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## The experimental study of the anti-inflammatory properties of combined Aerosols with the propolis phenolic hydrophobic drug

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### Abstract

The article presents the results of the experimental study of the anti-inflammatory properties of model samples of combined aerosol drugs with the cooling action, which contain the propolis phenolic hydrophobic drug (PPHD) and local anesthetics. The samples studied are recommended to use in order to prevent and treat acute injuries of muscles and joints exposed to increased physical activity mainly in sports medicine.

During the research it has been found that the aerosol containing 10% solution of PPHD in propylene glycol and 3% lidocaine hydrochloride shows the highest level of the anti-inflammatory activity (51.8 %). Therefore, the sample of this formulation is the most promising for further research with the purpose of creating a drug with the effective anti-inflammatory and local anesthetic action on its basis. In addition, the results obtained allow to conclude that it is the complex of biologically active substances of the propolis phenolic hydrophobic drug that contributes significantly to the final anti-inflammatory activity of the samples studied.

**Keywords:** aerosol, propolis phenolic hydrophobic drug, local anesthetics, anti-inflammatory action, sports medicine.

### 1. Introduction

Currently physical education and sports are an integral part of modern society. Undoubtedly, top records sport with its maximum physical and psychoemotional loads requires new adaptation levels from the human body, but their achievement is often extremely difficult without outside interference, and sometimes it is practically impossible<sup>[1]</sup>.

This aspect determines the role of sports medicine in planning the load for athletes, its limits, monitoring for the influence of physical exercises on the body, as well as prevention of possible negative impacts. Taking into account increasing loads and intensity of the exercise there is quite often a risk of injury in professional sports; in turn, it leads to the loss of training days, competition form, and in some cases to leaving sports at all<sup>[2, 3]</sup>. Hence the key tasks in sport medicine are prevention of sports injuries, their timely diagnosis and urgent treatment<sup>[4, 5]</sup>.

Injuries of large joints and the muscular tissue of organs of the locomotor apparatus are high on the list of all the variety of injuries occurring in top records sport, including athletic and strength sports<sup>[6]</sup>. Among the most common sports injuries there are often ruptures and inflammations (meniscal tear), dislocations and fractures (shoulder fracture, wrist fracture), hand injuries (dislocations of fingers), shank injuries, spinal injuries, knee injuries, etc. Therefore, pharmacotherapy of sports injuries should be mainly aimed at minimizing the inflammatory phase of the injured body area in order to accelerate the overall process of healing as quickly and effectively as possible<sup>[7]</sup>.

In view of the aforesaid, development of effective agents with the cooling, anti-inflammatory and analgesic action in order to use them in sports medicine is of scientific interest. For this purpose the experimental samples of combined aerosol drugs containing the propolis phenolic hydrophobic drug (PPHD), local anesthetics and coolants (a mixture of freons) have been developed and proposed for preclinical studies. The present paper is devoted to the study of the anti-inflammatory properties of the given pharmaceutical compositions with the purpose of substantiation of the most rational formulation and technology for the aerosol drug possessing a high pharmacological activity and a low toxicity.

## 2. Materials and Methods

A comparative study of the anti-inflammatory properties of the compositions under research were conducted in 70 white outbred rats of both genders with the body weight of 180.0-200.0 g. According to the standard health and safety regulations the experimental animals were kept on the appropriate food diet in the vivarium at the Central Research Laboratory (CRL) of the National University of Pharmacy certified by the State Enterprise "Centre for Drug Evaluation and Research at the Ministry of Public Health of Ukraine" as a base for research in experimental pharmacology [8]. The studies were conducted in accordance with EC Directive 86/609 EEC from November, 24, 1986 on compliance with laws, acts and regulations of the EU countries as to protection of animals used for experimental and other scientific purpose [9, 10].

As the study subjects the experimental samples of combined drugs in aerosol dosage forms (aerosol 1, 2, 3 and 4) were used; they contained various substances with the anti-inflammatory, analgesic and cooling action, including 10% solution of PPHD in propylene glycol, local anesthetics (lidocaine or articaine), as well as a mixture of freons as coolants. The formulations of all drugs studied are given in Table.

As reference drugs the medicine Proposal manufactured by "Zdorovya" Pharmaceutical company, Ltd. (Kharkiv, Ukraine), as well as the aerosol containing a mixture of freons without other active pharmaceutical ingredients were used.

In the course of the experiment all experimental animals were divided into 7 groups, there were 10 rats in each group. The groups were as follows:

Group 1 – control pathology;

Group 2 – rats with the paw edema receiving aerosol 1 as skin application;

Group 3 – rats with the paw edema receiving aerosol 2 as skin application;

Group 4 – rats with the paw edema receiving aerosol 3 as skin application;

Group 5 – rats with the paw edema receiving aerosol 4 as skin application;

Group 6 – rats with the paw edema receiving the reference drug Proposal as skin application;

Group 7 – rats with the paw edema receiving the mixture of freons as skin application.

Previously all animals (excluding the control group) were applied the test samples of aerosols as a single cutaneous dose on the rear right paw in the part of the limb from the beginning of hair-covering, including the ankle joint and below, in the conditionally therapeutic dose of 20 mg. Aerosols were applied with a tampon, preliminary placing the contents of the vial in a glass container under conditions of thorough rubbing-in and prevention of their licking by the animals from the surface of the skin at least for 15 min.

In an hour after aerosol application the aseptic exudative inflammation of the rear right paw was modeled in all the animals by subplantar introduction of 0.1 ml of 1 % solution of

$\lambda$ -carrageenan (Fluka, Switzerland) [11]. The size of edema was measured in 3 h after pathology modeling, it corresponded to 4 h after the application of the aerosols studied with the help of digital plethysmometer (IITC Life Science, USA) [12, 13]. Then the anti-inflammatory activity (AA) was calculated by degree of the edema decrease in animals receiving aerosols under research compared to the control pathology group:

$$AA = \frac{\Delta V_c - \Delta V_0}{\Delta V_c} \times 100\%$$

Where,

$\Delta V_c$  – is the average percentage of the paw edema in the control pathology group;

$\Delta V_0$  – is the percentage of the paw edema for each animal in the experimental group under the action of the aerosol studied.

The values of the anti-inflammatory activity obtained were subjected to statistical processing by standard methods of variation statistics with the computer programs, as well as using Student-Fischer test [14]; they were presented in the form of comparative tables with the results of different groups.

## 3. Results and Discussion

The main element of the anti-inflammatory action of medicines is their influence on the exudative phase of the inflammatory response. In this regard, for an objective assessment of their anti-inflammatory properties in the experiment the study of the anti-exudative effect on the model of aseptic exudative inflammation is generally accepted [15].

For this purpose in the course of the research presented the model of the carrageenan-induced paw edema in rats was used as the most common and representative model of the inflammatory response in experimental pharmacology [11].

While studying the anti-inflammatory properties of the model samples of pharmaceutical compositions in aerosol dosage forms the results presented in Table were obtained.

Analysis of the data obtained shows that within 3 h after the inflammatory response modeling the intensification of exudative processes in the limbs of animals occurred in the control pathology group. Thus, in comparison with the initial values the size of the rats' paw increased on average by 1.0 cm<sup>3</sup> (from 1.61 to 2.61 cm<sup>3</sup>), and it was 63.5 % (Table).

According to the data provided the most pronounced anti-inflammatory effect was recorded under the action of aerosol 2. Thus, under its influence there was a significant decrease in the size of the paw edema in animals compared to the control pathology group and rats receiving aerosol 4 or the mixture of freons. The size of the paw edema was 30.6% from the initial level, it stipulated the value of AA – 51.8% (Table).

At the same time, aerosols 1 and 3 revealed a slightly lower level of activity – 49.4% and 46.8%, respectively, however, without significant differences from the sample of aerosol 2. Thus, under their influence the size of the paw edema in rats was 32.1% from the initial level for the sample of aerosol 1 and 33.8% for the sample of aerosol 3 (Table). It should be noted that the level of AA of aerosols 1, 2 and 3 had no significant differences from the activity of the reference drug Proposal, and was significantly higher compared to the group of rats receiving the mixture of freons or the sample of aerosol 4 (Table). This phenomenon is explained by the presence of the common component in the formulations of aerosols 1, 2 and 3 – the solution of PPHD in propylene glycol in the concentration of 10%, which stipulates the anti-inflammatory

properties of the drugs studied.

The results obtained indicated that under the effect of the reference drug Proposal the size of the rats' extremity increased by 36.1% from the initial values (from 1.62 to 2.21 cm<sup>3</sup>), and the value of AA was 43.2%. It significantly exceeds the activity of the sample of aerosol 4 or the mixture of freons (Table).

Among the aerosol compositions containing local anesthetics the sample of aerosol 4 exhibited the least pronounced anti-inflammatory properties – its AA value was only 25.9%, and

the size of the extremity in rats increased by 47.1% compared to the initial level. This insignificant level of AA can be explained by the absence of phenolic compounds of propolis in the formulation of the given sample.

During the experiment it was also found that in animals receiving the mixture of freons without the active substances the size of the paw edema was 51.8%; it allowed to record the lowest level of AA – 18.4%, which was pharmacologically insignificant for medicines with the anti-inflammatory action.

**Table 1:** A comparative anti-inflammatory activity of pharmaceutical compositions in aerosol dosage forms

Name	Object The content of active pharmaceutical ingredients, %							The size of the extremity, cm <sup>3</sup>		The size of edema, %	AA, %
	PPHD solution in PG	Articaine	lidocaine	Menthol	Rosemary Oil	Buckthorn Oil	Allantoin	Outcome	in 4 h after the drug application		
Control pathology	—	—	—	—	—	—	—	1.61±0.05	2.61±0.05	63.5±5.7	—
Aerosol 1	10.0	3.0	—	1.0	1.0	5.0	—	1.60±0.05	2.11±0.05	32.1±1.7 <sub>1,2,4</sub>	49.4±2.7 <sub>2,4</sub>
Aerosol 2	10.0	—	3.0	1.0	1.0	5.0	—	1.54±0.04	2.01±0.05	30.6±2.5 <sub>1,2,4</sub>	51.8±3.9 <sub>2,4</sub>
Aerosol 3	10.0	5.0	—	2.0	2.0	—	1.0	1.57±0.04	2.10±0.05	33.8±1.7 <sub>1,2,4</sub>	46.8±2.7 <sub>2,4</sub>
Aerosol 4	—	—	3.0	1.0	1.0	—	—	1.52±0.04	2.24±0.06	47.1±1.4 <sub>1,3</sub>	25.9±2.2 <sub>3</sub>
Proposal	10.0	—	—	—	—	—	—	1.62±0.04	2.21±0.05	36.1±1.3 <sub>1,2,4</sub>	43.2±2.1 <sub>2,4</sub>
Mixture of freons	—	—	—	—	—	—	—	1.54±0.05	2.34±0.08	51.8±1.5 <sub>3</sub>	18.4±2.4 <sub>3</sub>

Notes:

- 1 – Differences were significant compared to the control pathology group ( $p \leq 0.05$ );
- 2 – Differences were significant compared to the animals receiving aerosol 4 ( $p \leq 0.05$ );
- 3 – Differences were significant compared to the animals receiving the reference drug Proposal ( $p \leq 0.05$ );
- 4 – Differences were significant compared to the animals receiving the mixture of freons ( $p \leq 0.0$ )

#### 4. Conclusion

1. When studying the anti-inflammatory properties of the given compositions in aerosol dosage forms it has been found that aerosol 2 containing 10% solution of PPHD in propylene glycol and 3% lidocaine hydrochloride shows the highest level of the anti-inflammatory activity (51.8%).
2. When studying the anti-inflammatory properties no significant differences in the activity levels of the drug samples were found. The data obtained allow to conclude that it is the complex of biologically active substances of the propolis phenolic hydrophobic drug that contributes significantly to the final anti-inflammatory activity of the samples studied. It also indicates the expedience of introduction of the PPHD solution into the formulation of the aerosol samples studied as an anti-inflammatory component in the concentration of 10%.
3. According to the results of a comparative study of the anti-inflammatory activity of the experimental samples of the aerosols studied it has been found that the sample of aerosol 2 containing 10% solution of PPHD in propylene glycol and 3% lidocaine hydrochloride is the most suitable for further development of an aerosol drug since it is exactly this pharmaceutical composition has exhibited the highest level of the anti-inflammatory activity – 51.8%.

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