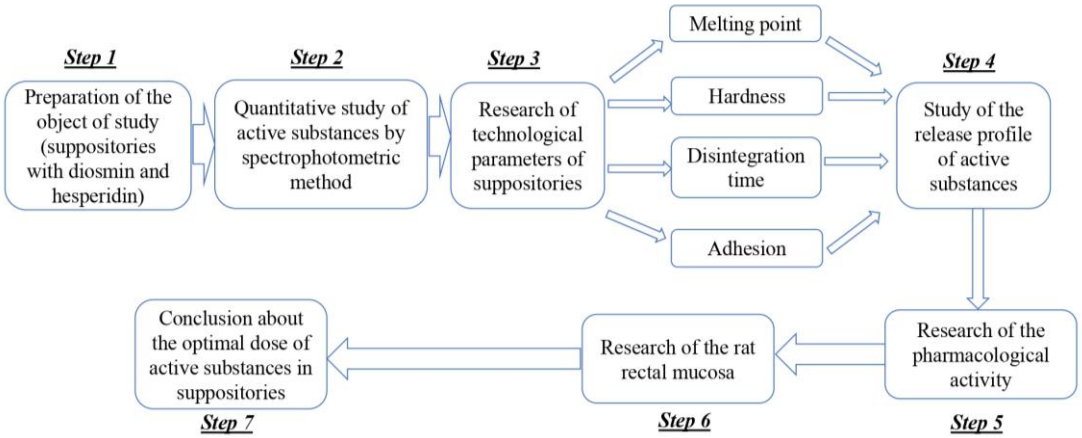


Fig. 1. Algorithm of the research

3. Material and methods

Preparation of suppositories

Samples of biphasic-type suppositories with a mass of 4.0 were objects of the study. Suppositories were used for the study 1 day after manufacture. This period of time was necessary for the formation of a structured system of suppositories. Stabilization with surfactants was a necessary requirement for the formation of biphasic rectal forms [15, 16]. Quantity, type and ratio of surfactants were determined by literature study the hydrophilic-lipophilic balance between the hydrophobic and hydrophilic phases [17]. It was established that the optimal stabilization of the system and reduction of interfacial surface tension was provided by the surfactants Montanov L and sorbitan oleate with the ratio 5:1 and amount of 7 % of the total sample mass [18].

Quantitative parameters of sodium alginate and calcium stearate were equal in all suppository samples [19].

For research samples of suppositories were made as follows. Sodium alginate (constant value – 1.5 % of the total mass of the sample – 0.06 g) was added to the purified water (changing value – amount of hydrophilic phase) with constant stirring. The resulting gel was left for a certain period to form a homogeneous system (hydrophilic medium), followed by the addition of diosmin and hesperidin. Then, Witepsol W-35 (changing value) and emulsifiers (montanov L and sorbitan oleate in a ratio of 5:1, changing value) were melted in a laboratory reactor made of stainless steel, with constant stirring (200 turnover/minute) at a temperature of 60–70 ºC (hydrophobic medium); after that, it was cooled to 40 ºC and calcium stearate (constant value – 0.5 % of the total mass of the sample – 0.02 g) was added. Both parts of the base were combined with constant stirring. The total weight of the one suppositories is 4.0 g.

The quantitative content of the test samples presented in Table 1.

Table 1

Quantitative content of suppository samples

Name of ingredient	Sample No. 1, mg/per suppository	Sample No. 2, mg/per suppository	Sample No. 3, mg/per suppository
Diosmin	135	270	450
Hesperidin	15	30	50
Witepsol W32	1800	1600	1350
Purifed water	1690	1740	1790
Sorbitane oleat	56	56	56
Montanov L	224	224	224
Sodium alginate	60	60	60
Calcium stearate	20	20	20

Quantitative determination of active substances by spectrophotometric method

1.0 of the sample was placed on the bottom of a beaker (capacity 100 ml). 25 ml of 96 % ethanol was added and gradually heated in a water bath (t=40 °C) until it was fully melted. After that, the sample was left for 20 minutes before curing of the suppository base, and the solution remaining on the surface of the base was decanted into a funnel on a paper filter "white tape" (measuring flask for filtrate – 100 ml). The described procedure was repeated twice with portions of 25 ml of ethanol. Then 40 ml of 0.1M sodium hydroxide was added. Obtained solution was shaken for 5 minutes, settled and decanted into 100 ml volumetric flask. The described procedure was repeated twice with portions of 40 and 15 ml of 0.1M sodium hydroxide. The volume of the solution was diluted to 100 ml with 0.1M sodium hydroxide. An aliquot of the obtained solution (2 ml) was taken, transferred to a 100 ml volumetric flask and diluted to 100 ml with 0.1M sodium hydroxide. The optical density of the resulting solution was determined on a spectrophotometer Specord 200 Plus (Analytik Jena, Germany) at a wavelength of 370 nm. At the same time, the optical density of a standard sample of total combination of diosmin and hesperidin was determined.

Research of technological parameters of suppository samples

The Erweka ST 32 device (ERWEKA GmbH, Germany) was used to determine the disintegration time of the suppositories. The study was performed according to the method 2.9.2 "Disintegration of suppositories and pessaries" EP [20]. The sample was considered to have passed the test if the disintegration time did not exceed 60 minutes.

Studies of hardness and adhesion parameters were performed on the device TA.XTExpressC (Stable Micro Systems Ltd, Godalming, Surrey, UK). The study of adhesion characteristics was carried out by the system of "back extrusion" [21]. A metal flat disk with a diameter of 50 mm was used as a working platform for the study. Suppository samples were melted at 37 °C (volume of test samples – 50±2 ml) and placed in a container (capacity 100 ml), which was under the disk. The study parameters (speed 2 mm/sec and distance – 10 mm) were selected according to previous calibration measurements. The study of the hardness of suppository samples was carried out according to the system "cylindrical probe". A standard needle (P / 5N) with a constant load force of 5 kg was used as a working platform. The sample was placed vertically under the working platform (the distance of penetration of the needle into the sample – 3 mm). Criteria of hardness and adhesion did not apply to pharmacopoeial parameters for determining the quality of suppositories but were important for the pharmacodynamics of the drug. According to the study of literature sources, it was found that the sample considered to have passed the test if the hardness >2500 g, adhesion >300 g×sec. The obtained results were interpreted using the software "Exponent Connect".

The study of the melting point of the samples was carried out by the open tube capillary method. An Erweka SSP tester (ERWEKA GmbH, Germany) was used as device. The determination was performed according to the method 2.2.15 "Melting point - open tube capillary method" SPhU [22]. The sample considered to have passed the test if the melting point was ≤ 37.2 ° C.

Release profile of active pharmaceutical ingredients

USP dissolution apparatus II (Erweka DT light series, GmbH, Germany) was used as a device to determine the release profile of active pharmaceutical ingredients [23]. 0.1M phosphate buffer (pH 7.3) in volume of 900 ml was used as dissolution medium. The temperature was 37±0.5°C and the rotation speed was 100 rpm. 2 ml of samples were selected manually with syringe fitted with membrane filter tip after 5, 10, 15, 20, 25, 30, 35, 40 and 45 min and replaced with fresh buffer. The withdrawn samples were suitably diluted and assayed for active pharmaceutical ingredients content using spectrophotometric analysis at 370 nm against a sample from a blank suppository treated similarly.

Research of the pharmacological activity of active pharmaceutical ingredients

The study was performed with 48 outbred male rats aged 3 months. Experimental animals were kept in a vivarium at the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy (Kharkiv, Ukraine) in accordance with sanitary and hygienic standards in the conditions recommended for this species of animals. The animals were housed in separate polypropylene cages in a room with natural light regime "day and night" at a temperature of 19–24 °C and a humidity of 50–60 %. The animals were on a standard diet for this species, had free access to water and food [24]. Each stage of the experiment was conducted in compliance with the principles of Directive 2010/63/EU of the Council of the EU "On the protection of animals used for scientific purposes" (Brussels, 2010) and "General ethical principles of animal experiments" (Kyiv, 2001) The research was approved at a meeting of the commission on bioethics issues of National University of Pharmacy No. 3 of 25.03.2021.

Randomization by experimental groups was performed through minimizing the difference in animal

weight. Experimental animals were divided into 6 experimental groups of 8 animals each:

1) Intact control – no experimental pathology, rectal administration of purified water;

2) Control pathology – modelling of experimental hemorrhoids, lack of treatment, rectal administration of purified water;

3) Control pathology + sample of suppositories No. 1 – modelling of experimental hemorrhoids, the presence of treatment in the listed daily dose of 50 mg/kg of animal weight;

4) Control pathology + sample of suppositories No. 2 – modelling of experimental hemorrhoids, the presence of treatment in the listed daily dose of 75 mg/kg of animal weight;

5) Control pathology + sample of suppositories No. 3 – modelling of experimental hemorrhoids, the presence of treatment in the listed daily dose of 100 mg/kg of animal weight;

6) Control pathology + reference sample – modelling of experimental hemorrhoids, the presence of treatment with a reference sample in the listed daily dose of 90 mg/kg of animal weight.

Sea buckthorn suppositories (PJSC "Monpharm", Ukraine; ATX code A16AX) were used as reference samples, the main indications for which were hemorrhoids and other diseases of the rectum. Daily doses of test and reference agent equivalent to this species were determined according to FDA recommendations for preclinical studies, considering the average therapeutic daily doses for humans and the interspecific difference in body weight and surface area.

Experimental hemorrhoids were induced in animals of all groups except the intact control group by applying an aqueous-ethereal solution of croton oil. Using sterile cotton swabs soaked in 0.1 ml of croton oil solution, experimental animals were applied on an empty stomach to the rectoanal area (distance – 20 mm; diameter – 4 mm; time – 10 sec). The onset of pathology was observed 7 hours after application (indicator – development of edema).

24 hours after induction of hemorrhoids, animals of the experimental groups began to receive treatment. Every day for 5 days after the reconstruction of the model pathology, the animals were injected with the test agents (once a day, time of administration: 9.00–10.00) on an empty stomach. The samples were injected in a mild form (t=37 °C) with a syringe (without needle), and after that anus was closed with a medical patch (time-30 min). To maintain equal experimental conditions, the positive and negative control animals received an appropriate amount of purified water (listed dose – 10 ml/kg) by a similar route of administration.

On the 5th day of treatment (2 hours after the last sample injection), the animals were euthanized. Animals were removed from the experiment by decapitation under chloroform anesthesia and biological material was collected. In experimental animals, anorectal tissues were surgically removed.

Considering the probable pharmacological action of the sample, the permeability of the vascular wall was considered as a response parameter in the screening study. Evaluation of the quantitative characteristics of exudation was performed by determining the amount of blue Evans dye. The dye solution was administered (dose 30 mg/kg) 30 minutes before the induction of pathology to the experimental animals into the tail vein. Dye extraction with formamide was performed on 5th day after postmoral tissue removal (amount – 1 ml/kg, t=55 °C, time – 24 hours). A semi-automatic Map Lab Plus biochemical analyzer (Biochemical Systems International Srl, Italy) was used to determine the absorption of formamide (wavelength – 620 nm) [25].

Research of the rat rectal mucosa

Tissue fragments for the study were fixed in 10 % formaldehyde solution. Slices were obtained from paraffin blocks using a sled microtome. Then, they were mounted on a glass slide and stained with hematoxylin and eosin [26]. Study of micropreparations was performed under a light microscope Granum L30 Bino (Granum, China). Photography of microscopic images was performed with a digital video camera Granum DCM 310 (Granum, China). Photographs were processed using software Toup Vie.

Statistical processing of results

Statistical processing of the obtained data was performed using IBM SPSS with one-way analysis of variance for several independent groups (ANOVA). Differences were considered significant at a significance level of $p<0.05$.

4. Results

At the first stage of our study, we studied the quantitative content of diosmin and hesperidin in suppositories by the spectrophotometric method. The obtained adsorption spectrum of the sample presented in Fig. 2. Spectrum corresponds to the normal overlane of the spectra fraction of diosmin and hesperidin at the quantitative study.

According to the results of the study, it was found that in all samples the quantitative content of the fraction of diosmin and hesperidin was not less than 98 % (sample No. 1: 147±0.3 mg; sample No. 2: 294±0.6 mg; sample No. 3: 490±0.98 mg). The obtained results allowed to conclude about the compliance of the quantitative content of rectal suppositories with diosmin and hesperidin to the regulatory documentation.

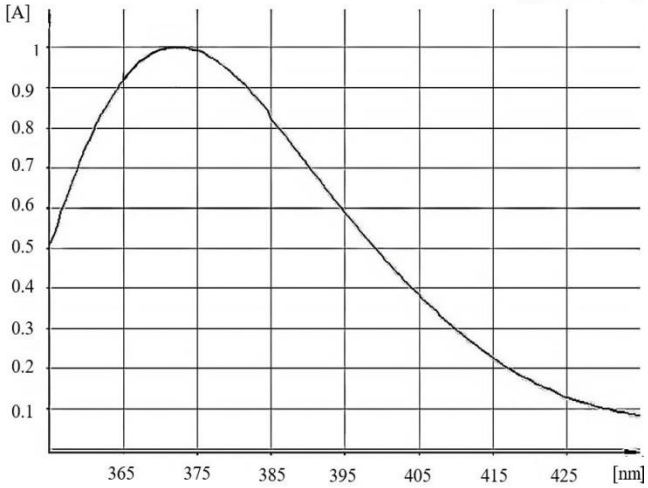


Fig. 2. Adsorption spectrum of samples with diosmin and hesperidin

The next step was to determine the technological parameters of suppositories and confirmed their compliance with quality standards. The results presented in Table 2. According to the obtained results, samples met quality indicators requirements and did not exceed the established parameters of hardness >2500 g, adhesion >300 g×sec, disintegration time <60 min, melting point ≥37.0 °C, but ≤37.2 °C.

Table 2

Technological characteristics of samples of suppositories

Sample number	Disintegration time/min	Hardness/g	Adhesion/g*sec	Melting point/°C
No. 1	35±0.5	2788±36	411±19	37.0±0.1
No. 2	28±0.5	2640±33	449±19	37.1±0.2
No. 3	28±0.5	2620±31	451±38	37.1±0.1

Furtherwork has been devoted to studying the release profiles of active pharmaceutical ingredients from suppositories. This was shown in Fig. 3.

According to the results of the study, it was found that the most complete and fastest release was observed in sample 2. About 99.8 % of the API was released from this sample in 45 minutes of the experiment. For samples 1 and 3, at the end of the experiment, the percentage of released API did not exceed 95 %.

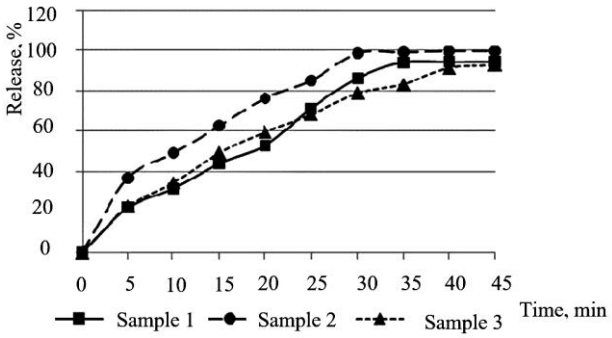


Fig. 3. Release profile of active pharmaceutical ingredients

In the next stage, the experimental model of hemorrhoids in rats was created. During the study, an increase in the permeability of the vascular wall of the tissues around the anorectal area was determined. Significant extravasation of blue Evans dye bound to albumin into the extravascular space was considered an indicator of permeability. The results of the study presented in Table 3.

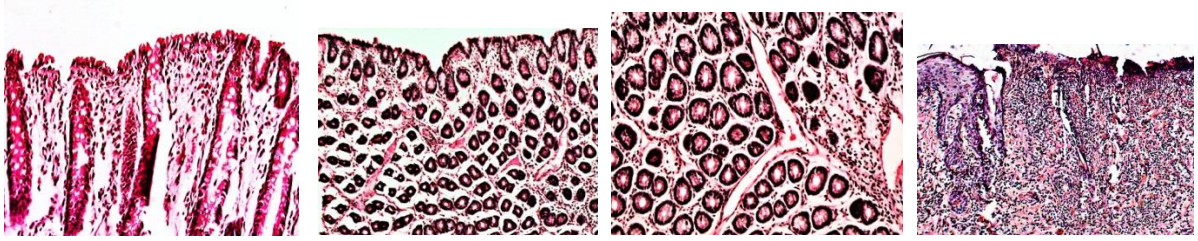
Table 3

Evaluation of vascular permeability of the rectoanal zone of rats with experimental hemorrhoids (M±SEM, n=8)

Experimental groups	Concentration of blue Evans in rectoanal tissue extract areas of rats, μg / mg
Intact control	0.037±0.002
Control pathology	0.137±0.006 *
Control pathology + test sample, 50 mg/kg	0.083±0.008 */**
Control pathology + test sample, 75 mg/kg	0.052±0.006 */**/#
Control pathology + test sample, 100 mg/kg	0.055±0.006 */**/#
Control pathology + reference sample	0.081±0.010 */**

*Note: * – differences are likely with respect to animals of the intact group control (p≤0.05); ** – differences are likely with respect to the animals of the control group pathology (p≤0.05); # – differences are likely with respect to the animals of the reference group (p≤0.05)*

The results of the study of the mucous membrane of rats (5 days after modeling the control pathology of hemorrhoids) are presented in Fig. 4.

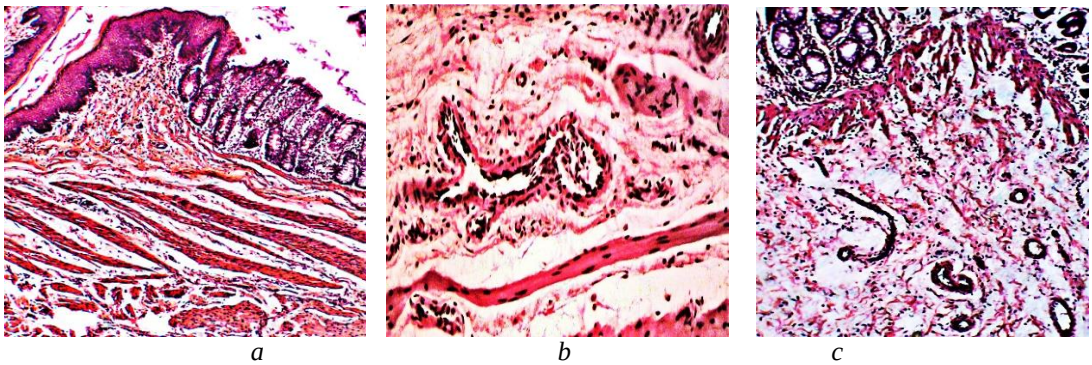


a *b* *c* *d*

Fig. 4. Proximal wall of the rectal of the rat for 5 days after modeling of experimental hemorrhoids, stained with Hematoxylin and Eosin (×200 magnification): *a* – expansion and fullness of the subepithelial capillary network; *b* – expansion and fullness of venous vessels; *c* – expansion and fullness of the stroma of the mucous membrane, edema of the stroma; *d* – ulcerative defect of the mucous membrane

In rats, ulcerative defects of the mucous membrane were found in the proximal part of the anal canal wall. Along the edges of the defect, the epithelial layer is thickened, its migration to the surface of the defect is not observed. The defect itself spread to the entire depth of the mucous membrane, filled with a few destructively altered intestinal crypts and swollen collagen fibers of the stroma. Remains of necrotic masses are visible on the surface of the defect. In the underlying submucosa, as well as in the stroma of the defect, the cellular content is clearly increased. The own plate of a mucous membrane of investigated department out of zones of defects is swollen. The capillary subepithelial network, venous stroma vessels and blood vessels of the arterial and venous plexuses of the submucosal layer are dilated, full-blooded.

After administration of suppositories with diosmin and hespiridine to animals, the mucous membrane of the wall of the examined part of the anal canal becomes practically undamaged (Fig. 5).



a *b* *c*

Fig. 5. Proximal wall of the rectal of the rat for 5 days after modeling of experimental hemorrhoids, stained with Hematoxylin and Eosin: *a* – normal condition of the mucous membrane, hematoxylin and eosin ×100; *b, c* – lack of expansion and fullness of vessels of different caliber of the submucosal layer, hematoxylin and eosin ×250

5. Discussion

In the analysis of literature sources, it was found that the satisfactory technological performance of suppositories contribute to increasing the bioavailability of the APIs [27]. Also, due to formatation of biphasic system, there is a possibility of increasing the rate of solubilization of diosmin and hesperidin with physiological fluid [28].

However, even with satisfactory structural parameters of the suppository, there is a possibility of correlation of the rate of absorption of API through the mucous membrane of the rectum [29]. This process could be most rationally provided if sample:

1) easily overcame the resistance of the internal sphincter of the rectum (average hardness of the sample) [30];

2) rapidly disintegrated in the cavity of the anorectal zone (minimum disintegration time and maximum melting point) [31];

3) had a large area of distribution of the deformed suppository on the mucous membrane of the rectum (high adhesion) [32].

Considering the indicators that may directly affect the pharmacological activity of the suppositories we consider that samples No. 2 and No. 3 (Table 2) had the most acceptable technological parameters.

We assume that the decrease in the percentage of API release in samples 1 and 3 (Fig. 2) is associated with incorrect inclusion of diosmin and hesperidin in the formed sodium alginate-calcium stearate complex [33]. According to the results of the study of literary sources, this can be observed with excessive addition of the APIs to the biphasic medium (sample 3 for 4.0 mass suppositories had 500 mg of API) or formation of excessively strong alginate-calcium complex with active substances [34].

The results of pharmacological study was found that in groups of animals of control pathology the model of experimental hemorrhoids the permeability of the vessels of the anorectal zone increased 3.5 times compared with the same indicator in intact animals ($p \leq 0.05$). In animals injected with a test sample at a dose of 50 mg / kg was a probable decrease in extravasation of blue Evans into the intercellular space by 39.4 % compared with control pathology ($p \leq 0.05$). In addition, the strength of the effect did not differ statistically from the reference group, in which buckthorn suppositories were used as a therapeutic agent ($p \leq 0.05$).

The best effect was demonstrated by a test sample with a dose of 75 mg/kg, which significantly reduced the vascular permeability of the anorectal area of experimental animals. The positive result was manifested through a reduction of the content of blue Evans in the affected tissues by 62.0 % ($p \leq 0.05$ against control pathology). At the same time, the effectiveness of the test sample at this dose was probably higher than that of the reference agent ($p \leq 0.05$). The activity of the test sample at a dose of 100 mg/kg did not differ statistically from the results of the previous dose. Thus, in this group there was a decrease in the permeability of the vascular wall in the anorectal area by 59.9 % ($p \leq 0.05$ against control pathology). This may be due to the limited properties of flavonoids in passing through the intestine and vascular wall [35]. It should be noted that the reference suppositories with sea buckthorn oil concentrate also significantly reduced the extravasation of blue Evans into the tissue space of the anorectal area by 40.9 % compared with the corresponding indicator in the control pathology group ($p \leq 0.05$), which was significantly inferior to the study test sample at doses of 75 and 100 mg/kg.

According to the results of the study of the mucous membrane of the anal zone of rats, it can be concluded that significantly reduced blood saturation of vessels of different calibers of the mucous membrane and venous plexuses of the submucosal layer. The microscopic picture of the proximal wall of the anal canal of rats retained a normal histological structure [36].

Study limitations. Studies conducted to establish the optimal technological parameters for suppositories do not reflect all possible risks associated with the implementation of the technology in the factory and require additional research on the industrial equipment available for the manufacturer.

Prospects for further research. A promising direction for further research is development of technological regulatory documentation for suppositories with diosmin and hesperidin, verification of the coherence of the developed drug with quality parameters, as well as justification of the technological process for industrial production.

6. Conclusions

The results obtained during the pharmacological study proved that diosmin and hesperidin help to reduce the permeability of the vascular wall and protect the capillaries from damage. We failed to build a probit-model for further in-depth pharmacological studies, because no linear dose-effect relationship was observed during the screening study. Therefore, based on the data obtained, the most effective dose was 75 mg/kg (in terms of a human dose of 300 mg per suppository). The drug in the selected dose showed a significant therapeutic effect, which significantly exceeded that of the test sample at a lower dose and the reference agent.

According to the results of technological studies, it was determined that the drug had satisfactory structural and mechanical properties.

Studies of the histological structure of the mucous membrane of rats proved that there is a positive effect of treatment with suppositories with diosmin and hesperidin due to the improvement of the normal condition of the mucous membrane, the absence of edema and ulcerative defect.

Research of the release profile of active pharmaceutical ingredients showed that the best percentage of release is characteristic of sample 2 (99.8 %)

Therefore, suppositories with diosmin and hesperidin in above-mentioned qualitative and quantitative composition can be used for further research and will be of interest in the treatment of hemorrhoids of both acute and chronic forms.

The obtained results are part of the PhD thesis «Development of the composition and technology of suppositories with diosmin and hesperidin» and are going to be used in further research.

Conflict of interests.

The authors declare that they have no conflicts of interest.

Financing.

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