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Study of the anti-inflammatory effect of the combined extract BNO 1016 in a leukotriene-dependent in vivo inflammation model

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Abstract

A significant role in the pathogenesis of rhinosinusitis is played by an inflammatory reaction in the maxillary sinuses of the nasal cavity. Leukotrienes are the most powerful mediators of inflammation, especially at the early stages. The effect of combined dry extract BNO 1016 could be useful for the inhibition of the inflammation in rhinosinusitis. Thus, it appears reasonable to study the anti-inflammatory activity of the combined dry extract BNO 1016.

Materials and methods: The tested drug is the combined dry extract BNO 1016 produced by "Bionorica SE". The reference drug is Ibuprofen. Leukotriene inflammation was induced by subplantar injection of zymozan into the right hind paw of male and female Wistar rats. Volumes of the resulting edema were measured and levels of anti-inflammatory activity together with mean effective dose (ED50) were calculated.

Results: The highest anti-inflammatory activity was observed at a dose level of 500 mg/kg of BNO 1016 during all time-points of the experiment and reached 65.2% 2 h after induction of inflammation. The anti-inflammatory activity of BNO 1016 at 500 mg/kg was credibly higher than that of Ibuprofen at all time-points of the experiment. Based on the data obtained from this leukotriene-dependent inflammation experiment the mean effective dose (ED50) for anti-inflammatory activity of dry combined extract BNO 1016 was calculated with help of Probit analysis.

Conclusion: The combined dry extract BNO 1016 shows a high level of anti-inflammatory activity in leukotriene-dependent inflammation which is very promising for the improvement of the treatment of patients. However, additional preclinical and clinical research on this drug should be carried out.

Keywords: Leukotriene, Inflammation, Paw edema, Zymozan, Dry combined extract BNO 1016, Ibuprofen, Anti-inflammatory activity

Introduction

Sinupret extract is a herbal medicinal product containing a hydroethanolic dry extract (BNO 1016; Dry Extract Ratio (DER): 3–6:1) of a combination of five herbs: gentian root, primula flowers, sorrel herb, elder flowers and verbena herb (1:3:3:3:3) and a well-known herbal drug commonly used for rhinosinusitis therapy [1]. Significant role in rhinosinusitis pathogenesis is played by inflammatory reaction in maxillary sinuses of nasal cavity [1]. To date, the mechanisms of action of dry combined extract BNO 1016 on the course of this pathology have not been fully determined, yet. Anti-inflammatory activity of BNO 1016 in prostaglandine-dependent inflammation induced with carrageenan studied in vivo, in which inhibition of COX-2 expression together with decreasing of prostaglandin E_2 level were revealed [2]. Leukotrienes are some of the most powerful mediators of the inflammation, especially at the early stages [3]. At the initial stage of any inflammation the largest impact is caused by leukotrienes – strong phlogogens which induce the exudation in response to damage and thereby starting the cascade of processes of other inflammatory mediators release [2]. The ability of dry combined extract BNO 1016 pharmacodynamics to affect the leukotriene link of

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inflammation might lead to high rate of the anti-exudative effects Therefore, it is reasonable to study the anti-inflammatory effect of dry combined extract BNO 1016 on the model of zymozan-induced inflammatory exudative paw edema in rats. In which the key role of the activation of lipoxygenase pathway of arachidonic acid metabolism and, as a result, leukotrienes production in the pathogenic mechanism development is played.

The study goal is to investigate experimentally the effect of dry combined extract BNO 1016 on the course of leukotriene-dependent inflammation for substantiation of its use as a drug for pathogenetic therapy of rhinosinusitis.

Materials and methods

There were taken albino laboratory Wistar-rats both males and females weighing 150–180 g in total number of 60. Rats were distributed into equal experimental groups with 10 animals each, 5 males and 5 females. Experimental animals were held in vivarium of the Central Scientific-Research Laboratory of the National University of Pharmacy (Ukraine) according to the standard sanitary requirements providing necessary diet [4–7]. All studies were performed in accordance with Directive 2010/63/EU about meeting laws, regulations and administrative provisions of the EU states concerning protection of animals used for experimental and other scientific purposes [8, 9].

The tested drug is dry combined extract BNO 1016 produced by "Bionorica SE" under the trade name "Sinupret extract", containing dry extract (DER 3–6:1) of Gentian root (Gentianae radix) consists of the dried roots of *Gentiana lutea L.*, Primula flower (Primula flos) consists of the dried flowers of *Primula veris L./Primula elatior (L.) Hill.*, Sorrel herb (Rumicis herba) consists of the dried aerial parts of *Rumex species*, Elder flower (Sambuci flos) consists of the dried flowers of *Sambucus nigra L.*, Verbena herb (Verbenae herba) consists of the dried aerial parts of *Verbena officinalis L.* (1:3:3:3:3). It can be suspended in water; thus, it was administered to animals in the form of water suspensions prepared with physiological saline. Test drugs solutions were made at such concentrations that all animals received it at the same dose by volume - 10 ml/kg.

The reference drug is ibuprofen in film-coated tablets for oral use containing 200 mg. The test samples of ibuprofen were also administered once intragastrically.

The animals were divided into 6 study groups (10 animals per group, 5 males and 5 females) in the following way:

Group 1 – control, rats with zymozan paw edema, administered physiological saline in volume 10 mg/kg intragastrically;
Group 2 – rats with zymozan paw edema, administered dry combined extract BNO 1016 intragastrically at dose 15 mg/kg;
Group 3 – rats with zymozan paw edema, administered dry combined extract BNO 1016 at 50 mg/kg;
Group 4 – rats with zymozan paw edema, administered dry combined extract BNO 1016 at 150 mg/kg;
Group 5 – rats with zymozan paw edema, administered dry combined extract BNO 1016 at 500 mg/kg;
Group 6 – rats with zymozan paw edema, administered Ibuprofen intragastrically at 100 mg/kg (ED_{50} by analgesic activity [10]).

Assessment of each animal was performed in equal time intervals after pathology formation – 0.5, 1, 2, 3 and 6 h.

Then all rats received single intragastric administration of study drugs at corresponding doses. Animals of the control group received equivalent amount of physiological saline.

The baseline volume (cm^3) of the right hind paw was determined using a digital plethysmometer (IITC Life Science, USA) at the beginning of the experiment.

The pathology was induced in all rats after 1 h of study drug administration, by subplantar injection of 0.1 ml of 2% suspension of zymozan in the right hind paw ("Fluka", Switzerland) [11, 12].

Edema volume was measured on the right hind paw in order to see the trend after 0.5, 1, 2, 3 and 6 h after phlogogen injection using a digital plethysmometer (IITC Life Science, USA) and was represented in cm^3.

Anti-inflammatory activity (AIA) was evaluated in percentage by level of edema reduction in animals, which received study drug in comparison with animals of the control group. It was calculated by the following Eq. 1 [11]:

$$\text{Anti–inflammatory activity} = \frac{\Delta V_{control} - \Delta V_{test}}{\Delta V_{control}} \times 100\%, \quad (1)$$

where $\Delta V_{control}$ – mean % of edema volume in the control group;

ΔV_{test} – mean % of edema volume in the test group.

Then based on dependence of anti-inflammatory activity of the drugs on the administered dose the mean effective dose (ED_{50}) was calculated by Probit Analysis using MS Excel 2007 software [13, 14] in the next way. The percentages of activity in each group were converted into probits (y), and then their weighing factors (B) and dose variable (x) were determined with necessary calculations, using experimental data.

For further calculations the indicators ED_{16}, ED_{50}, and ED_{84} were determined. Equations reflecting the relationship between doses and probits are 2:

$$y = A_0 + A_1 x \quad (2)$$

Coefficients A_0 and A_1 were calculated by Eqs. 3 and 4:

$$A_0 = \frac{(\Sigma B) - (\Sigma xB)A_1}{\Sigma B} \quad (3)$$

$$\frac{\Sigma xB}{\Sigma B} \times [\Sigma yB - (\Sigma xB)A_1] + (\Sigma x2B)A_1 = \Sigma xyB \quad (4)$$

The solution of these equations allows to calculate the values A_0 and A_1, which made it possible to construct a chart of probit-analysis of the "activity-dose" dependence.

Then the values of dose variable were found (x) for ED_{16}, ED_{50} and ED_{84}, taking into account values of probits (y) which were ED_{16}–4, ED_{50}–5 and ED_{84}–6, respectively.

The standard error s value of ED_{50} was determined by the Eq. 5:

$$s = \frac{ED_{84} - ED_{16}}{2\sqrt{n}} \quad (5)$$

where n – number of observations;

ED_{84} – dose of the drug in which 84% activity is observed;

ED_{16} – dose of the drug in which 16% activity is observed.

Statistical processing of the obtained results was performed by ANOVA using Student's T-test and non-parametrical analysis methods (Mann-Whitney U Test) by Statistica 10.0, StatPlus 2009 and MS Exel 2007 [15–17].

Results

Considering the fact that the maximum level of pathological changes in aseptic zymozan inflammation model is observed in 1–2 h [11, 18], effectiveness of the test samples was evaluated in the same interval after pathology formation.

Experimental data show that within 2 h after the formation of the inflammatory reaction caused by zymozan there was the development of the exudative processes in animals' paws of all groups.

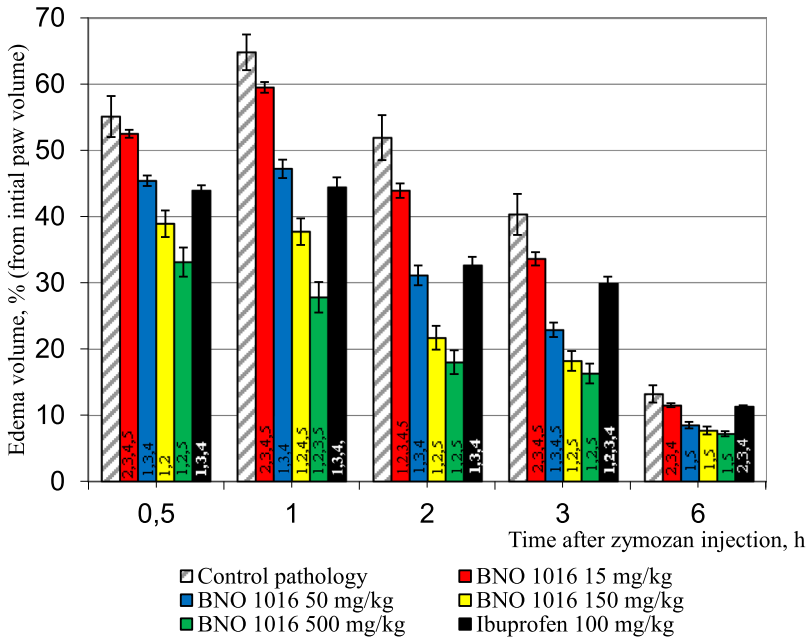
Thus, in comparison with the initial values, the volume of the paw after 30 min increased by 55.1% (Fig. 1), which caused the enlarge of the paw for 0.75 cm^3. Then, after the first hour of the experiment, paw volume increased to 64.8% (Fig. 1) of the initial volume, which was 0.88 cm^3, and then after 2 h it reduced to 51.9% (Fig. 1), which was 0.70 cm^3. With the time, the edema decreased to 40.3% in 3 h after the formation of the pathology and in 6 h — to 13.2% (Fig. 1).

According to the given data, dry combined extract BNO 1016 at dose of 15 mg/kg led to the development of 52.5% edema from the baseline in half an hour after the formation of pathology, 59.5% – after 1 h, 43.9% – after 2 h, 33.6% – after 3 h and 11.5% – after 6 h (Fig. 1). It should be noted, that the decrease in the volume of edema was not significant in comparison with the control group for only 2 h of observation. As a result of calculations the next indexes of AIA were obtained for dry combined extract BNO 1016 in dose of 15 mg/kg: 4.7% – after 30 min, 8.2% – after 1 h, 15.4% – after 2 h, 16.6% – after 3 h and 12.7% – after 6 h of the the pathology formation (Fig. 2). At the same time, this test sample was statistically lower than the level of activity of the dry combined extract BNO 1016 at doses of 50, 150 and 500 mg/kg, as well as the reference drug ibuprofen at dose of 100 mg/kg (Fig. 2).

When dry combined extract BNO 1016 was administered at dose of 50 mg/kg, there was a significantly higher activity level than in the previous group, which in turn, indicates the linear pharmacodynamics of this drug. Half an hour after the formation of the pathology, the volume of edema significantly declined in comparison with the control group and reached to 45.4% of the baseline values (0.61 cm^3), after 1 h – 47.2% (0.63 cm^3), after 2 h – 31.1% (0.42 cm^3), after 3 h – 22.9% (0.31 cm^3) and after 6 h – 8.5% (0.11 cm^3) (Fig. 1). Herewith the level of AIA was: 17.5%, 27.1%, 40.0%, 43.2% and 35.3%, respectively (Fig. 2).

More significant level of anti-inflammatory activity was observed with the use of dry combined extract BNO 1016 in a dose of 150 mg/kg. Thus, in comparison with the control group, at the 30th minute of observations, it significantly reduced the degree of edema of the animals' paw, while the volume of edema was 38.9% (0.52 cm^3), at the 1st hour – 37.7% (0.50 cm^3), at the 2nd hour – 21, 7% (0,29 cm^3), at the 3rd hour – 18.2% (0.24 cm^3) and at the 6th hour – 7.7% (0.10 cm^3) (Fig. 1). During the calculations, the AIA of this test sample was 29.3% – after half an hour, 41.8% – after 1 h, 58.1% – after 2 h, 54.8% – after 3 h and 41.8% – after 6 h (Fig. 2). It statistically exceeds the activity of dry combined extract BNO 1016 at a dose of 50 mg/kg after 30 min, 1, 2 and 3 h of pathology formation.

Under the influence of dry combined extract BNO 1016 at a dose of 500 mg/kg, there was a slightly higher level of activity than in the previous group but with significant differences only at the 1st hour of observation. So, 30 min later of the pathology formation the edema volume significantly decreased in comparison with the



Comments:
1 – Difference is credible compared to animals of the control group ($p \leq 0.05$);
2 – Difference is credible compared to animals received dry combined extract BNO 1016 at 50 mg/kg ($p < 0.05$);
3 – Difference is credible compared to animals received dry combined extract BNO 1016 at 150 mg/kg ($p < 0.05$);
4 – Difference is credible compared to animals received dry combined extract BNO 1016 at 500 mg/kg ($p < 0.05$);
5 – Difference is credible compared to animals received reference drug Ibuprofen ($p < 0.05$);

Fig. 1 Effect of dry combined extract BNO 1016 on edema volume

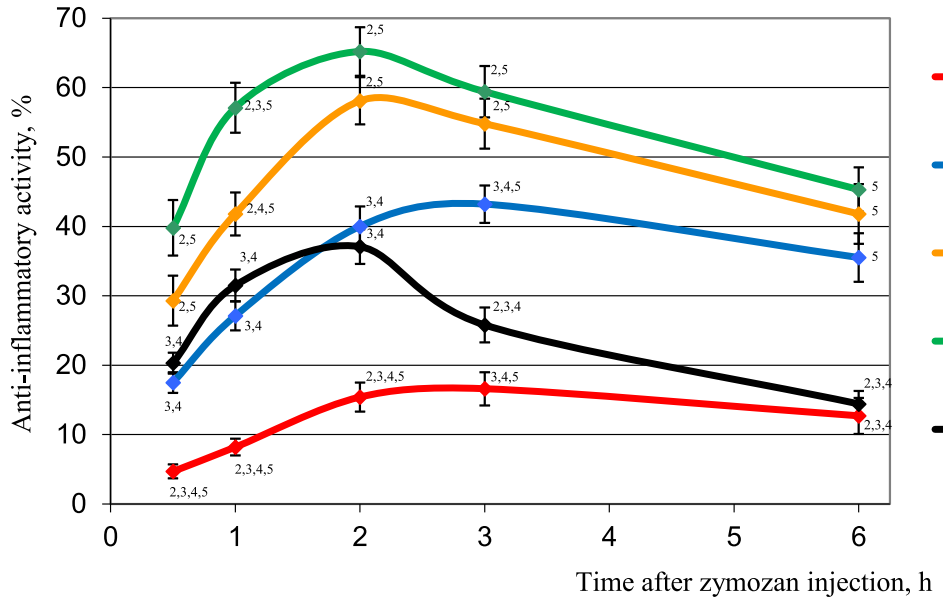
control group and was 33.1% from the original values, which was on average 0.45 cm^3, after 1 h – 27.8% (0.37 cm^3), after 2 h – 18.0% (0.24 cm^3), after 3 h – 16.3% (0.22 cm^3) and after 6 h – 7.2% (0.10 cm^3) (Fig. 1). Herewith the level of AIA was the following: after 30 min – 39.8%, after 1 h – 57.1%, after 2 h – 65.2%, after 3 h – 59.4% and after 6 h – 45.3% (Fig. 2). It should be noted, that the obtained result is the highest level of AIA among all test samples of dry combined extract BNO 1016 at this stage of experiment. However, according to the degree of anti-inflammatory effect, the tested drug at a dose of 500 mg/kg statistically exceeded the effect of 150 mg/kg dose only as for the first hour of the observation (Fig. 2).

In the course of the study, the reference drug ibuprofen was expected to show low anti-inflammatory activity, due to its predominant influence on the cyclooxygenase pathway of the arachidonic acid transformation, rather than on lipoxygenase. Thus, it significantly reduced the degree of edema of the animals' paw in comparison with the control group as for the 30th minute of observation, while the volume of edema was 43.9% (0.60 cm^3) (Fig. 1), and AIA index – 20.3% (Fig. 2). After 1 h the edema volume was 44.4% (0.61 cm^3), AIA index – 31.5%, after 2 h – 32.6% (0.45 cm^3) and 37.1%, and after 3 h – 29.9% (0.41 cm^3) and 25.8% accordingly. As for the 6th hour of observation, ibuprofen insignificantly reduced the volume of edema by 11.3% (to 0.16 cm^3) (Fig. 1), while the AIA index was 14.4% (Fig. 2).

Then we calculated ED_{50} for the drug dry combined extract BNO 1016 for anti-inflammatory action based on dose-dependent drug activity by the Probit analysis [13, 14].

As on the previous step, the ED_{50} indicators were calculated at the 1st and 2nd hours after the pathology formation, since during this time interval the highest activity of the test drug and the highest degree of pathological changes in the development of experimental inflammation were observed (Fig. 1).

For proper calculations, it was sufficient to use the results for three doses of 15, 50 and 150 mg/kg (Table 1). In the calculations, 500 mg/kg dose activity parameters



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3 – Difference is credible compared to animals received dry combined extract BNO 1016 at 150 mg/kg ($p < 0.05$);
4 – Difference is credible compared to animals received dry combined extract BNO 1016 at 500 mg/kg ($p < 0.05$);
5 – Difference is credible compared to animals received reference drug Ibuprofen ($p < 0.05$);

Fig. 2 Anti-inflammatory activity of dry combined extract BNO 1016

Table 1 Dose values and activity level for determining ED_{50} of dry combined extract BNO 1016 in rats according to anti-inflammatory activity by Probit analysis

Dose, mg/kg	Activity, %	Dose variable, x	Probit, y	Weighing factor, B	xB	x^2B	YB	xyB
after 1 h								
15.0	8.0	3	3.6	2.3	6.9	20.7	8.28	24.84
50.0	27.0	10	4.39	4.3	43.0	430.0	18.88	188.77
150.0	42.0	30	4.80	4.8	144.0	4320.0	23.04	691.20
Sum				11.4	193.9	4770.7	50.20	904.81
after 2 h								
15.0	15.0	3	3.96	3.5	10.5	31.5	13.86	41.58
50.0	40.0	10	4.75	4.8	48.0	480.0	22.80	228.00
150.0	58.0	30	5.20	4.8	144.0	4320.0	24.96	748.80
Sum				13.1	202.5	4831.5	61.62	1018.38

were not used to obtain a value of ED_{50} that could be statistically checked.

The solution of Eqs. 2, 3 and 4 allows to calculate the values A_0 and A_1, which made it possible to construct a chart of probit-analysis of the "activity-dose" dependence shown in Fig. 3.

The final results of the calculations are presented in Table 2.

As a result of the conducted calculations, the parameters of ED_{50} of dry combined extract BNO 1016 by anti-inflammatory activity were determined: ED_{50} at the 1st hour of observation was 171.2 ± 26.4 mg/kg, and ED_{50} at 2nd hour — 115.5 ± 23.6 mg/kg, which is 1.5 times lower.

Discussion

The obtained results indicate that dry combined extract BNO 1016 has a significant anti-inflammatory effect on the model of zymozan edema, which has a statistically significant dose-dependent nature in most of the time points of the observation period, and the degree of activity at dose levels of 50, 150 and 500 mg/kg exceeds the activity of the reference drug Ibuprofen. This high level of AIA caused by combination of the effective plants: gentian root, primula flowers, sorrel herb, elder flowers, verbena herb, which were combined in the one tablet through the phytoneering technology of the "Bionorica SE".

A comparative analysis of the anti-inflammatory effect of tested drug in the studied doses and the reference drug ibuprofen suggests, that the dry combined extract BNO 1016 at doses of 150 and 500 mg/kg significantly exceeds ibuprofen over all observation period, 50 mg/kg does not inferior to it for 30 min, 1 h and 2 h and exceeds as for the 3rd and 6th hours after the pathology formation (Fig. 1). Administration of dry combined extract BNO 1016 at a dose of 15 mg/kg is inferior to the anti-inflammatory effect of ibuprofen over all observation periods, except for the 6th hour, when no statistic differences were observed (Fig. 1).

According to the results of the observations, the duration of the anti-inflammatory effect of dry combined extract BNO 1016 is at least 7 h after administration, with the maximum activity observed at the range of 1–3 h (Fig. 1). Calculated indexes of ED_{50} of tested drug according to anti-inflammatory action on the model of zymozan-induced edema were as for the 1st hour of observation - 171.2 mg/kg, and at the 2nd hour - 115.5 mg/kg, which allow to recommend these doses for preclinical studies on models of experimental pathology, caused by the action of leukotrienes or in clinical practice, taking into account conversion factors for humans [19].

While extrapolating the obtained indicators of ED_{50} dry combined extract BNO 1016 for human following the recommendations of the FDA [19], there were made the calculations during which the daily dose of the drug was determined for the maximum anti-inflammatory effect on the leukotriene-induced model after 2 and 3 h of post-administration:

- after 2 h: $171.2\,mg/kg\ x\ 60\,kg/6.2 = 1657\,mg$
- after 3 h: $115.5\,mg/kg\ x\ 60\,kg/6.2 = 1118\,mg$

The obtained results are too large and exceed the recommended daily dose of dry combined extract BNO 1016 of 480 mg (3 tablets of 160 mg per day) to 2.3–3.5 times. Otherwise, it is well known, that increase of drug

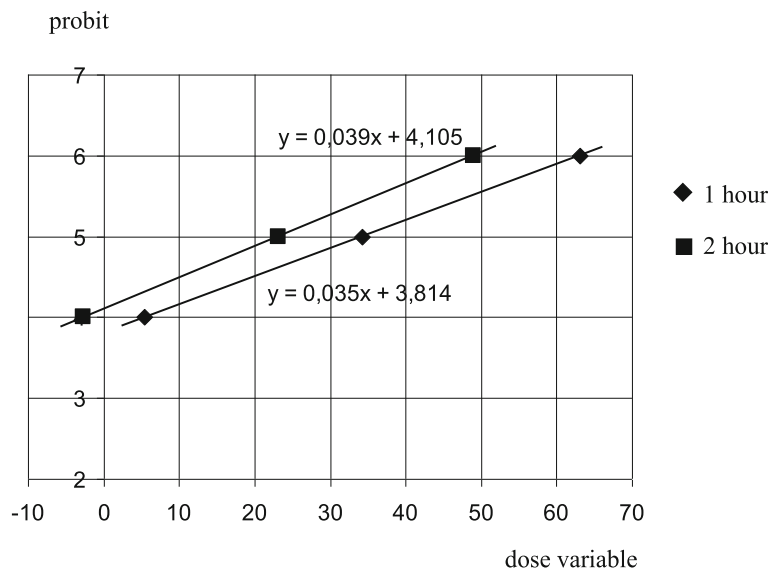

Fig. 3 Chart of Probit Analysis of the "activity-dose" dependence

Table 2 Results of calculations for determining ED_{50} of dry combined extract BNO 1016 according to anti-inflammatory activity by Probit Analysis method

A_1	A_0	Equation „probit-dose" dependence	Dose variable ED_{50}	Dose variable ED_{16}	Dose variable ED_{84}	ED_{50}, mg/kg	s, mg/kg
after 1 h							
0.035	3.814	y = 0.035x + 3.814	34.23	5.37	63.10	171.2	26.4
after 2 h							
0.039	4.105	y = 0.039x + 4.105	23.11	−2.72	48.94	115.5	23.6

dose may lead to more increase of its toxicity rather than efficacy. The tested drug is BNO 1016 quite safety drug [1], but estimating of its safety in this dose level will require additional toxicological researches, both preclinical and clinical. On other hand, recommended daily dose of dry combined extract BNO 1016 480 mg (or 50 mg/kg converting for administration for rats in experiment) possesses high level of AIA the same with reference drug ibuprofen and exceeded it at the 3rd and 6th hours after zymozan injection. This, in turn, can ensure the healthcare provider specialists and patients that Sinupret extract provides its positive effect on leukotriene link of inflammation, when it used for treatment [20].

Thus, based on the results of the study, as well as data on the pharmacokinetics and toxicology of dry combined extract BNO 1016, it can be assumed that in order to ensure a high anti-inflammatory effect at the initial stages of the development of rhinosinusitis, in particular to suppress the leukotriene-induced link of inflammation of the nasal cavity and around the nasal sinuses, it is advisable to use dry combined extract BNO 1016 in a recommended daily dose 480 mg, which corresponds to 3 tablets per day.

Conclusions

1. The drug dry combined extract BNO 1016 on the model of zymozan-induced rat paw inflammation at doses 15–500 mg/kg has an anti-inflammatory effect, which for most of the intervals of observation within 7 h after administration has a statistically significant dose-dependent feature with a maximum activity within 1–3 h. BNO 1016 at a dose of 15 mg/kg has a poor anti-inflammatory effect. However, at 50 mg/kg (corresponding to the 1-fold equivalent of the recommended human daily dose) it has a credible anti-inflammatory effect, which exceeded the effect of Ibuprofen at 3 and 6 h after zymozan injection. The degree of activity at 150 and 500 mg/kg statistically exceeds the activity of the reference substance Ibuprofen at a dose 100 mg/kg.
2. During the course of the study the anti-inflammatory activity of BNO 1016 in the model of zymozan-induced paw edema, ED_{50} indexes were calculated. They were at the 1st hour of observation – 171.2 mg/kg, and at the 2nd hour – 115.5 mg/kg. It enables to recommend these doses for preclinical studies in animal models of experimental pathology determined by the action of leukotrienes or in clinical trials, taking into account the conversion factors for humans after all necessary toxicological studies.
3. The results of the research on anti-inflammatory properties of BNO 1016 in models of exudative inflammation due to the influence of leukotrienes seem to suggest the high efficacy of this agent on the leukotriene-dependent link of the pathogenesis of rhinosinusitis due to combination of the effective plants: gentian root, primula flowers, sorrel herb, elder flowers, verbena herb. Credible AIA was observed from a dose level of 50 mg/kg and increased along with higher dose levels. Consequently, the high rate of the pharmacological effects development, which exceeds the activity of the reference drug Ibuprofen, was considered as a significant advantage in the treatment of this pathology.
4. To confirm the above assumptions, it is rational to conduct a preclinical study of the effect of BNO 1016 on the course of experimental rhinosinusitis in animals.

Abbreviations

AIA: Anti-inflammatory activity; BNO 1016: Conditional name of the tested drug; COX-2: Cyclooxygenase-2; DER: Dry Extract Ratio; ED_{16}: Dose of the drug in which 16% activity is observed; ED_{50}: Mean effective dose; ED_{84}: Dose of the drug in which 84% activity is observed

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Authors' contributions

All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of National University of Pharmacy.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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