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EVALUATION OF THE ANTI-EXUDATIVE EFFECT OF OINTMENT WITH ONONIS SPINOSA L. EXTRACT IN MODELS OF INFLAMMATION OF VARIOUS ORIGIN

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Experimental studies on the antiexudative activity of Ononis spinosa L. extract were conducted in models of trypsin and zymosan-induced inflammation. To assess the activity of the plant extract, ointments of varying concentrations (1.5 %, 2.5 %, 3.5 %) in terms of polyphenolic compounds were prepared for transdermal application. According to the study's results, the 3.5 % ointment exhibited the most pronounced anti-exudative effect, effectively suppressing the focus of inflammation and contributing to an accelerated reduction in the morphological indicators of the affected limbs in rats. The ointment with Ononis spinosa L. extract did not differ significantly, but was not inferior in effect to the comparison drug - Dolgit cream. The obtained data indicate the feasibility of using Ononis spinosa L. extract in the treatment of inflammatory processes, particularly those of infectious origin. The identified pharmacological activity justifies the need for further preclinical and clinical studies. The results obtained may serve as the basis for the development of effective local anti-inflammatory agents of plant origin.

Key words: antiexudative effect, inflammation, Ononis spinosa L. extract.

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ОЦІНКА АНТИЕКСУДАТИВНОЇ ДІЇ МАЗІ З ЕКСТРАКТОМ ONONIS SPINOSA L. НА МОДЕЛЯХ ЗАПАЛЕННЯ РІЗНОГО ҐЕНЕЗУ

Проведено експериментальні дослідження антиексудативної активності екстракту Ononis spinosa L. на моделях трипсинового та зимозанового запалення. З метою оцінки активності рослинного екстракту були виготовленні мазі різних концентрацій (1,5 %, 2,5 %, 3,5 %) в перерахунку на поліфенольні сполуки для трансдермального нанесення. Згідно результатів дослідження встановлено, що найбільш виражену антиексудативну дію проявляла 3,5 % мазь, яка ефективно пригнічувала осередок запалення та сприяла пришвидшеному зменшенню морфологічних показників уражених кінцівок щурів. Мазь з екстрактом Ononis spinosa L. суттєво не відрізнялась, та не поступалась дії препарату порівняння – Долгіт крему. Отримані дані свідчать про доцільність використання екстракту Ononis spinosa L. у терапії запальних процесів, зокрема інфекційно-опосередкованого ґенезу. Виявлена фармакологічна активність обґрунтовує потребу в подальших доклінічних і клінічних дослідженнях. Отримані результати можуть стати основою для створення ефективних місцевих протизапальних засобів рослинного походження.

Ключові слова: антиексудативна дія, запалення, екстракт Ononis spinosa L.

The study is a fragment of the research project "Pharmacological correction of simulated pathological conditions through the use of developed drugs", state registration No. 0122U200545.

Inflammatory processes are among the most common pathological conditions that accompany both acute and chronic diseases, affecting various organs and body systems. They arise in response to tissue damage, exposure to toxic substances, infectious agents, or immunological disorders, performing a primarily protective function. However, in cases of prolonged or excessive inflammation, the development of severe complications and secondary pathologies is possible.

In Ukraine, the primary means of treating inflammation remains synthetic pharmacological drugs, in particular non-steroidal anti-inflammatory drugs (NSAIDs), the effectiveness of which, however, is often

accompanied by numerous adverse reactions, especially with prolonged use. In this regard, a pressing task for modern pharmacology and pharmacy is the development of new, safe drugs of natural origin, particularly herbal preparations, which are characterized by lower toxicity, good tolerability, and a comprehensive therapeutic effect.

One of the promising sources of such remedies is the medicinal plant Ononis spinosa L. (spiny wolfberry), a member of the Fabaceae family, which has been widely used in folk medicine across many European countries [5, 7, 9–11].

Ononis spinosa L. is a promising object of phytochemical and pharmacological research due to its multi-vector therapeutic action and rich phytochemical composition. The plant contains a whole complex of biologically active compounds [5, 6, 9, 10]: flavonoids (quercetin, kaempferol), isoflavonoids (formononetin, onogenin), triterpenes (α-onocerin), phenolic acids (caffeic, ferulic), essential oils, and tannins, etc. Due to this combination of compounds, the plant exhibits anti-inflammatory, analgesic, antioxidant, diuretic, and antispasmodic activity [4, 7, 11]. Expanding the scientific base on this plant will contribute to its introduction into modern pharmaceutical practice as a source of safe and effective medicines.

The purpose of the study was to investigate the anti-exudative activity of ointments with varying concentrations of Ononis spinosa L. extract in various inflammation models.

Materials and methods. Experimental studies were conducted on sexually mature, non-linear, white rats maintained on a standard vivarium diet with free access to food and water. The Bioethics Commission of the I.I. Mechnikov ONU has established that the scientific research conducted on experimental animals meets the ethical requirements as per the order of the Ministry of Health of Ukraine No. 231 dated November 1, 2005. The studies were carried out by the principles of the Declaration of Helsinki, adopted by the General Assembly of the World Medical Association (2000), the Council of Europe Convention on Human Rights and Biomedicine (1997), the relevant provisions of the WHO, the International Council of Medical Scientific Societies, the International Code of Medical Ethics (1983), the "General Ethical Principles of Experiments on Animals", approved by the First National Congress on Bioethics (Kyiv, 2001) under the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Educational Purposes" (Strasbourg, 18.03.1986) [3].

The object of the study was Ononis spinosa L., which grew in the botanical garden of the I.I. Mechnikov ONU. The plant raw materials were extracted with a 70 % water-ethanol mixture at a raw material to solvent ratio of 1:8 in a Soxhlet apparatus (for 4 hours). For screening studies of the antiexudative properties of the plant extract, a series of ointments of different concentrations (0.5, 1.5, 2.5, 3.5 %) were created in terms of the sum of polyphenolic compounds. Polyethylene glycol (PEG) 1500, polyethylene oxide (PEO) 400, and 1,2-propylene glycol (PG), in a ratio of 4:2:3, were chosen as the ointment base.

Antiexudative activity was studied in zymosan and trypsin inflammation models. The inflammatory reaction was induced by subplantar injection of phlogogens under the plantar aponeurosis of the hind limb of the animals: 0.1 ml of 0.5 % aqueous trypsin solution for the trypsin model and 0.1 ml of 2 % zymosan solution to reproduce the zymosan model. A 5 % ibuprofen ointment – Dolgit cream (Manufacturer: DOLORGIT GmbH & Co.KG, Germany) was chosen as the comparison drug [1, 8].

For each inflammation model, 6 groups of animals, 10 individuals each, were created:

1. group of animals – control (without baseline treatment);
2. received applications of ointment base;
3. group of animals – received applications of 1.5 % ointment with Ononis spinosa L. extract.
4. group of animals – 2.5 % ointment with Ononis spinosa L. extract.
5. group of animals – 3.5 % ointment with Ononis spinosa L. extract.
6. group of animals – received transdermal applications of the comparison drug 5 % ibuprofen ointment (Dolgit-cream).

The dynamics of changes in the inflammatory process were assessed before the administration of the phlogogen and 1, 3, 6, 9, and 24 hours after the administration of the phlogogenic agent by measuring the volume and thickness of the affected limb. Ointments with Ononis spinosa L. extract were applied immediately after phlogogen administration and after each measurement at a dose of 100 mg/cm^2 of the surface of the affected limbs. Volume gain and antiexudative activity were calculated for all animal groups [1].

All obtained data were processed using generally accepted statistical analysis methods in biomedical research using standard computer software packages. Mathematical processing included calculations of arithmetic mean values (M) and their errors (±m). The significance of group differences in

the pain threshold was determined using the parametric Student's t-test, the Wilcoxon Rank-Sum test, the Mann-Whitney test, and the one-way analysis of variance (ANOVA) method. Differences were considered statistically significant at a level of $p \leq 0.05$. Before applying parametric criteria, the hypothesis of a normal distribution of random variables was tested [2].

Results of the study and their discussion. The results of the study showed that subplantar administration of trypsin phlogogen caused the development of inflammation in all experimental groups, with peak values of tissue volume and thickness increase at the third hour after induction (Table 1, Fig. 1).

The most intense and prolonged inflammatory process was observed in the control group and in animals treated with the ointment base and 1.5 % ointment containing Ononis spinosa L. extract, as confirmed by similar morphological characteristics of the affected tissues.

Among the studied concentrations of ointments based on Ononis spinosa L. extract, the best antiexudative effect was observed with the highest concentration of the ointment. Namely, 3.5 % ointment, which, starting from the 6th hour of the experiment, contributed to a decrease in the volume of the affected limbs of the animals and subsequently contributed to their return to the limits of the physiological norm. The results of the antiexudative activity of the 3.5 % ointment based on Ononis spinosa L. extract (74.6 %) at 24 hours of the experiment were similar to those of the reference preparation (76.3 %), confirming the high effectiveness of the selected ointment sample.

Table 1

Antiexudative activity of ointments with Ononis spinosa L. extract in a trypsin inflammation model

No.	Group		Observation time				
			1	3	6	9	24
Volume increment, in % of initial values							
1	Control		36.8±3.1	44.6±4.2	42.8±4.7	39.3±3.1	32.7±3.5
2	Ointment base		37.4±3.4	44.9±3.6	43.2±4.1	41.4±3.9	34.6±3.3
3	Ointments with Ononis spinosa L. extract	1.5 % ointment	37.8±3.2	45.2±4.2	42.7±3.9	40.3±3.7	33.5±3.4
4		2.5 % ointment	38.1±3.5	43.4±4.8	39.7±3.6	36.3±3.4	27.2±2.9
5		3.5 % ointment	37.9±3.3	42.5±4.1	37.4±3.4	31.3±3.4	11.6±1.7*
6	Dolgit cream		38.4±3.5	42.6±4.6	36.8±3.7	29.6±2.3*	9.8±0.7*
Antiexudative activity in % relative to the control							
3	Ointments with Ononis spinosa L. extract.	1.5 % ointment	-	-	-	-	-
4		2.5 % ointment	-	-	1.4±0.3	2.2±0.2	2.5±0.3
5		3.5 % ointment	-	1.2±0.2	13.1±1.4	17.6±1.3*	74.6±4.2*
7	Dolgit cream		-	1.2±0.1	14.7±1.5	18.5±1.4*	76.3±3.9*

Note: * – statistically significant difference compared to the control group ($p \leq 0.05$).

Similar dynamics were observed when measuring the thickness of the affected limbs of animals. Among all the studied samples of ointments based on Ononis spinosa L. extract, the 3.5 % ointment demonstrated the most pronounced anti-inflammatory effect (Fig. 1).

The use of 3.5 % ointment, as well as the comparator drug, administered immediately after the phlogogenic agent, resulted in a reduction in the inflammatory reaction as early as the third hour of the study. Later, at the 6th, 9th, and 24th hours of the experiment, the animals showed a gradual decrease in the thickness of the inflamed areas, indicating suppression of the exudative process. Statistical analysis confirmed the significance of these changes compared to the control group ($p \leq 0.05$).

On the other hand, in the groups where the ointment base was applied, as well as in the groups treated with 1.5 % and 2.5 % ointments, changes in the morphometric parameters of the limbs did not differ significantly from the control values, indicating the absence of a significant effect on the course of the inflammatory process.

The study of the effect of antiexudative activity on the zymosan inflammation model showed that the maximum increase in morphological indicators of the affected limbs of animals was observed in the first hour of the experiment and contributed to an increase in limb volume by an average of 65 % compared to the initial values (Table 2).

Starting from the 3rd hour of the study and during subsequent observation time points, a gradual decrease in the intensity of the inflammatory process was observed in all experimental groups. However, in the control group, as well as in animals treated with the ointment base and 1.5 % ointment containing Ononis spinosa L. extract, the dynamics of inflammation reduction were similar and exhibited inflated morphometric indicators, indicating a lack of significant therapeutic efficacy compared to other groups.

In groups 5 and 6 of animals, the most pronounced anti-exudative activity was observed, and the applied preparations demonstrated a gradual decrease in the increase in the volume of the inflammatory

focus. At 9 and 24 hours of observation, the antiexudative activity of 3.5 % ointment was 57.6 % and 41.6 %, while that of the comparator was 36.8 % and 53.1 % ($p \leq 0.05$), respectively. The obtained data indicate statistically significant effectiveness of both agents (Table 2).

It should be noted that at the end of the experiment, Dolgit cream demonstrated slightly higher activity, i.e., it was more effective at the later stages of the experiment, while the ointment based on Ononis spinosa L. extract showed a more pronounced effect at the beginning of the study, namely at 6 and 9 hours (Table 2, Fig. 2).

Table 2

Antiexudative activity of ointments with Ononis spinosa L. extract in a zymosan inflammation model

No.	Group		Observation time				
			1	3	6	9	24
Volume increment, in % of initial values							
1	Control		64.7±5.2	60.6±5.7	52.4±5.9	46.8±3.7	41.2±4.2
2	Ointment base		65.2±5.4	59.7±4.2	53.6±4.7	45.9±3.3	40.8±3.5
3	Ointments with Ononis spinosa L. extract	1.5 % ointment	66.4±6.1	62.9±5.7	53.4±5.0	46.3±4.1	41.6±3.9
4		2.5 % ointment	65.7±4.3	57.3±4.2	39.6±4.2	29.7±2.6*	32.4±3.6
5		3.5 % ointment	66.3±5.3	50.7±5.8	28.7±2.5*	20.6±2.7	15.0±1.1*
6	Dolgit cream		64.2±4.6	53.9±4.3	30.1±3.8*	23.7±2.1*	8.4±0.6*
Antiexudative activity in % relative to the control							
3	Ointments with Ononis spinosa L. extract.	1.5 % ointment	-	-	-	-	-
4		2.5 % ointment	-	-	10.6±1.2	22.8±1.4	9.4±1.1
5		3.5 % ointment	-	16.3±1.2	33.4±3.7	57.6±4.3*	41.6±3.9*
7	Dolgit cream		-	22.2±2.5	35.2±3.4	36.8±3.1*	53.1±4.7*

Note: * – statistically significant difference compared to the control group ($p \leq 0.05$).

Assessment of antiexudative activity based on the thickness of affected limbs in rats in the zymosan inflammation model showed that a significant reduction in inflammation ($p \leq 0.05$) was observed in the groups where 3.5 % ointment based on Ononis spinosa L. extract and Dolgit cream were used. A decrease in the thickness of affected limbs was observed already at the 9th hour of the experiment. This effect persisted for up to 24 hours, indicating a prolonged impact of the products.

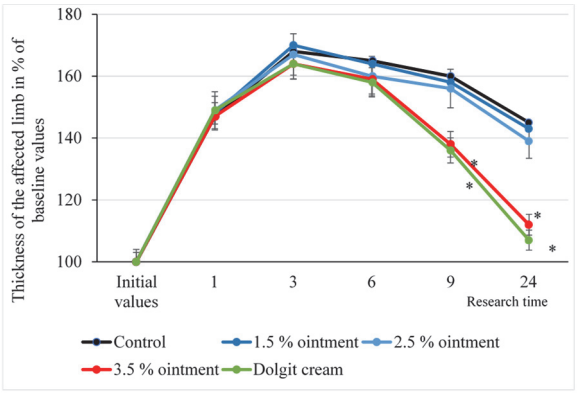

Fig. 1. Thickness indicators of affected limbs (in %) to baseline values in the trypsin inflammation model.

Note: * – statistically significant difference compared to the control group ($p \leq 0.05$).

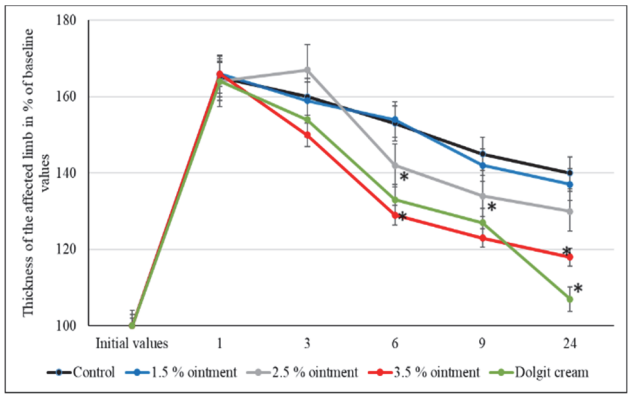

Fig. 2. Thickness indicators of affected limbs (in %) to baseline values in the zymosan inflammation model.

Note: * – statistically significant difference compared to the control group ($p \leq 0.05$).

Trypsin-induced inflammation is characterized by a serous-exudative response, accompanied by increased vascular permeability, the activation of mediators (histamine, serotonin, bradykinin, and prostaglandins), and the migration of neutrophils. The pronounced effect of 3.5 % ointment with Ononis spinosa L. extract in this model may be due to the ability of its biologically active components to inhibit cyclooxygenase-2 activity, reduce the secretion and effects of pro-inflammatory compounds, and provide an anti-exudative local impact. This is the key to treating acute inflammatory processes with a predominance of exudation.

Zymosan-induced inflammation reflects an immune-dependent acute reaction driven by the activation of macrophages, neutrophils, and other immunocompetent cells under the influence of β-glucan. This is accompanied by the release of cytokines (TNF-α, IL-1β, IL-6), nitric oxide, and prostaglandins. The marked reduction in exudation under the influence of 3.5 % ointment may indicate a potential immunomodulatory or immunosuppressive activity of the extract, targeting the early stages of the

inflammatory response. This effect demonstrates its ability to influence cytokine-dependent mechanisms characteristic of systemic and infection-mediated forms of inflammation.

Thus, the study found that 3.5 % ointment with Ononis spinosa L. extract exhibits pronounced antiexudative activity in models of trypsin and zymosan inflammation, with reductions in the volume of affected tissues of 74.6 % and 57.6 %, respectively, which is comparable to the action of the comparator drug - Dolgit cream. Similar results were obtained in a study by Spiegler et al. [9], where isoflavonoids and triterpenes of O. spinosa reduced the production of pro-inflammatory cytokines (IL-8, TNF-α) and neutrophil activity. Studies by Ergene Öz et al. [5] also confirmed the anti-inflammatory effect of the extract when used in topical preparations, reducing vascular permeability and edema. An essential role in the implementation of the pharmacological effect is played by α-onokerin and formononetin, which have COX-2-inhibitory properties [7]. In the zymosan inflammation model, the efficacy of the ointment may be related to the immunomodulatory effects of the extract components, which affect the NF-κB and MAPK signaling pathways [11]. Thus, the results of the study are consistent with current scientific data on the anti-inflammatory potential of Ononis spinosa L. and confirm the feasibility of further studying it as a promising phytocomponent in local anti-inflammatory agents.

Conclusions

1. Experimental study of ointments based on Ononis spinosa L. of different concentrations showed that the highest antiexudative activity was exhibited by 3.5 % ointment in terms of polyphenolic compounds under conditions of transdermal application.

2. It was found that 3.5 % ointment significantly ($p \leq 0.05$) reduced exudation in conditions of trypsin- and zymosan-induced inflammation, which is essential in the treatment of acute and infection-dependent inflammatory processes.

3. The study's results, which demonstrate the pronounced antiexudative effect of Ononis spinosa L. extract, confirm the feasibility of further investigation in preclinical and clinical conditions to expand its therapeutic indications.

Prospects for further research include continuing studies on Ononis spinosa L. extract and an in-depth examination of the mechanisms underlying its anti-inflammatory action, particularly its impact on various aspects of inflammation. It is also advisable to conduct preclinical and clinical trials to assess the effectiveness and safety of the drug in various forms of inflammatory diseases accompanied by exudation.

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