DETERMINATION OF THE DYNAMICS OF EXTRACTION OF BAS FROM THE «OPRONOPHYT» COLLECTION AND ITS PHARMACOLOGICAL ACTIVITY

Tetiana Oproshanska[1], Olha Khvorost[1], Oleksandr Kukhtenko[1], Natalia Voloshchuk [2], Kateryna Screbtsova[1]

1 - National University of Pharmacy, Ukraine
2 - National Pirogov Memorial Medical University, Vinnytsya

Introduction. Phytomedicines are one of the components in the complex therapy of various diseases as they exhibit a wide range of pharmacological activity with significantly fewer side effects. Also important role plays the availability of plant raw materials and the range of dosage forms that can be obtained using various technological processes.

We paid attention to phytomedicines which are used in the complex therapy of inflammatory processes of the musculoskeletal system, because these diseases take the 3rd place in the world as a most common and the second as a factor in permanent disability [1] and are accompanied by severe pain [2-4]. Having analyzed the treatment protocols for musculoskeletal diseases, we pay attention to the fact that the basis is drug therapy which, depending on the disease, includes analgesics, nonsteroidal anti-inflammatory drugs, antirheumatic drugs, muscle relaxants and chondroprotectors [5-8]. Using of phytomedicines is not provided for in the treatment protocols, but despite this, an analysis of the pharmaceutical market for agents used in diseases of the musculoskeletal system indicates a wide range of soft and liquid forms based on extracts from plant row materials, plant preparations, plant teas, which are used in adjuvant therapy to reduce inflammation, swelling and pain [9].

In previous studies, we developed the composition of the original plant collection «Opornofit» with predicted analgesic, anti-inflammatory and anti-exudative activity and the technology for obtaining a liquid extract based on it [10]. This technology involves the use of the maceration method at room temperature for 48 h, the ratio of plant raw material to liquid extract is 1:1, the extractant is 50% ethanol. This method is simple, easily reproducible in any conditions, and does not require significant costs. In industrial production, the rational use of equipment with a reduction in time spent on the production itself is of great importance. Today, the appropriateness of using more advanced methods is often studied, such as: process intensification through the use of dynamic conditions [11-12], ultrasonic extraction, which significantly shortens the process time [13], and filtration extraction [14]. Also, the content of phenolic compounds is used as parameters that prove the appropriateness of the process [15].

Therefore, taking this into account, it is advisable to conduct research on the application of the filtration extraction method to obtain extracts from the developed original plant collection «Opornofit», which makes it possible to speed up the extraction process with simultaneous maximum extraction of biologically active substances from the plant raw materials. In addition, it is appropriate to confirm the types of pharmacological activity predicted by us by determining some of their aspects.

The aim is to establish the dynamics of the extraction of polyphenols and hydroxycinnamic acids from the original plant collection «Opornofit» by the filtration extraction method and to study some aspects of its pharmacological activity.

Materials and methods. The plant collection «Opornofit» was used to study. The plant raw materials for which were harvested in the Kharkiv and Vinnytsia regions during the corresponding growing seasons in 2024. According to the design of this study, the extraction of the plant collection «Opornofit» was carried out by filtration extraction in a laboratory extractor to a total ratio of plant raw material:extract (DER) of 1:5 [16]. The dry residue and the quantitative content of total polyphenols and total hydroxycinnamic acids were determined in the obtained samples [17]. Extraction was carried out with 50% ethanol at room temperature.

To study the analgesic and antiexudative activity of the liquid extract «Opornofit» 28 male Wistar rats were used, which were randomly divided into 4 groups of 7 animals. The study of the liquid extract at a dose of 100 mg/ml was carried out on the adjuvant arthritis (AA) model, which is an experimental model of rheumatoid arthritis in humans and is caused by the administration of Freund's complete adjuvant. The comparison solution was diclofenac sodium at a dose of 8 mg/ml [18].

The degree of nociception and analgesic effect were determined using a dolorimeter (Dolorimeter Baseline, USA) by assessing the pain threshold (PT) on days 1, 14 and 21. The inflammatory reaction of the foot and anti-inflammatory effect were determined by measuring the volume of the affected limb using a plethysmometer (Ugo Basile, Italy) at similar study times.

Pharmacological studies were performed on the basis of the certified «Research Laboratory for Preclinical Study of Medicinal Products of the Department of Pharmacology» of National Pirogov Memorial Medical University, Vinnytsya (certificate of technical competence № 171/23 dated 12.6.2023, valid until 12.5.2028).

During the experimental studies, the standards of current legislation and international recommendations on conducting biomedical research using animals were observed («European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1986, as amended, 1998) and the Law of Ukraine № 3447-IV of February 21, 2006, as amended, «On the Protection of Animals from Cruelty»).

Results and discussion. It was obtained 10 drains of 50 ml from the plant collection «Opornofit» by the method of filtration extraction. In each drain, the dry residue and the quantitative content of total polyphenols and total

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hydroxycinnamic acids were determined. The results are given in Table 1.

As can be seen from the data in Table 1, with an increase in the ratio of plant raw material:extractant in drains 1-6, a gradual decrease in the dry residue, the quantitative content of total polyphenols and total hydroxycinnamic acids is observed. In drain 7, the dry residue and the content of biologically active substances are somewhat higher than in drain 6. In drains 9 and 10, the dry residue becomes stable, while it is observed a slight increase total polyphenols in drain 10 compared to drain 9. Therefore, given the amount of dry residue, the content of total polyphenols and total hydroxycinnamic acids, effective and rational extraction occurs up to drain 5 inclusive.

The second stage of the study was the determination of the analgesic and anti-inflammatory activity of the liquid extract «Opornofit» within the framework of the preclinical study of the original plant medicine. The results are given in Table 2 and 3.

Table 1. Dynamics of extraction of a number of compounds from the collection «Opornofit»

Drain	ω_n – dry residue in a separate portion of the extract V_n, %	The content of total polyphenols in a single portion of the extract, V_n, %	The content of total hydroxycinnamic acids in a single portion of the extract V_n, %
1	6,55	2,04	1,24
2	3,69	1,77	1,04
3	2,87	1,51	0,89
4	2,49	1,23	0,87
5	2,14	1,12	0,88
6	1,35	1,04	0,62
7	1,49	1,06	0,64
8	0,87	0,41	0,28
9	0,79	0,39	0,21
10	0,79	0,41	0,19

Table 2. Analgesic activity of the liquid extract «Opornofit» in comparison with diclofenac sodium under the conditions of their intragastric administration in the model of adjuvant arthritis in rats (M±m)

Groups, n=7	PT, г/мм^2			Action, %
	1st day	14th day	21st day	
Control	420.0±8.16	417.1±4.21	421.4±8.29	-
AA without treatment	410.0±6.17	292.8±4.21*# -28.5 %	321.4±10.33*#-21.6 %	-
AA + liquid extract «Opornofit» (100 mg/kg)	418.6±8.57	340.0±4.88*# -18.7 %	371.43±22.7 -11.3 %	46.8
AA + diclofenac sodium (8 mg/kg)	400.0±9.26	358.6±7.69* -10.3 %	368.7±8.00 -7.82 %	64.5

Notes: * – statistically significant differences ($p<0.05$) compared to 1 day in the corresponding group;
– statistically significant differences ($p<0.05$) compared to diclofenac sodium in the same study period.

Table 3. Antiexudative activity of the liquid extract «Opornofit» in comparison with diclofenac sodium under conditions of their intragastric administration in the model of adjuvant arthritis in rats (M±m)

Groups, n=7	Volume of the affected foot, mm^3			Action, %
	1st day	14th day	21st day	
Контроль	539.2±12.6	540.5±13.0	539.6±12.5	-
Control	515.5±10.5	729.4±6.40*# + 41.7 %	695.9±13.9*#+ 35.0 %	-
AA without treatment	502.2±3.65	587.4±11.4*# +16.9 %	576.2±5.48* +14.8%	59.0
AA + liquid extract «Opornofit» (100 mg/kg)	504.6±5.30	609.5±9.61* +20.8 %	567.1±11.1* +12.4 %	65.4

Notes: * – statistically significant differences ($p<0.05$) compared to 1 day in the corresponding group;# – statistically significant differences ($p<0.05$) compared to diclofenac sodium in the same study period.

Two-week treatment of rats with the liquid extract, similar to diclofenac sodium, significantly reduced the manifestations of hyperalgesia and other signs of inflammatory reaction, as evidenced by a statistically significant increase in PBC in group 3 compared to the negative control. At the same time, on days 14 and 21, against the background of the introduction of the liquid extract, the drop in PT was 18.7 % and 11.3 %, respectively ($p<0.05$), which was 55 % and 69 % higher than with the

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introduction of the reference solution of diclofenac sodium (the drop in PT was 10.3 % and 7.82 %, respectively).

Prophylactic and therapeutic administration of the studied liquid extract significantly inhibited the manifestations of the inflammatory reaction, which is confirmed by the dynamics of changes in the volume of the injured rat foot. Thus, on the 14th and 21st day of administration of the liquid extract, the elevation of the studied indicator was 16.9 % and 14.8 %, respectively, compared with the initial value. At the same time, in the group of animals receiving diclofenac sodium, a decrease in the volume of the injured foot was observed by 20.8 % on the 14th day and 12.4 % on the 21st day.

Thus, the liquid extract «Opornofit» in the model of adjuvant arthritis showed significantly higher analgesic activity on the 14th and 21st day of use than the reference drug diclofenac sodium. At the same time, its antiexudative activity was slightly inferior to diclofenac sodium on the 14th day of use and was higher on the 21st. Thus, it can be noted that the analgesic and antiexudative activity of the liquid extract «Opornofit» reaches its maximum peak in the second and third weeks of treatment, which corresponds to the acute and peak phase of inflammation. At the same time, the reference drug diclofenac sodium is not recommended for use for such a long period due to the development of severe adverse reactions, which are absent with prolonged use of the liquid extract.

Conclusions.
For the first time, the dynamics of the extraction of total polyphenols and total hydroxycinnamic acids from the original plant collection «Opornofit» was studied using the filtration extraction method. For the first time, the previously predicted analgesic and anti-inflammatory activity was confirmed for a liquid extract based on the collection «Opornofit». It was found that in the model of adjuvant arthritis, the liquid extract exhibits higher analgesic activity on the 14th and 21st day of use and anti-exudative activity on the 21st day than the reference drug diclofenac sodium. These studies became another step in the process of introducing the new original plant collection «Opornofit» and a number of phytomedicines based on it into the modern practice of the domestic healthcare system.

Conflict of interest. Missing.

Determination of the dynamics of extraction of BAS from the «Opronophyt» collection and its pharmacological activity

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We paid attention to phytomedicines which are used in the complex therapy of inflammatory processes of the musculoskeletal system, because these diseases take the 3rd place in the world as a most common and the second as a factor in permanent disability and are accompanied by severe pain. **The aim** is to establish the dynamics of the extraction of polyphenols and hydroxycinnamic acids from the original plant collection «Opornofit» by the filtration extraction method and to study some aspects of its pharmacological activity. **Materials and methods.** The plant collection «Opornofit» was used to study. The plant raw materials for which were harvested in the Kharkiv and Vinnytsia regions. The extraction of the plant collection «Opornofit» was carried out by filtration extraction in a laboratory extractor to a total ratio of plant raw material:extract (DER) of 1:5. The dry residue and the quantitative content of total polyphenols and total hydroxycinnamic acids were determined in the obtained samples. Extraction was carried out with 50% ethanol at room temperature. To study the analgesic and antiexudative activity of the liquid extract «Opornofit» 28 male Wistar rats were used, which were randomly divided into 4 groups of 7 animals. The study of the liquid extract at a dose of 100 mg/ml was carried out on the adjuvant arthritis (AA) model, which is an experimental model of rheumatoid arthritis in humans and is caused by the administration of Freund's complete adjuvant. The comparison solution was diclofenac sodium at a dose of 8 mg/ml . **Results and discussion.** It was obtained 10 drains of 50 ml from the plant collection «Opornofit» by the method of filtration extraction. In each drain, the dry residue and the quantitative content of total polyphenols and total hydroxycinnamic acids were determined. Given the amount of dry residue, the content of total polyphenols and total hydroxycinnamic acids, effective and rational extraction occurs up to drain 5 inclusive. The liquid extract «Opornofit» in the model of adjuvant arthritis showed significantly higher analgesic activity on the 14th and 21st day of use than the reference drug diclofenac sodium. At the same time, its antiexudative activity was slightly inferior to diclofenac sodium on the 14th day of use and was higher on the 21st. Thus, it can be noted that the analgesic and antiexudative activity of the liquid extract «Opornofit» reaches its maximum peak in the second and third weeks of treatment, which corresponds to the acute and peak phase of inflammation. At the same time, the reference drug diclofenac sodium is not recommended for use for such a long period due to the development of severe adverse reactions, which are absent with prolonged use of the liquid extract.
Conclusions. For the first time, the dynamics of the extraction of total polyphenols and total hydroxycinnamic acids from the original plant collection «Opornofit» was studied using the filtration extraction method. For the first time, the previously predicted analgesic and anti-inflammatory activity was confirmed for a liquid extract based on the collection «Opornofit». It was found that in the model of adjuvant arthritis, the liquid extract exhibits higher analgesic activity on the 14th and 21st day of use and anti-exudative activity on the 21st day than the reference drug diclofenac sodium. These studies became another step in the process of introducing the new original plant collection «Opornofit» and a number of phytomedicines based on it into the modern practice of the domestic healthcare system.

Keywords: filtration extraction method, liquid extract, analgesic activity, anti-exudative activity

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