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INVESTIGATION OF THE ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF ANHYDROUS GEL "HYPERICUM-DERM"

Actuality. *Purulent wound is one of the thorniest, difficult and urgent issues in clinical practice. Due to the start of full-scale military actions in Ukraine in 2022, the number of purulent-necrotic wounds has increased several times, almost 40% of all wounds are purulent-*

necrotic. Purulent-inflammatory wounds are very acute and often lead to generalized infections, the development of sepsis and also the death of patients. Thus, the development of new anti-inflammatory and antioxidant drugs in the form of soft dosage forms is relevant today.

The aim of the study. The purpose of our work was investigate in silico, in vivo anti-inflammatory and in vitro antioxidant activity of anhydrous gel "Hypericum-Derm".

***Material and methods**. The objects of the study were anhydrous gel "Hypericum-Derm", which includes α-arbutin, clotrimazole, lidocaine hydrochloride, Hypericum perforatum herb extract, Crataegus monogyna leaf and flower extract. Molecular docking was performed using AutoDockTools 1.5.6; anti-inflammatory effect was assessed by the model of carrageenan induced paw edema in rats; antioxidant activity was determined by potentiometric method.*

***Research results.** The combination compounds of "Hypericum-Derm" anhydrous gel was high selective inhibited all targets of proinflammation targets: cyclooxygenase-2 (COX-2), phospholipase A2, NF-kB, 5-lypooxygenese (5-LOX), and prooxidants targets: myeloperoxidase, xanthine oxidase, NADPH oxidase**.** Experimental research was demonstrated that anhydrous gel "Hypericum-Derm" significantly decrease edema after 1, 2, 3 and 4 hours by 94,0, 94,0, 99,0, 100,0%, respectively, compared to the control group. Anhydrous gel "Hypericum-Derm" at dose of 0,02 had a high antioxidant activity (86,13±1,00 mmol-eqv./$m_{dry\ res.}$) that was 4,6 times higher than a "gold standard" epigallocatechin-3-O-gallate.*

***Conclusion.** Theoretical and practical studies of the composition of the main components of the anhydrous gel "Hypericum-Derm" were conducted. According to theoretical studies, it was shown that neither the "gold standard" sodium diclofenac nor the natural compound is capable of highly selectively inhibiting all key targets of proinflammatory enzymes, but in the case of prooxidant enzymes, one substance was found that highly selectively inhibits all targets – hyperecin. Experimental results showed that the anhydrous gel "Hypericum-Derm" actively inhibits all stages of inflammation on the carrageenan model. Also, it was found that "Hypericum-Derm" has high antioxidant activity.*

***Key words:** anhydrous gel, molecular docking, inflammation, antiradical effect, wound.*

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ДОСЛІДЖЕННЯ ПРОТИЗАПАЛЬНОЇ ТА АНТИОКСИДАНТНОЇ АКТИВНОСТІ БЕЗВОДНОГО ГЕЛЮ «ГІПЕРИКУМ-ДЕРМ»

Актуальність. *Гнійні рани є одними з найгостріших, складних і актуальних питань у клінічній практиці. У зв'язку з початком повномасштабних воєнних дій в Україні у 2022 році кількість гнійно-некротичних ран зросла в кілька разів, майже 40% усіх ран є гнійно-некротичними. Гнійно-запальні рани мають дуже гострий перебіг та часто призводять до генералізованих інфекцій, розвитку сепсису, а також смерті пацієнтів. Отже, розроблення нових протизапальних і антиоксидантних препаратів у вигляді м'яких лікарських форм є актуальним.*

Мета дослідження. *Метою нашої роботи було дослідження in silico, in vivo протизапальної та in vitro антиоксидантної активності безводного гелю «Гіперикум-Дерм».*

Матеріал і методи*. Об'єктами дослідження були безводний гель «Гіперикум-Дерм», у склад якого входить α-арбутин, клотримазол, лідокаїну гідрохлорид, екстракт трави звіробою звичайного, екстракт листя та квіток глоду одноклітинного. Молекулярний докінг проводили за допомогою AutoDockTools 1.5.6; протизапальний ефект оцінювали на моделі набряку лапи, індукованого карагенаном, у щурів; антиоксидантну активність визначали потенціометричним методом.*

Результати дослідження. *Основні компоненти безводного гелю «Гіперикум-Дерм» мали високу селективність, пригнічувати всі мішені прозапальних ферментів, циклооксигеназу-2 (ЦОГ-2), фосфоліпазу А2, NF-kB, 5-ліпооксигенезу (5-ЛОГ), а також прооксидантні мішені: міелопероксидазу, ксантиноксидазу, НАДФН-оксидазу. Експериментальні дослідження показали, що безводний гель «Гіперикум-Дерм» значно зменшував набряк через 1, 2, 3 та 4 години на 94,0, 94,0, 99,0, 100,0% відповідно, порівняно з контрольною групою. Безводний гель «Гіперикум-Дерм» у дозі 0,02 моль мав високу антиоксидантну активність (86,13±1,00 ммоль-екв./$м_{сух. реч.}$), що в 4,6 раза перевищувало «золотий стандарт» епігалокатехін-3-О-галлат.*

Висновок. *Були проведені теоретичні та практичні дослідження складу основних компонентів безводного гелю «Гіперикум-Дерм». Згідно з теоретичними дослідженнями, було виявлено, що ні «золотий стандарт» диклофенак натрію, ні природна сполука не здатні високоселективно інгібувати всі ключові мішені прозапальних ферментів, але щодо прооксидантних ферментів було виявлено одну речовину, яка високоселективно інгібує всі мішені – це гіперецин. Експериментальні результати показали, що безводний гель «Гіперикум-Дерм» активно інгібує всі стадії запалення на карагенановій моделі. Також було встановлено, що «Гіперикум-Дерм» має високу антиоксидантну активність.*

Ключові слова: *безводний гель, молекулярний докінг, запалення, антирадикальний ефект, рана.*

Introduction. Actuality. Hostilities in eastern Ukraine, ongoing since 2014 and intensifying into full-scale war in February 2022, continue to the present day. UN Human Rights Office data records over 40 000 casualties between April 2014 and late 2020, including more than 4 000 military fatalities and 12 000 injured personnel. The nature of combat injuries follows a clear distribution: the majority (up to 60%) are blast-related, followed by combined injuries (20–22%) and thermal burns (10–13%). These statistics highlight the ongoing relevance of improving treatment for wounds and burns, a significant challenge for the state's civilian and military medical infrastructure (Gumeniuk, 2023, p. 1–15).

A wound is a complex inflammatory response of the body to tissue damage. Effective wound healing directly depends on the immune response, proinflammatory cytokines, and growth factors. Wound healing is a complex process consisting of three phases: inflammation, proliferation, and remodeling (Shah and Amini-Nik, 2017, p. 1068). Immediately after bleeding stops following injury, the immune system activates neutrophils. Neutrophils are the first line of defense against microorganisms. Also, within the first few hours, killer T cells begin to actively infiltrate the wound along with neutrophils. The main function of killer T cells is to detect and destroy foreign agents. Then, on the third day of inflam-

mation, macrophages actively begin to work, replacing neutrophils in the wound. Macrophages, in turn, release proinflammatory cytokines into the wound, namely tumor necrosis factor α (TNF-α), and interleukin-1 (IL-1), IL-6 (Öhnstedt, 2019, p. 485–497).

These cytokines indirectly activate nuclear factor kB and, among other things, stimulate the release of metalloproteinases. Metalloproteinases destroy damaged cells, and growth factors such as vascular endothelial growth factor (VEGF), tissue growth factor β (TGF-β), basic fibroblast growth factor (bFGF), and keratinocyte growth factor (KGF) are released from the cells. Activation of growth factors is the proliferation stage; the key task of this stage is to stimulate fibroblasts and keratinocytes. During the remodeling stage, a complete reorganization of collagen fibers from immature type III to mature type I fibers occurs, which allows for the maximum reproduction of normal tissue (Han, 2001, p. 131–139).

In our recent study (Maslov, 2025, p. 175–194), we proposed and developed the composition of the anhydrous gel "Hypericum-Derm", which theoretically and practically exhibits antimicrobial activity against resistant gram-negative strains of bacteria and fungi, including Acinetobacter baumani, Pseudomonas aeruginosa, Klebsiela pneumoniae, Enterobacter cloacae, and Candida albicans. The anhydrous gel contains polyethyleneglycol 1 500 and 400, St. John's wort extract, hawthorn leaf and flower extract, clotrimazole, arbutin, and lidocaine hydrochloride. This anhydrous gel composition is theoretically optimal for combating resistant strains of bacteria and fungi. To understand how the developed gel would act in phase I of the wound, we decided to evaluate the anti-inflammatory and antioxidant effects of the new anhydrous gel.Thus, the aim of our study was investigate *in silico*, *in vivo* anti-inflammatory and *in vitro* antioxidant activity of anhydrous gel "Hypericm-Derm".

Materials and research methods. ***Reagents.*** "Levomekol" (Pharmaceutical Factory "Viola") – series number LMK-03-250722-01-UA; gel "Diclofenac" (Pharmaceutical Factory "Viola") – series number DIC-01-250823-01-UA.

Experimental animals. The study involved 24 male rats of the outbred white strain, weighing between 180 and 220 grams. These rats were sourced from the vivarium of the National University of Pharmacy (NUPh). Throughout the experiment, the rats were housed in macrolon boxes with five animals in each box. Rats had unrestricted access to water and food, which were provided on a daily basis. The bedding was replaced on a three-day cycle. The rats were maintained under specific conditions, including a temperature of 22±2 °C, relative humidity of 60±5%, and a daily light cycle of 12 hours of light and 12 hours of darkness.

All procedures carried out during the study adhered to the guidelines set by the National Institute of Health for the care and use of laboratory animals, as well as the European Council Directive on 24 November 1986 for the Care and Use of Laboratory Animals (86/609/EEC). The study protocol was approved by the Local Ethics Committee.

Antioxidant activity. The antioxidant activity of anhydrous gel "Hypericum-Derm" was assessed using the potentiometric method (Maslov, 2024, p. 105–122). Antioxidant activity was calculated according to the following equation and expressed as mmol-equiv./$m_{dry\ res.}$:

$$AOA = \frac{C_{OX} - \alpha \times C_{red}}{1+\alpha} \times K_{dil} \times 10^3 \times \frac{m_1}{m_2}, \text{ where}$$

$\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - E\text{ethanol})nF/2.3RT}$; C_{ox} – concentration of $K_3[Fe(CN)_6]$, mol/l; C_{red} – concentration of $K_4[Fe(CN)_6]$, mol/l; $E_{ethanol}$ – $0{,}0546 \cdot C_{\%} - 0{,}0091$; $C_{\%}$ – concentration of ethanol; ΔE – change of potential; F = 96 485,33 C/mol – Faraday constant; n = 1 – number of electrons in electrode reaction; R = 8,314 J/molK – universal gas constant; T – 298 K; K_{dil}– coefficient of dilution; m_1 – mass of dry residue; m_2 – mass of dry residue in 1,0 g of gel.

As a standard was chosen epigallocatechin-3-O-gallate.

Anti-inflammatory activity. The anti-exudative activity of extract was studied on 24 white outbred male rats weighing 180–220 g, in which a model of acute inflammation induced by subplantar injection of 0,1 mL of 1% carrageenan (Fluka, Switzerland) into the right hind paw of rats, measurement of paw edema in rats was carried out after 1, 2, 3, 4 hours (Stefanov, 2001, p. 500).

The anhydrous gel and comparison drugs were applied to the right hind foot twice: 40 minutes before injections of the phlogotropic agent and 20 minutes after its administration. Animals of the positive control groups were not treated.

All animals were divided into 4 groups. The first group was control pathology (animals of the positive control groups were not treated.), the second group – polyethyleneglycol base (PEG 400 and PEG 1500 (8:2)), the third group – gel of "Diclofenac", the fourth group – anhydrous gel "Hypericum-Derm".

Molecular docking. A molecular docking study was conducted using the tool known as AutoDockTools 1.5.6 (Morris, 2008, p. 1–8).

COX-2 (PDB ID: 1ddx), phospholipase A2 (PDB ID: 3hsw), 5-LOX (PDB: 2q7m), NF-kB (1svc), myeloperoxidase (PDB: 3f9p), xanthine oxidase (PDB: 1fiq), NADPH oxidase (PDB ID: 5o0X) structures were obtained from PDB database (Burley, 2025,

D564 – D574). The resolution of 1ddx was 3,00 Å whereas 5o0X – 2,20 Å, 2q7m – 4,25 Å, 1svc – 2,60 Å, 3f9p – 2,93 Å, 1fiq – 2,50 Å. The ligand structures of rutin (CID_5280805); α-arbutin (CID_158637); clotrimazole (CID_2812); hypericin (CID_3663); hyperoside (CID_5281643); lidocaine hydrochloride (CID_6314), vitexin (CID_5280441), isovitexin (CID_162350); quercetin (CID_5280343); diclofenac sodium (CID_5018304) were obtained from PubChem database (Kim, 2025, p. D1516 – D1525). The active site of the docking protein was identified utilizing the Computed Atlas for Surface Topography of Proteins (Tian, 2018, p. W363 – W367). "

Statistical analysis. To obtain statistical results, the Statistica 10 program was used, the results of anti-inflammatory activity were analyzed using Mann-Whitney test, whereas the results of antioxidant activity were analyzed using analysis of variance (ANOVA) post-hoc Tukey's HSD test. Differences were considered significant at $p < 0,05$.

Research results and their discussion. The next stage of our research was molecular docking of the identified phenolic compounds of St. John's wort herb, hawthorn leaves and flowers, as well as α-arbutin, clotrimazole and lidocaine hydrochloride for potential anti-inflammatory and antioxidant activity. In our latest work (Maslov, 2025, p. 175–194), we studied the polyphenolic profile of the extract of St. John's wort and the leaves and flowers of hawthorn, and we used the obtained identified substances for molecular modeling.

There are a large number of enzymes in the body that have pro-inflammatory and pro-oxidant properties. We selected, according to our point of view, the key pro-inflammatory enzymes: COX-2, 5-LOG, phospholipase A2, and NF-kB; as well as important pro-oxidant enzymes: myeloperoxidase, NADPH and xanthine oxidase. For comparative analysis of the level of enzyme inhibition, we took the "gold standards" – sodium diclofenac as a standard of anti-inflammatory activity, and epigallocatechin-3-O-gallate as a standard of antioxidant activity. To understand the level of selectivity of inhibition of the studied substances to the active centers of bacterial enzymes, we applied the following classification of selectivity (Konda, 2021, p. 215–219): $IC50 < 0,001$ mM (high selective); $0,05 > IC50 > 0,01$ (medium selective); $IC50 > 0,05$ mM (low selective).

According to the results shown in Table 1, it was shown that highly selective inhibitors of the COX-2 enzyme include clotrimazole, hyperoside, rutin, isovitexin, arbutin, lidocaine hydrochloride and catechin, while sodium diclofenac, chlorogenic acid, caffeic acid, vitexin, quercetin, gallic acid are low-selective inhibitors, and in the case of hypericin, it is inactive with respect to the COX-2 enzyme. The active center of the COX-2 enzyme structure is the following series of amino acids: ALA199, ALA202, THR206, TYR385, GLU203, HIS388, LEU391, LEU390, TRP387 (fig. 1).

Next, we assessed the ability of the main components of the anhydrous gel to inhibit phospholipase A2. We found that rutin, hyperecin, hyperoside, vitexin, isovitexin, clotrimazole, chlorogenic acid, arbutin, catechin and apigenin are highly selective inhibitors, while lidocaine hydrochloride, sodium diclofenac, quercetin and ferulic acid are moderately selective inhibitors. And the lowest results in seleutivity were shown by caffeic and gallic acids. The active center for inhibiting phospholipase A2 are amino acids: LYS147, VAL145, HIS144, TYR 60, THR146, PHE22, TYR28 (fig. 1).

The next most important proinflammatory enzyme is 5-LOG. According to the results of the study, we found that hyperecin, isovitexin, vitexin, hyperoside, clotrimazole, apigenin and arbutin are highly selective inhibitors. Quercetin and diclofenac sodium have medium selectivity, and low-selectivity include catechin, lidocaine hydrochloride, gallic acid, ferulic acid and caffeic acid. It was also found that rutin and chlorogenic acid are completely inactive with respect to the 5-LOG enzyme. Amino acids VAL81, ALA84, LEU11, ILE14, VAL34, and LEU88 acted as an active center for binding the enzyme structure of 5-LOG (fig. 1).

The next important enzyme, which acts especially in chronic inflammation is NF-kB. According to the results of the study, not a single highly selective inhibitor was found, only three medium-selective inhibitors were established – hyperecin, vitexin and isovitexin. In turn, the remaining compounds showed low selectivity for inhibition of the active center of NF-kB, which may indicate the complexity and, in particular, the importance of inhibition of this enzyme. The active center of NF-kB was represented by the following amino acids: LYS147, LYS148, THR146, TYR60, LEU210, HIS144 (fig. 1).

The next part of the molecular docking study is a theoretical assessment of the inhibition of prooxidant enzymes. The first important enzyme, which is the basis for the emergence of free radicals, is the enzyme NADPH. According to the results of the study shown in table 2, hyperecin, isovitexin, clotrimazole, hyperoside are highly selective inhibitors of NADPH, while vitexin, catechin, arbutin, lidocaine hydrochloride, rutin, epigallocatechin-3-O-gallate act as medium-selective inhibitors. Low-selective inhibitors include chlorogenic acid, quercetin, ferulic acid, gallic acid, caffeic acid, and sodium diclofenac. The active center of the NADPH enzyme was the amino acids PRO521, PHE693, ASN692, FAD801, TRP695, PRO694 and THR520 (fig. 2).

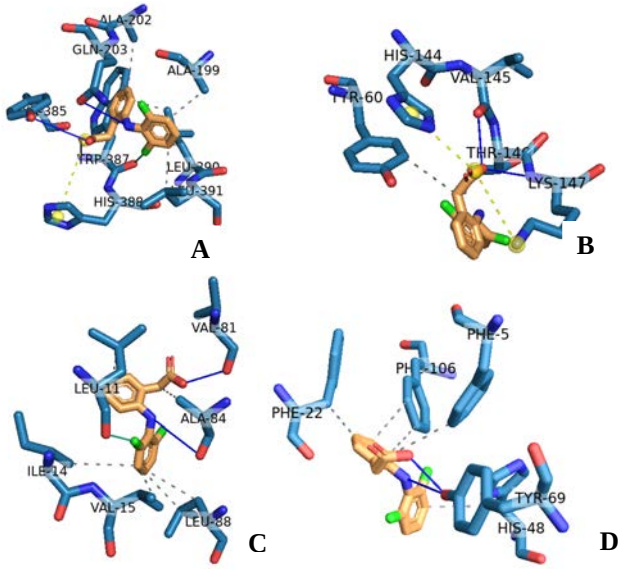

Fig. 1. Molecular interaction analysis of diclofenac sodium with active center of COX-2 (A), phospholipase A2 (B), 5-LOX (C) and NF-kB (D) structures

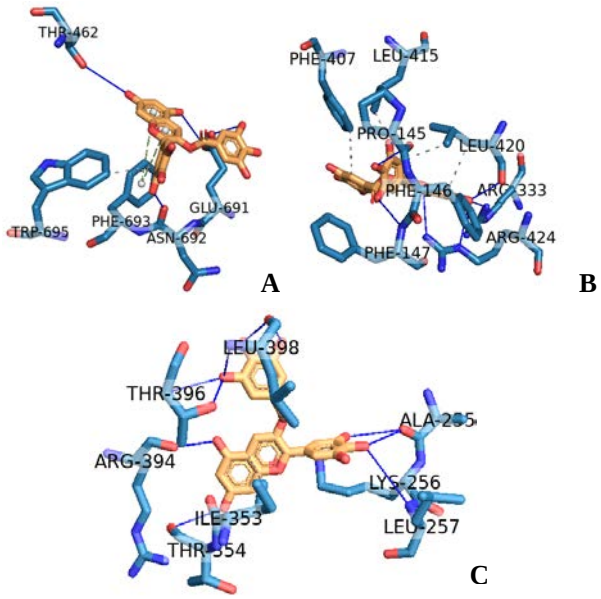

Fig. 2. Molecular interaction analysis of epigallocatechin-3-O-gallate with active center of NADPH oxidase (A), myeloperoxidase (B), xanthine oxidase (C) structures

One of the most important enzymes that acts for the occurrence of oxidative stress is myeloperoxidase. The active center of this enzyme is the following amino acids: LEU420, PHE407, PHE146, GLU116 and ALA421. According to the obtained data shown in Table 4, it was found that hyperecin is the only highly selective inhibitor, the medium-selective ones included hyperoside, vitexin, rutin, clotrimazole and isovitexin. Low-selective inhibitors of myeloperoxidase included arbutin, catechin, apigenin, chlorogenic acid, lidocaine hydrochloride, ferulic acid, sodium diclofenac, caffeic acid, epigallocatechin-3-O-gallate, gallic acid and quercetin (table 2).

The next prooxidant enzyme that we decided to test is xanthine oxidase. Most literature sources say that this enzyme is responsible for the metabolism of hypoxanthine to casanthine, and is one of the causes of gout. However, during the oxidation of hypoxanthine to xanthine, secondary reaction products are released – free radicals, which are responsible for the initiation of oxidative stress. The study found that highly selective inhibitors include isovitexin, vitexin, rutin, clotrimazole, hyperecin, hyperoside. Medium-selective inhibitors include arbutin, catechin, epigallocatechin-3-O-gallate, apigenin and lidocaine hydrochloride, while quercetin, gallic acid, chlorogenic acid, sodium diclofenac, caffeic acid and ferulic acid are low-selective inhibitors. The active center of the enzyme a xanthine oxidase was represented by the amino acids: FAD606, GLY260, THR262, ILE266, GLY265, ASN261, GLY260 (fig. 2).

The antioxidant activity of obtained anhydrous gel "Hypericum-Derm" was assessed by the potentiometric method with a "gold standard" – epigallocatechin-3-O-gallate. As result of the study, it was found that at a dose of 0,02 mole of phenolic compounds gel, the antioxidant activity of anhydrous gel "Hypericum-Derm" was 86,13±1,00 mmol-eqv./$m_{dry\ res.}$ that in 4.6 times higher of standard solution at same concentration epigallocatechin-3-O-gallate. According to the developed conditional classification of antioxidant activity according to Maslov (Maslo, 2021, p. 215–219), we determined that the anhydrous gel "Hypericum-Derm" at a dose of 0,02 mole of gel had a high level of antioxidant activity, whereas epigallocatehin-3-O-gallate had lower middle level of antioxidant power (table 3).

Anhydrous gel "Hypericum-Derm" significantly reduced paw edema by 94% compared to the control group from the first hour. Thereafter, paw edema was decreased by 93,00, 99,00 and 100,00% at 2, 3, and 4 hours, respectively, compared with control group. Treatment with anhydrous gel "Hypericum-Derm" was showed a significant reduction in edema at 1, 2, 3 and 4 hours compared with "Diclofenac" gel. Anhydrous gel "Hypericum-Derm" was reduced paw edema better than polyethylenglycol base. In the first hour, paw edema better at 42%, at the second hour – 32%, at the third hour – 31%, and at the fourth hour – 19% (table 4).

Table 1

Molecular docking of the identified compounds and anti-inflammatory drug standard diclofenac sodium with the COX-2, phospholipase A2, 5-LOX, Nf-kB

№	Ligand	Binding energy ΔGbind[a] (kcal/mol)	Ki[b] mmol	Binding site	Level of selectivity
1.	Clotrimazole	−13,57	0,00000011377	A: ALA199, ALA202, TYR385, TRP387, HIS388, LEU390, LEU391	High selectivity
2.	Hyperozide	−12,36	0,0000009	A: ALA199, LEU391, TRP387, TYR385, GLU203	High selectivity
3.	Rutin	−10,98	0,00000898	A: CYS37, ASN39, GLY45, CYS47, GLY135, GLY135, VAL155, ALA156	High selectivity
4.	Isovitexin	−10,89	0,00001042	A: TYR385, HIS386, TRP387, HIS388, TRP387, LEU390, LEU391, VAL447	High selectivity
5.	α-arbutin	−9,48	0,000113	A: ALA199, GLN203, TYR385, TRP387, LEU391, LEU390	High selectivity
6.	Lidocaine hydrochloride	−8,91	0,00029606	A: TYR385, TRP387, LEU390, LEU391	High selectivity
7.	Apigenin	−8,73	0,000398	A: ALA199, ALA202, GLN203, THR206, TRP387, LEU390, LEU391, TYR385	High selectivity
8.	(+)-catechin	−8,40	0,00069535	A: ALA199, ALA202, GLN203, TRP387, LEU390, THR206, TYR385	High selectivity
9.	Diclofenac sodium	−5,76	0,05977	A: ALA199, ALA202, GLN203, TRP387, LEU390, LEU390, TYR385, HIS388	Low selectivity
10.	Chlorogenic acid	−5,66	0,07041	A: GLN203, TYR385, HIS388, SER451, GLN454, HIS207, HIS386, HIS388	Low selectivity
11.	Caffeic acid	−5,48	0,09663	A: ILE124, THR149, ARG376, ALA378, ASP125, SER126, THR129, THR149, ARG150, ARG376, LYS532	Low selectivity
12.	Vitexin	−5,28	0,13459	A: VAL447, HIS207, THR212, TYR385, HIS388, SER451, GLN454, HIS386, HIS388	Low selectivity
13.	Quercetin	−4,59	0,42855	A: CYS36, ASN39, CYS41, PRO154, ALA156	Low selectivity
14.	Gallic acid	−4,2	0,83022	A: ILE124, SER126, GLN370, GLN 372, LYS532	Low selectivity
15.	Ferulic acid	−3,2	5,55	A: LEU391, PHE395, PHE404, LEU408, GLN203	Low selectivity
16.	Hypericin	–	–	–	Inactive
№	phospholipase A2				
1.	Rutin	−15,51	0,0000000043	A: ILE9, PHE22, LEU31, TYR69, PHE106, PRO18, LEU19, ASN23, GLY30, CYS45, HIS48, ASP49	High selectivity
2.	Hypericin	−15,34	0,00000000564	A: PHE22, PHE106, AS N23, TYR28, HIS48, ASP49, TYR69, PHE5	High selectivity
3.	Hyperozide	−14,05	0,00000005045	A: PHE5, PHE22, ASN23, GLY30, CYS45, HIS48, ASP49, TYR69	High selectivity
4.	Vitexin	−13,89	0,00000006539	A: PRO18, PHE22, LEU31, CYS45, HIS48, ASP49, TYR69, HIS48, PHE22, ASN23	High selectivity

5.	Isovitexin	−12,36	0,00000087765	A: ARG6, GLY30, HIS48, ASP49, TYR69, LEU2, PHE5, ARG6, LEU19, ASN23	High selectivity
6.	Clotrimazole	−12,15	0,00000125	A: PHE22, PHE106, HIS48, ASP49, PHE5, HIS48	High selectivity
7.	Chlorogenic acid	−10,09	0,00004421	A: PHE5, ILE9, ASN23, TYR69, PRO18, TYR28, GLY30, GLY32, HIS48, ASP49, TYR69	High selectivity
8.	α-arbutin	−9,65	0,00008488	A: PHE22, PHE106, GLY30, CYS45, HIS48, TYR69	High selectivity
9.	(+)-catechin	−9,34	0,00014196	A: PHE5, ILE9, PHE22, HIS48, PHE106, PRO18, TYR28, GLY30, ASP49	High selectivity
10.	Apigenin	−8,57	0,00052654	A: PHE5, ILE9, PHE22, GLY30, CYS45, HIS48, ASP49	High selectivity
11.	Lidocaine hydrochloride	−8,05	0,00127	A: ILE9, PHE22, ASN23, HIS48, PHE106, PHE106	Middle selectivity
12.	Diclofenac sodium	−7,65	0,00248	A: PHE5, PHE22, HIS48, PHE106, TYR69	Middle selectivity
13.	Quercetin	−6,79	0,01062	A: PHE5, ILE9, PHE22, GLY30, CYS45, HIS48, ASP49	Middle selectivity
14.	Ferulic acid	−5,92	0,04545	A: PHE5, HIS48, PHE106, PHE22, GLY30, CYS45, HIS48, ASP49	Middle selectivity
15.	Caffeic acid	−5,83	0,05354	A: PHE5, ASN23, TYR28, GLY30, HIS48, ASP49	Low selectivity
16.	Gallic acid	−5,25	0,05452	A: PHE5, PHE106, PHE22, TYR28, GLY30, CYS45, HIS48, ASP49	Low selectivity
№	5-LOX				
1.	Hypericin	−11,92	0,00000182	A: THR66, ILE119, PHE123	High selectivity
2.	Isovitexin	−11,62	0,00000305	A: VAL70, ILE119, PHE123	High selectivity
3.	Vitexin	−11,44	0,00000412	A: VAL70, ILE119, PHE123	High selectivity
4.	Hyperozide	−11,12	0,00000707	A: PHE123, VAL70, ILE119, THR66	High selectivity
5.	Clotrimazole	−10,79	0,00001243	A: PHE123, VAL70, ILE119, THR66	High selectivity
6.	Apigenin	−7,38	0,00392	A: PHE123, ILE110, THR66	High selectivity
7.	α-arbutin	−7,33	0,00422	A: VAL70, ILE119, THR66	High selectivity
8.	Quercetin	−6,45	0,01857	A: ILE119, THR66	Middle selectivity
9.	Diclofenac sodium	−6,00	0,03982	A: VAL81, ALA84, LEU11, ILE14, VAL15, LEU88	Middle selectivity
10.	(+)-catechin	−4,55	0,46333	A: TYR64, LEU68, VAL61, PRO65	Low selectivity
11.	Lidocaine hydrochloride	−4,36	0,63676	A: ASP62, ALA63, TYR112, ILE113, LYS116, ILE119, LEU120, PHE123	Low selectivity
12.	Gallic acid	−4,20	0,83022	A: ASN23, PHE26, VAL61, ASN23, ASN57, CYS60, VAL61, TYR64, ARG94	Low selectivity
13.	Ferulic acid	−3,20	5,55	A: VAL70, ILE119, PHE123, THR66	Low selectivity
14.	Caffeic acid	−2,72	10,17	A: LEU68, PRO65, VAL61, TYR64	Low selectivity
15.	Rutin	–	–		Inactive
16.	Chlorogenic acid	–	–		Inactive
№	Nf-kB				
1.	Hypericin	−7,24	0,00497	A: ARG57, GLU63, PHE58, LYS244, ASP242, HIS144, TYR60, LYS244	Middle selectivity
2.	Vitexin	−7,06	0,00668	A: LYS244, TYR60, HIS144, THR146, LYS147	Middle selectivity

3.	Isovitexin	−6,42	0,0198	A: LYS244, TYR60, HIS144, THR146, LYS147	Middle selectivity
4.	Rutin	−5,16	0,16479	A: LYS244, PRO246, ALA245, TYR60, HIS144, SER211, LEU210, LYS147, ASP209, MET208	Low selectivity
5.	Clotrimazole	−5,11	0,17947	A: PRO51, ASP238, LYS52, ALA73, GLN50, ASP238, ARG54, GLN53	Low selectivity
6.	α-arbutin	−5,05	0,19708	A: LYS52, ALA73, PRO51, GLN50, ALA 73, ASP238, ARG53, ARG54	Low selectivity
7.	(+)-catechin	−4,82	0,29324	A: LEU210, LYS147, HIS144, LYS148, TYR60,	Low selectivity
8.	Apigenin	−4,61	0,41778	A: LEU210, LYS147, LYS148, HIS144, THR146, TYR60,	Low selectivity
9.	Ferulic acid	−4,45	0,55163	A: LYS147, LEU210, THR146, HIS144, TYR60	Low selectivity
10.	Gallic acid	−4,43	0,56509	A: LEU210, TYR60, HIS144, LYS147	Low selectivity
11.	Caffeic acid	−4,25	0,77275	A: VAL145, HIS144, TYR60, THR146, LYS147, LYS148	Low selectivity
12.	Hyperozide	−4,24	0,77668	A: ASP238, PRO51, GLN53, GLU341, LYS52, ALA73, GLU341	Low selectivity
13.	Lidocaine hydrochloride	−4,00	1,18	A: ASP238, PRO51, GLN53, GLU341, LYS52, ALA73, GLU341	Low selectivity
14.	Diclofenac sodium	−3,90	1,38	A: TYR50, HIS144, LEU210, VAL145, THR146, LYS147	Low selectivity
15.	Quercetin	−3,61	2,28	A: LYS145, LYS147, LEU210, THR146, THR60, HIS144	Low selectivity
16.	Chlorogenic acid	−3,38	3,32	A: LYS244, TYR60, HIS144, THR146, LYS147	Low selectivity

Notes: ΔGbind – free-binding energy, Ki – concentration inhibited 50% of enzyme activity, green colour – high selective, yellow colour – medium selective, red colour – low selective.

Table 2

Molecular docking of the identified compounds and anti-antioxidant drug standard epigallocatechin-3-O-gallate with the NADPH oxidase, myeloperoxidase, xanthine oxidase

NADPH oxidase					
№	**Ligand**	**Binding energy**	**Ki[b]**	**Binding site**	**Level of selectivity**
		ΔGbind[a] (kcal/mol)	**mmol**		
1.	Hypericin	−11,64	0,00000208	A: PRO694, TRP698, FAD801	High selectivity
2.	Isovitexin	−11,51	0,00000366	A: ARG573, SER425, ARG473, TRP580, ASN572, GLY537, ALA536, TRP695, LEU696, PRO670	High selectivity
3.	Clotrimazole	−11,10	0,00000736	A:PRO460, FAD801, TRP695, PRO694	High Selectivity
4.	Hyperozide	−9,81	0,00006497	A: ARG573, SER425, ARG473, TRP580, ASN572, GLY537, ALA536, TRP695, LEU696, PRO670	High selectivity
5.	Vitexin	−7,71	0,00224	A: ARG573, SER425, ARG473, TRP580, ASN572, GLY537, ALA536, TRP695, LEU696, PRO670	Medium selectivity
6.	(+)-catechin	−7,15	0,00572	A: CYS668, PHE667, PHE693, PRO542, FAD801, THR462, GLU691, TYR445, THR520, PRO520, SER522, PRO521, THR462, FAD801	Medium selectivity
7.	α-arbutin	−6,97	0,0078	A: THR520, PRO542, CYS668, PHE693, ASN692, GLU691, THR520, TRP695, ASN692	Medium selectivity

8.	Apigenin	−6,80	0,01043	A: LYS548, TRP580, PHE581, GLU579, VAL582, LEU584, GLU583	Medium selectivity
9.	Lidocaine hydrochloride	−6,43	0,01934	A: LYS548, TRP580, PHE581, GLU579, VAL582, LEU584, GLU583	Medium selectivity
10.	Rutin	−6,37	0,002154	A: PRO521, PHE693, FAD801, ASN692, TRP695, PRO694, THR520	Medium selectivity
11.	Epigallocatechin-3-O-gallate	−5,97	0,04237	A: PRO521, PHE693, FAD801, ASN692, TRP695, PRO694, THR520	Medium selectivity
12.	Chlorogenic acid	−5,75	0,06077	A: THR520, PRO542, PHE693, THR462, FAD801, ASN692	Low selectivity
13.	Quercetin	−5,51	0,09072	A: TRP580, GLU579, PHE 581, LEU584, LYS548, GLY583, GLY579	Low selectivity
14.	Ferulic acid	−4,99	0,21929	A: LYS548, LEU584, PHE581, TRP580, GLU579, GLU583	Low Selectivity
15.	Gallic acid	−4,86	0,27261	A: GLU579, TRP580, PHE 581, VAL582, GLU583, LEU584, LYS546	Low selectivity
16.	Caffeic acid	−4,85	0,27626	A: GLU579, TRP580, PHE 581, VAL582, GLU583, LEU584, LYS546	Low selectivity
17.	Diclofenac sodium	−4,76	0,3245	A: PHE581, HIS476, TRP580, LEU423, PHE581, LEU584, GLU583	Low selectivity
№	myeloperoxidase				
1.	Hypericin	−9,19	0,0001850	C: LEU420, RHE407, RHE146, GLU116, SER110	High selectivity
2.	Hyperozide	−8,06	0,00123	C: LEU420, RHE407, RHE146, GLU116, SER110	Medium selectivity
3.	Vitexin	−7,78	0,00200	C: LEU420, RHE407, RHE146, GLU116, SER110	Medium selectivity
4.	Rutin	−7,6	0,00269	C: LEU420, RHE407, RHE146, GLU116, ALA421	Medium selectivity
5.	Clotrimazole	−7,59	0,00274	C: LEU420, RHE407, RHE146, GLU116, ALA421, SER110	Medium selectivity
6.	Isovitexin	−6,42	0,00277	C: LEU420, RHE407, RHE146, GLU116	Medium selectivity
7.	α-arbutin	−6,08	0,03515	C: LEU420, RHE407, RHE146, GLU116	Low selectivity
8.	(+)-catechin	−5,57	0,08306	C: LEU420, RHE407, RHE146, GLU116	Low selectivity
9.	Apigenin	−5,39	0,11279	C: LEU420, RHE407, RHE146, GLU116	Low selectivity
10.	Chlorogenic acid	−5,19	0,15696	C: LEU420, RHE407, RHE146, GLU116, ALA220,	Low selectivity
11.	Lidocaine hydrochloride	−4,72	0,34692	C: LEU420, RHE407, RHE146, ALA220, GLU116	Low selectivity
12.	Ferulic acid	−4,65	0,3912	C: LEU420, RHE407, RHE146, GLU116	Low selectivity
13.	Diclofenac sodium	−4,65	0,3873	C: LEU420, RHE407, RHE146, ALA115, GLU116	Low selectivity
14.	Caffeic acid	−4,53	0,48171	C: LEU420, RHE407, RHE146, GLU116, ALA115	Low selectivity
15.	Epigallocatechin-3-O-gallate	−4,52	0,49	C: LEU420, RHE407, RHE146, ALA115, GLU116	Low selectivity
16.	Gallic acid	−4,41	0,58473	C: LEU420, RHE407, RHE146, GLU116	Low selectivity
17.	Quercetin	−3,3	3,79	C: LEU420, RHE407, RHE146, GLU116, ALA115	Low selectivity
№	xanthine oxidase				
1.	Isovitexin	−11,31	0,00000515	A: Arg426, ILE358, FAD606, SER356, ARG394, ILE266, GLU263	High selectivity
2.	Vitexin	−10,82	0,00001172	A: ARG426, ILE358, SER359, FAD606, SER356, ASN351, THR282, GLU263, ILE266	High selectivity

3.	Rutin	−10,43	0,00002253	A: FAD606, GLU263, ARG394, THR354, ILE353, LEU396, THR396, LYS395	High selectivity
4.	Clotrimazole	−9,58	0,00009497	A: GLU263, ARG426, SER356, SER359, ILE358, FAD606, SER359	High selectivity
5.	Hypericin	−9,41	0,00012756	A: SER356, ILE358, FAD606, ARG426, GLU263, ILE266	High selectivity
6.	Hyperozide	−8,61	0,00048606	A: VAL345, VAL259, ALA282, ALA302, CYS303, ASN288, ALA304, SER307	High selectivity
7.	α-arbutin	−7,68	0,00235	A: FAD606, ASN351, THR354, SER356, ARG426, THR262, GLU263	Medium selectivity
8.	(+)-catechin	−7,43	0,0036	A: FAD606, LEU257, LYS256, ALA255, LYS249, GLY399, LEU398, GLU402	Medium selectivity
9.	Epigallocatechin-3-O-gallate	−7,30	0,00445	A: FAD606, LEU257, LYS256, ALA255, LYS249, GLY399, LEU398, GLU402	Medium selectivity
10.	Apigenin	−6,84	0,00968	A: THR262, GLU263, THR354, ASN351, ARG426, THR262, SER356, FAD606	Medium selectivity
11.	Lidocaine hydrochloride	−6,52	0,01666	A: FAD606, THR396, LEU257, LYS256, ALA255, LYS249, PRO400, GLY399, LEU398, THR396	Medium selectivity
12.	Quercetin	−5,8	0,05629	A: FAD606, THR396, LEU257, LYS256, ALA255, LYS249, PRO400, GLY399, LEU398, THR396	Low selectivity
13.	Gallic acid	−4,86	0,27261	A: FAD606, LEU257, LYS256, ALA255, LYS249, THR396, LEU398, ILE353, LYS395	Low selectivity
14.	Chlorogenic acid	−4,7	0,35986	A: SER306, SER307, ALA304, SER344, ASN288	Low selectivity
15.	Diclofenac sodium	−4,17	0,88351	A: FAD606, GLY260, THR262, ILE266, GLY265, ASN261, GLY260	Low selectivity
16.	Caffeic acid	−4,03	1,11	A: ARG426, SER356, ILE358, SER359, FAD606, ASN351	Low selectivity
17.	Ferulic acid	−4,01	1,16	A: LYS343, LYS340, GLU309, SER306, LEU313	Low selectivity

Notes: ΔGbind – free-binding energy, Ki – concentration inhibited 50% of enzyme activity, green colour – high selective, yellow colour – medium selective, red colour – low selective

Inflammation and oxidative stress are always found in many chronic diseases: diabetes mellitus, hypertension, atherosclerosis, Alzheimer's disease, cancer and aging (Biswas, 2016, p. 1–9). During the inflammatory process, neutrophils and macrophages produce a large number of free radicals to kill the invading foreign agent (Fan, 2013, p. 176–185). In the course of studies, it was found that in addition to phagocytic cells, non-phagocytic cells are also capable of producing free radicals. Thus, recent studies have shown that IL-6 produces free radicals due to the expression of NADPH oxidase (Li, 2015, p. 1031–1048). Therefore, the "ideal" anti-inflammatory drug should inhibit not only pro-inflammatory enzymes, but also pro-oxidant ones.

The next step of our research was to sum up obtained data mentioned before. All compounds of anhydrous gel "Hypericum-Derm" were conditionally divided into two categories (Table 5, 6). The first category included compounds that had a high selectivity for the active site, and the second category included compounds that had medium

Table 3

Antioxidant activity of the anhydrous gel "Hypericum-Derm", n = 5 (M±SD)

Drug	Dose, molar concentration	Antioxidant power, mmol-eqv./$m_{dry\ res.}$	Conditional terms of antioxidant activity
anhydrous gel "Hypericum-Derm"	0,02[a]	86,13±1,00[1]	High
Epigallocatechin-3-O-gallate	0,02[a]	20,26±0,10[2]	Lower middle

Notes: M – average number, SD – standard deviation, a – molar concentration calculated in gallic acid, different numbers indicate significant effect at the significance level of $p \leq 0,05$.

and low selectivity. This compound separation approach was necessary to clearly identify compounds that interact highly effectively with proinflammation and prooxidant mechanisms and which compounds work below this level. According to the obtained data shown in Table 5, it can be concluded that no compound is capable of blocking all proinflammatory enzymes, only a combination of compounds has the ability to stop the development of the inflammatory process. Isovitexin, hyperoside, clotrimazole, arbutin, and apigenin are the main compounds that are capable of blocking most of the proinflammatory enzymes, except for the mechanism – NF-kB. In second place are compounds that block 2 of the 4 selected enzymes, namely: vitexin, rutin, hyperecin, catechin. Also, during the molecular docking, it was found that quercetin, caffeic acid, ferulic acid, chlorogenic acid and gallic acid are not presented anywhere as highly selective inhibitors of proinflammatory enzymes. This list of compounds also includes the popular "gold standard" in medicine – sodium diclofenac.

According to the results of the study shown in table 5, it was found that none of the compounds is a highly selective inhibitor of NF-kB. NF-kB is activated by procytokines IL-1 and TNF-α, and is the body's response to the development of chronic diseases such as rheumatoid arthritis, ulcers, asthma, multiple sclerosis (Fan, 2013, 176–185). A wound is an acute inflammatory process, of course, there is a case when it goes into a chronic stage, for example, with diabetes mellitus. In this case, systemic highly selective inhibitors of NF-KB should be used, in acute inflammatory processes this intake of inhibitors is inappropriate. Thus, the composition of the anhydrous gel "Hypericum-Derm" is theoretically capable of inhibiting all key targets of acute inflammation.

Table 6 shows the summarized results of molecular docking of prooxidant enzyme inhibition of the main components of the anhydrous gel "Hypericum-Derm". The study found that hyperecin is the only compound that blocks all key prooxidant enzymes, in second place are isovitexin, hyperoside, which blocks two of the three targets, in third place are rutin, quercetin, lidocaine hydroloride and clotrimazole, which block one of the three mechanisms. All other compounds are not capable of highly selectively inhibiting any prooxidant enzyme, including the popular "gold standards" in medicine and science – sodium diclofenac and epigallocatechin-3-O-gallate.

Therefore, we can conclude that the composition of the non-ionic gel "Hypericum-Derm" not only inhibits all targeted pro-inflammatory enzymes, but also pro-oxidant ones, which indicates theoretical evidence of blocking all important mechanisms of inflammation and oxidative stress development in a wound.

We have evaluated the antioxidant effect of the anhydrous gel "Hypericum-Derm" and the "gold standard" – epigallocatechin-3-O-gallate in one molar concentration of 0,02 mol. Some readers may have a question about the use of molar concentrations, and not "%" or "mg / ml" when comparing antioxidant activity. This is due to the fact that when comparing in one concentration, for example in "%", the compounds under study will have a different number of molecules, since each compound has its own individual molecular weight, and therefore, such a comparison of pharmacological activity will be "incorrect" from our point of view, since one compound will have more molecules, and the other – less.

Based on the results of the study, it was found that the anhydrous gel "Hypericum-Derm" has a higher antioxidant activity than the solution of epigallocatechin-3-O-gal-

Table 4

Anti-inflammatory activity of the anhydrous gel "Hypericum-Derm" on the carrageenan edema model, n = 6 (M±m)

Experimental conditions	Parameter	Dynamics of inflammation development, hours			
		1	2	3	4
Control pathology (CP)	ΔV	0,47±0,05	0,84±0,08	1,10±0,11	1,16±0,12
Polyethyleneglycol base	ΔV	0,21*±0,02	0,32*±0,03	0,35*±0,04	0,23*±0,02
	AA, %	55,0	63,0	68,0	81,0
Diclofenac gel, 5%	ΔV	0,44*/**±0,04	0,78*/**±0,08	0,65*/**±0,07	0,48*/**±0,05
	AA, %	6,38	7,14	41,00	58,0
anhydrous gel "Hypericum-Derm"	ΔV	0,03*/**/#±0,01	0,06*/**/#±0,01	0,01*/**/#±0,01	0,00*/**/#
	AA, %	94,0	93,0	99,0	100,0

Notes: 1* p ≤ 0,05 – the level of statistical significance of the CP group;

2. ** p ≤ 0,05 – reliable values for the preparation: polyethyleneglycol base;

3. # p ≤ 0,05 05 – reliable values of the drug diclofenac gel;

4. AA – anti-inflammatory activity;

5. ΔV – size of the edema;

6. n – number of animals in group.

Table 5

Schematic classification of anti-inflammatory drug standards alongside main compounds of anhydrous gel "Hypericum-Derm" into two categories

№	Compound	COX-2	phospho lipase A2	5-LOX	Nf-kB	Number of closed key enzyme of inflammation
Drug standard						
1.	Diclofenac sodium					0
Compounds of anhydrous gel "Hypericum-Derm"						
1.	Vitexin					2
2.	Isovitexin					3
3.	Hyperoside					3
4.	Rutin					2
5.	Quercetin					0
6.	Hyperecin					2
7.	(±)-catechin					2
8.	Caffeic acid					0
9.	Ferulic acid					0
10.	Chlorogenic acid					1
11.	Lidocaine hydrochloride					1
12.	Clotrimazole					3
13.	α-arbutin					3
14.	Apigenin					3
15.	Gallic acid					0

Notes: green colour – high level of selectivity; red colour – lower and medium of selectivity.

Table 6

Schematic classification of antioxidant drug standards alongside main compounds of anhydrous gel "Hypericum-Derm" into two categories

№	Compound	NADPH oxidase	myeloperoxidase	xanthine oxidase	Number of closed key enzyme of antioxidant protection
Drug standard					
1.	Diclofenac sodium				0
2.	Epigallocatechin-3-O-gallate				0
Compounds of anhydrous gel "Hypericum-Derm"					
1.	Vitexin				1
2.	Isovitexin				2
3.	Hyperoside				2
4.	Rutin				1
5.	Quercetin				1
6.	Hyperecin				3
7.	(±)-catechin				0
8.	Caffeic acid				0
9.	Ferulic acid				0
10.	Chlorogenic acid				0
11.	Lidocaine hydrochloride				1
12.	Clotrimazole				1
13.	α-arbutin				0
14.	Apigenin				0
15.	Gallic acid				0

Notes: green colour – high level of selectivity; red colour – lower and medium of selectivity.

late in the same concentration. In our opinion, this is due to the fact that the anhydrous gel is a complex preparation that contains different groups of phenolic compounds from flavonoids to anthracene derivatives, so the complex of phenolic compounds more actively restores Fe^{3+} ions than epigallocatechin-3-O-gallate alone.

Inflammation is the body's response to an external or internal stimulus. This reaction is quite complex and always goes hand in hand with oxidative stress. To assess the anti-inflammatory activity, we chose the carrageenan model on the rat paw, since this model reveals the main mechanisms of the inflammatory process. According to the canonical pathway of inflammation development on the carrageenan model, it is known that serotonin and histamine are active in the first hour of inflammation, cytokines in the second hour, and prostaglandins, namely COX-2, from the 3rd to the 5th hour. Based on the above results, we can say for sure that the anhydrous gel "Hypericum-Derm" inhibits all inflammation mechanisms, and it was also found that the polyethylene oxide base itself works well at all stages of inflammation.

When comparing theoretical and experimental results, one can say about the comparison of "theories" with "practice", what to say about the importance of the developed concepts of theoretical evaluation of anti-inflammatory and antioxidant activity of new drugs, as well as further development of concepts not only in the creation of external dosage forms, but also for internal systemic administration.

Conclusions. 1. According to theoretical studies, it was shown that neither the "gold standard" sodium diclofenac nor the natural compound are capable of highly selectively inhibiting all key targets of proinflammatory enzymes, but in the case of prooxidant enzymes, one substance was found that highly selectively inhibits all targets – hyperecin.

2. Experimental results showed that the anhydrous gel "Hypericum-Derm" actively inhibits all stages of inflammation on the carrageenan model, and possess a high antioxidant activity.

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