# CLINICAL STUDY PROTOCOL

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| **Study title** | Evaluation of anti-inflammatory properties of herbal medicines in patients with tonsillopharyngitis – interventional, open, randomized, one center comparative trial in parallel groups |
| **Study ID:** | Место для ввода текста. |
| **Protocol version:** | 2.0 |
| **Version date:** | 11.09.2019 |
| **Investigational drug:** | Tonsilgon N |
| **International non-proprietary name:** | Not applicable |
| **Dosage form, strength:** | Oral drops 100 ml |
| **Study phase:** | Место для ввода текста. |
| **Sponsor-investigator** | Prof. Kozlov Vladimir Sergeevich<br>FBSI PPE Central State Medical Academy of Russian Federation Department of Presidential Affairs<br>121359, Moscow, Marshala Timoshenko st., 19, b. 1A<br>Phone.: +7 499 140 1876<br>E-mail: org@cgma.su |

**Confidentiality statement**

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# Study sponsor's approval page

**Protocol title:** Evaluation of anti-inflammatory properties of herbal medicines in patients with tonsillopharyngitis – interventional, open, randomized, one center comparative trial in parallel groups

**ID:** Место для ввода текста.

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**Sponsor-investigator**:

DMedSci, professor, Kozlov Vladimir Sergeevich
(Ф.И.О. сотрудника)

Head, Department of Otolaryngology
(должность)

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Phone: +7 499 140 1876
E-mail: org@cgma.su

_____ «____»_____ 201__
Signature Date

## Investigator's statement of compliance to protocol

I hereby confirm that I have read and understood this protocol (version 2.0 of 11.09.2019), the Investigator's brochure, including the potential risks and side effects of the drug, and other information about the drug and the study, provided by the Sponsor.

I agree to conduct this study in accordance with the requirements of this protocol, as well as to protect the rights, safety, confidentiality, well-being and health of patients in accordance with the ethical requirements set forth in the Helsinki Declaration of the World Medical Association (WMA), Federal Law No. 61-ФЗ "On the Circulation of Medicines" of April 12, 2010; the requirements of Order No. 200n "On Approval of Guidelines for Good Clinical Practice" of the Ministry of Health (MoH) of the Russian Federation of April 4, 2016; principles of the National Standard of the Russian Federation GOST 52379-2005 "Good Clinical Practice" (GCP) and other regulatory requirements of the Russian Federation.

I agree to make changes to the protocol only after notifying the Sponsor, unless it is necessary to protect the safety, rights and well-being of patients. I fully understand that any changes made by the Investigator(s) without prior discussion with the representative of the Sponsor will constitute a violation of the protocol (except for the procedures that are necessary to protect the health of patients).

I agree to personally conduct or monitor the described study.

I agree to inform patients that the drugs are used for research purposes; I will ensure compliance with the requirements for obtaining informed consent after approval by the Ethics Council and the local Independent Ethics Committee (NEC) and in compliance with GCP principles.

In accordance with the GCP principles, I agree to inform the Sponsor of all adverse events (AEs) that developed during the study.

I agree to ensure that all employees, colleagues, and individuals involved in the study are informed of their obligations to comply with the above agreements. I agree to keep adequate and accurate records, and to provide these records for analysis in accordance with the GCP principles.

I will ensure that the local NEC, which operates in accordance with the GCP requirements, is responsible for conducting the ethical review as well as for approving the study. I also agree to quickly report to the local NEC about all changes in research activities and all unexpected problems, including risk for patients and other aspects. In addition, I will not make any changes to the study without the approval of the Ethics Council/local NEC, unless necessary to address a clear, unexpected threat to the life and health of patients.

I am willing to provide direct access to primary documents and agree to be audited by representatives of the Sponsor and regulatory bodies. I guarantee that the investigational drug(s) supplied by the Sponsor will only be used as described in this protocol.

I agree to comply with all other requirements regarding the responsibilities of clinical investigators, as well as all other important requirements of Good Clinical Practice.

Investigator:
Signature: _____
Date: «____»_____ 202__
Full name:
Position:
Institution:
Address:

## Table of contents

# List of abbreviations

| Abbreviation | Definition |
| --- | --- |
| AE | Adverse event |
| ANCOVA | Covariate analysis |
| APC | Andtibody-Producing Cell |
| ARD | Acute respiratory disease |
| ATC | Anatomical-Therapeutical-Chemical classification |
| BP | Blood pressure |
| CD | Cluster of differentiation |
| DNA | Desoxyribonucleic acid |
| EAEU | Eurasian Economic Union |
| eCRF | Electronic Case Report Form |
| GCP | Good Clinical Practice |
| GI | Gastrointestinal |
| ICH | International Conference on Harmonization |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IL | Interleukin |
| INN | International non-proprietary name |
| $LD_{50}$ | Median lethal dose |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MH | Ministry of Health |
| MIC | Minimal Inhibitory Concentration |
| NF-κB | Nuclear factor-κB |
| SAE | Serious adverse event |
| $_{S}$IgA | Secretory Immunoglobulin A |
| SOP | Standard operating procedure |
| TNF-α | Tumor necrosis factor-α |
| TSS | Tonsillopharyngitis Severity Score |
| v/v % | Volume percentage |
| VRI | Viral respiratory infection |
| WMA | World Medical Association |

# Synopsis

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| **EudraCT number** | Not applicable |
| **Study ID** | Not applicable |
| **Study title** | Evaluation of anti-inflammatory properties of herbal medicines in patients with tonsillopharyngitis – interventional, open, randomized, one center comparative trial in parallel groups |
| **Short title** | Comparison of Tonsilgon N and local herbal anti-inflammatory medicine in patients with tonsillitis |
| **Study drug т** | Tonsilgon N |
| **Study drug route of administration** | Oral, 30 minute before/after meal. Keep in mouth for 30 seconds before swallowing. |
| **Study drug dosing regimen** | 25 drops 6 times/day until complete 7 days. |
| **Reference product** | Shalfej, orodispersible tablet (“Valeant” LLC) |
| **Reference product route of administration** | Keep in mouth until completely dispersed. Take 30 minutes before/after meal. |
| **Reference product dosing regimen** | 6 tablets/day every 2 hours until complete 7 days. |
| **Salvation therapy** | In case of pronounced pharyngeal pain, paracetamol at 500 mg up to 4 times/day is allowed after consultation with investigator. |
| **Duration of treatment** | 7 days (1 week) |
| **Diagnosis for inclusion** | 1. Acute viral tonsillopharyngitis (mild to moderate)<br>2. Chronic tonsyllopharingitis exacerbation. |
| **Study timeline** | Supposed time of study conduct: 03.2019-12.2019 |
| **Количество исследовательских центров** | One-center study. |
| **Number of patients** | It is planned to enroll 70 patients aged 18 to 55 years:<br>1. Control and reference groups – 10 healthy subjects<br>2. Tonsilgon N drops treatment group – 30 patients<br>3. Shalfej, orodispersible tablets, treatment group – 30 patients |
| **Study objectives** | 1. To evaluate influence of Tonsilgon N drops intake on throat local immunity parameters (secretory immunoglobulin A [$_{S}$IgA], interferon (IFN)-α, tumor necrosis factor (TNF), lysozyme, lactoferrin, (IL)-1, -6, -8, -10, and -17);<br>2. To evaluate clinical efficacy of tonsillopharyngitis treatment with Tonsilgon N drops (Bionirica SE) compared to Shalfej, orodispersible tablets (“Valeant” LLC) |

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| | 3. To evaluate seriousness and probability of adverse events (AEs) and serious adverse events (SAEs) in study groups. Pregnancy cases data will be collected in separate. |
| **Study design** | Interventional, open-label, randomized, single-center comparative trial in parallel groups.<br><br>Study will enroll adult patients (aged 18 to 55 years) during 24 hours after symptom onset of acute viral tonsillopharyngitis or viral exacerbation of chronic tonsillopharyngitis.<br><br>Patients fulfilling inclusion criteria without non-inclusion criteria will be randomized in groups for treatment with investigational drug or comparator. Treatment will continue for 7 days. |
| **Study methods** | In present study efficacy of tosillopharyngitis treatment with Tonsilgon N will be evaluated as well as its' impact on selected parameters of oropharyngeal local immunity. To assess efficacy of treatment routine clinical methods will be used: pharyngoscopy, physical examination, patient survey, and dynamic evaluation of tosillopharyngitis symptoms by patient. To assess influence of Tonsilgon N on local oropharyngeal immunity invasive diagnostic method of upper mucosa layer of tonsils and pharynx back scarification will be used with following enzyme-linked immunosorbent assay (ELISA) for ($_{S}$IgA, IFN-α, TNF, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17. To evaluate normal values of indicated parameters in tonsil and back pharynx mucosa samples of 10 healthy volunteers will be examined once.<br><br>As a primary endpoint, dynamic of Tonsillopharyngitis Severity Score (TSS) points will be used. Questionnaire is used in clinical practice to assess dynamic in symptom pronunciation during infectious-inflammatory process in throat [1]. TSS scale version of contract research organization Appletree AG was adapted for purposes of current study.<br><br>TSS is a questionnaire for evaluation of presence and severity of following symptoms: throat pain, difficulty to swallow, salivation, pharynx mucosa hyperemia, hyperthermia by 4-point scale:<br><br>• 0 = no symptom;<br>• 1 = mild symptom;<br>• 2 = moderate symptom;<br>• 3 = significant symptom.<br>Axillar body temperature measurements will be classified as following:<br><br>• 0 points < 37.5 °C;<br>• 1 point from 37.5 to < 38.5 °C; |

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| | • 2 points from 38.5 to < 39.5 °C;<br>• 3 points ≥ 39.5 °C.<br><br>TSS will be filled out by study doctor based on patients' complaints and physical examination. Study doctor will summarize every symptom score to count TSS total score. Sum of TSS points will be evaluated at screening to assess inclusion criteria and baseline tonsillopharyngitis severity, and at visits 2 and 3 as well (Day 4 and Day 8 ± 1) to assess efficacy of treatment in both study groups.<br><br>Thus, the maximum duration of participation in the study for one patient will be 39 days |
| **Patient visits** | **Visit 1 (day 1)**<br>• Signing the informed consent form to participate in the study<br>• Demographic, main disease medical history, past and concomitant diseases, prior therapy data collection<br>• Patient complaints collection<br>• Concomitant therapy registration<br>• Vital signs evaluation (blood pressure, heart rate, respiratory rate, body temperature)<br>• Physical examination<br>• Pharyngoscopy<br>• McIsaac scale assessment<br>• Streptatest<br>• Blood count<br>• Pregnancy test for females with preserved reproductive potential<br>• Inclusion/non-inclusion criteria evaluation<br>• Randomization<br>• Scarification sample collection of upper mucosa layer of tonsils and back pharynx[1]<br>• Dispensing of study drug/comparator drug<br>• Dispensing of patients' Diary<br>1 Registration of AEs/SAEs<br><br>**Visit 2**<br>• Patients' complaints collection<br>• Concomitant therapy registration<br>• Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature) |

[1] For evaluation of secretory immunoglobulin A, interferon-α, tumor necrosis factor, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and -17.

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| | • Physical examination<br>• Pharyngoscopy<br>• Scarification sample collection of upper mucosa layer of tonsils and back pharynx<br>• Evaluation of patients' Diary<br>• Compliance assessment by data from patients' diary<br>• Registration of AEs/SAEs<br>2 Exclusion criteria evaluation<br><br>**Visit 3 (day 8 ± 1)**<br><br>• Patients' complaints collection<br>• Concomitant therapy registration<br>• Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature)<br>• Physical examination<br>• Pharyngoscopy<br>• Blood count<br>• Scarification sample collection of upper mucosa layer of tonsils and back pharynx<br>• Return of unused drugs and used blisters (for comparator drug)<br>• Evaluation of patients' Diary<br>• Compliance assessment by data from patients' diary<br>• Registration of AEs/SAEs<br>• Exclusion criteria evaluation<br><br>**Visit 4 (day 37 ± 2)**<br>• Patients' complaints collection<br>• Concomitant therapy registration<br>• Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature)<br>• Physical examination<br>• Pharyngoscopy<br>• Scarification sample collection of upper mucosa layer of tonsils and back pharynx<br>• Return of patients' Diary<br>• Evaluation of patients' Diary<br>• Registration of AEs/SAEs<br><br>**Unscheduled visit**<br>• Patients' complaints collection<br>• Concomitant therapy registration |

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| | • Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature)<br>• Physical examination<br>• Pharyngoscopy<br>• Blood count[2]<br>• Evaluation of patients' Diary<br>• Registration of AEs/SAEs<br>• Evaluation of exclusion criteria<br><br>**Premature discontinuation visit**<br>• Patients' complaints collection<br>• Concomitant therapy registration<br>• Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature)<br>• Physical examination<br>• Pharyngoscopy<br>• Scarification sample collection of upper mucosa layer of tonsils and back pharynx<br>• Return of unused drugs and used blisters (for comparator drug)[3]<br>• Return of patients' Diary<br>• Evaluation of patients' Diary<br>• Compliance assessment by data from patients' diary<br>• Registration of AEs/SAEs ~~adverse events/serious adverse events Оценка Дневника пациента~~<br>• |
| **Primary efficacy and safety endpoints** | ***Primary efficacy endpoint:***<br><br>**Primary endpoint:**<br><br>Dynamic of tonsillopharyngitis severity by change of TSS score at day 4 compared to day 0 ($TSS_{d4} - TSS_{d0}$).<br><br>**Secondary endpoints:**<br><br>• Proportion of patients achieved clinically significant improvement (TSS score ≤ 5) at study day 4; |

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[2] In case of clinical indications (by Study Doctor's opinion)
[3] If visit is conducted prior to visit 3.

- Severity of pain ~~and~~ or discomfort in throat by TSS subscale – proportion of patients with every grade of severity at baseline and day 4;
- Time until complete resolution of every symptom evaluated by patients' diary;
- Proportion (%) of patients completely recovered by day 4 (disease outcome by objective evaluation of Study Doctor);
- Dynamic of every tonsillopharyngitis symptom severity by 4-point scale (0 to 3) at visit 2 compared to visit 1;
- Dynamic of every tonsillopharyngitis symptom severity by 4-point scale (0 to 3) at visit 3 compared to visit 1;
- Dynamic of local immunity markers (sIgA, TNF, IFN~~secretory immunoglobulin A, tumor necrosis factor, interferon~~-α, lysozyme, lactoferrin, ~~interleukin~~IL-1, -6, -8, -10, and-17) in mucosa of tonsils and back pharynx at visit 2 compared to visit 1;
- Dynamic of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 3 compared to visit 1;
- Dynamic of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 4 compared to visit 1;
- Proportion of patients (%), in which contents of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 2 was equivalent to values in healthy persons (if deviation was detected at visit 1);
- Proportion of patients (%), in which contents of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 3 was equivalent to values in healthy persons (if deviation was detected at visit 1);
- Proportion of patients (%), in which contents of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 4 was equivalent to values in healthy persons (if deviation was detected at visit 1).

***Primary safety endpoints:***

- Frequency of AEs and SAEs ~~adverse events and serious adverse events~~ registration in treatment groups;

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| | • Frequency of AEs and SAEs ~~adverse events and serious adverse events,~~ related to use of study drugs~~treatments~~;<br>• Evaluation of vital signs;<br>• Physical examination.<br><br>Description of AEs ~~adverse events~~ will be presented according to following scheme:<br><br>• Description of AE~~adverse event~~;<br>• Severity of presentation;<br>• Duration;<br>• Relation to study drugs~~treatment~~;<br>• Outcome. |
| **Inclusion criteria for patients/healthy subjects** | Study will enroll patients fulfilling all following criteria:<br>1. Males and females aged 18 to 55 (inclusive);<br>2. Diagnosis at inclusion – “mild acute tonsillopharyngitis” or “chronic tonsillopharyngitis exacerbation”;<br>3. Body temperature measured in armpit ≤ 37.5°C;<br>4. Tonsillopharyngitis severity by TSS ≥ 8 points;<br>5. Time from first symptoms’ onset until visit to physician – not more than 24 hours;<br>6. Signed informed consent form;<br>7. For females with childbearing potential and males – consent to use effective method of contraception across all study and following 1 month after its completion.<br><br>Healthy volunteers’ inclusion criteria:<br>1. Males and females aged 18 to 55 (inclusive);<br>2. Absence of acute disease signs;<br>3. Absence of chronic disease by medical history data;<br>4. Volunteer did not receive any medicines during 30 days prior to visit;<br>5. Absence of deviations from normal in physical examination and by vital signs assessment;<br>6. Absence of deviations from normal values in blood count. |
| **Non-inclusion criteria** | Patients applying to at least one of the following criteria will not be enrolled:<br>1. Any signs and symptoms of bacterial (streptococcal) tonsillopharyngitis (McIsaac scale score > 1);<br>2. Positive result of express-test Streptatest; |

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| | 3. Consumption of antibiotics during < 48 hours prior to inclusion;<br>4. Patients earlier received tonsillectomy or tonsillotomy;<br>5. Use of local treatments for oropharyngeal disease (aerosols, gargle solutions, tablets/orodispersible tablets/lozenges) during 24 hours prior to inclusion and/or impossibility to discontinue use of any local treatments, except used in study, during study course;<br>6. Use of systemic, inhaled or nasal glucocorticosteroids during 30 days prior to study start, injectable corticosteroids – during 3 months prior to study start and/or plans to use glucocorticosteroids (except topical dermal ones) during the course of study;<br>7. Impossibility to withdraw for study period any medicinal preparations that could influence result of current study, e.g., antiviral medicines, or preparations incompatible with study treatments (see Section “Prohibited concomitant therapies”);<br>8. Pharyngitis granulosa;<br>9. Signs of fungal oropharyngeal infection (white caseous plaques easy removable by pallet);<br>10. Clinical signs of diphtheria;<br>11. Presence of signs of sinusitis, otitis, eustachitis, laryngitis, tracheitis, bronchitis (since indicated conditions could demand indication of medicines, that could possibly affect evaluation of study results; it is acceptable to include patients with rhinitis with use of therapies permitted by the Protocol);<br>12. Vaccination of patient conducted in 30 days prior to inclusion;<br>13. Assumed low patients’ compliance with treatment or inability to undergo procedures and follow restrictions according to study protocol (e.g., as a result of psychiatric disorders);<br>14. Clinically meaningful deviations of blood count, including any of the following signs: leukocytosis > $9x10^9$/L, neutrophilia > 78%, band neutrophil content > 6% or presence of younger neutrophil forms, erythrocyte sedimentation rate > 30 mm/h;<br>15. Liver diseases;<br>16. History of craniocerebral injury;<br>17. Brain diseases;<br>18. Any cardiovascular, kidney, liver, gastrointestinal (GI), endocrine, and nervous system diseases or any other diseases/conditions that, by Study Doctor’s opinion, could lead to unsafety of patients participation in the study;<br>19. Any concomitant diseases that require use of medications influencing immune system (immune system modulators, stimulators, suppressors) or antibiotics;<br>20. Need to use medications that act through γ-aminobutyric acid receptors (e.g., barbiturates and benzodiazepines);<br>21. Pregnant, lactating women or women planning pregnancy during next two months; |

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| | 22. Women of reproductive age, that did not confirm use of highly effective contraception methods (combined oral contraceptives, double barrier method);<br>23. Misuse of alcohol, or use of other psychoactive substances<br>24. Known hypersensitivity for any component of Tonsilgon N (chamomile, althea, oak bark, taraxacum, horsetail, walnut, milfoil, plants of *Compositae* family) or salvia and other related herbs (*Asteraceae* family). |
| **Exclusion criteria** | Patient terminates participation in the study in following cases:<br>1. Negative dynamic of disease with signs of secondary bacterial infection (onset of any McIsaac scale criteria absent at inclusion or other signs that are considered as bacterial infection signs by the Study Doctor);<br>2. Need for surgical treatment;<br>3. Study Doctor decided to exclude patient for his/her good;<br>4. Withdrawal of informed consent (unwilling to continue participation in the study);<br>5. Serious deviation from the study protocol;<br>6. Individual intolerance of study drugs;<br>7. Development of AE or SAE event that requires diagnostics and/or treatment that could significantly alter current study procedures;<br>8. Patient does not follow the rules of study participation;<br>9. Erroneous inclusion (e.g., patient was enrolled with violation of inclusion/non-inclusion criteria of Protocol);<br>10. Presence of non-inclusion criteria during study conduct;<br>11. Patient receives/needs to receive additional treatment, that could influence study result or affect patients' safety (see Section «Prohibited concomitant medications");<br>12. Other conditions or events requiring discontinuation of patient from the study, by Study Doctors' opinion. |
| **Concomitant therapy of patients** | Use of any medication indicated for treatment of conditions other than current disease (acute tonsillopharyngitis or exacerbation of chronic tonsillopharyngitis) and not indicated in non-inclusion criteria, as well as in "Prohibited concomitant medications" section. |
| **Prohibited concomitant medications** | 1. Hypoglycemic medications;<br>2. Anticonvulsant medications;<br>3. Sedative medications, including barbiturate and benzodiazepines;<br>4. Medications, affecting γ-aminobutiric acid receptors; |

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| | 5. Glucocorticosteroids in any dosage form, except ones for dermal topical use;<br>6. Immune system modulators, stimulators;<br>7. Immune system suppressors;<br>8. Antivirals;<br>9. Antibiotic medications with systemic exposure or local effect at tonsils and pharynx;<br>10. Antifungal medications with systemic exposure or local effect on tonsils and pharynx;<br>11. Any medications for topical therapy (aerosols, sprays, inhaled medicines, gargles, tablets/orodispersible tablets/lozenges/troches) for pharyngeal or nasal application. |
| **Statistical data analysis** | All variables will be analyzed using descriptive and non-parametric methods. |
| **Safety assessment** | By reported AEs/SAEs. |
| **Sample size justification** | Sample size calculation was based on results of study NCT03095521, published at clinicaltrials.gov website (https://clinicaltrials.gov/ct2/show/study/NCT03095521). In this study comparison between Angal lozenges and Anti-Angin lozenges (both containing chlorhexidine and lidocaine) was performed. Change in total TSS score by day 4 was 6 point on average, while standard deviation for score change was 3 points. Based on the assumption that placebo effect at current disease is around 50% of medicine effect (i.e., 3 points), effect size is approximately equal to one.<br><br>In the present study it is expected that effect size will be less and at least 0,4, taking into account herbal nature of study medicine and comparator.<br><br>Method based on covariate analysis (ANCOVA) with correction for baseline TSS score was used for sample size calculation. All calculations were made in G*Power software version 3.1<br><br>**F tests –** ANCOVA: Fixed effects, main effects and interactions<br>**Analysis:** A priori: Compute required sample size<br>**Input:** Effect size f = 0.4<br>α err prob = 0.05<br>Power (1–β err prob) = 0.8<br>Numerator df = 2<br>Number of groups = 3 |

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| | Number of covariates = 1<br>**Output:** Noncentrality parameter λ = 10.2400000<br>Critical F = 3.1504113<br>Denominator df = 60<br>Total sample size = 64<br>Actual power = 0.8044215<br>Therefore, total size of three groups should be at least 64 complete cases in order to observe expected effect towards primary endpoint. Considering possibility of 10% patients' withdrawal during the study 70 patients should be enrolled: 10 patients will compose control group, 60 – treatment group, divided in two subgroups (30 patients each). |
| **Blinding. randomization** | Open-label randomized study conduct was planned. |

# 3 General information

## 3.1 Protocol title, identification number, and date:

**Title:** Evaluation of anti-inflammatory properties of herbal medicines in patients with tonsillopharyngitis – interventional, open, randomized, one center comparative trial in parallel groups
**Protocol ID:** Место для ввода текста.
**Version:** 2.0
**Version date:** 11.09.2019

## 3.2 Name and address of clinical study sponsor and monitor (if they differ)

Study sponsor: professor Kozlov Vladimir Sergeevich
Name of organization: FBSI PPE Central State Medical Academy of Russian Federation Department of Presidential Affairs
Address: 121359, Moscow, Marshala Timoshenko st., 19, b. 1A
Phone: +7 499 140 1876
E-mail: org@cgma.su

## 3.3 Name and position of persons authorized to sign the protocol and amendments on behalf of study sponsor:

Full name: _____
Position: _____
Organization name: _____
Address: _____
Phone: _____
E-mail: _____

# 4 Study justification

## 4.1 Investigation medicinal products

### 4.1.1 Investigational product

**Trade name:** Tonsilgon N

**International non-proprietary name (INN): –**

**Pharmaceutical form:** Oral drops

**Composition:**

100 g of product contain:

*Active ingredients:*

29 g of aqueous alcoholic extract of herbal medicinal stock material mixture:

*Althea* roots – 0.4 g

Chamomile flowers – 0.3 g

Horsetail grass – 0.5 g

Walnut leaves – 0.4 g

Milfoil grass – 0.4 g

Oak bark – 0.2 g

*Traxacum officinale* herb – 0.4 g

*Excipients:*

Purified water – 71 g

**Pharmacotherapeutic group**

Herbal antiseptic medicine.

**Anatomical-therapeutical-chemical classification code (ATC):** R02AA20

**Description**

Transparent or slightly cloudy fluid with yellow-brownish color and chamomile smell. Might precipitate in storage.

**Pharmacological effect**

Pharmacological properties are due to active substances in the medicine. Tonsilgon® N has anti-inflammatory and antiseptic effects. Active substances of chamomile, althea, and horsetail help to increase activity of body's non-specific protective factors. Polysaccharides, ether oils and flavonoids or chamomile, althea, and milfoil, tannins of oak barb exhibit anti-inflammatory action ant promote decrease of airway mucosa's edema.

**Therapeutic indications**

Acute and chronic diseases of upper respiratory tract (tonsillitis, pharyngitis, laryngitis).

Prevention of viral respiratory infection (VRIs) complications and as addition to antibacterial therapy in bacterial infection.

**Contraindications**

Hypersensitivity to the active substance or excipients of the medicine, in particular, herbs of *Compositae* family, alcoholism (including period after antialcohol treatment), child age (under 1 year) – since preparation contains ethanol.

**With caution:** liver disease, craniocerebral trauma, brain disease, child age older than 1 year (use is possible only after physician's consult) – since preparation contains ethanol.

**Use during pregnancy and breastfeeding**

Use of medicine is possible, if potential benefit from treatment for mother overcomes potential risks for fetus or child. Physicians' consult is needed.

**Posology and method of administration**

Oral. Oral drops are taken undiluted, keeping in mouth for some period of time before swallowing.

*Acute stage of disease:*

Adults: 25 drops 5–6 times per day.

Kids of school age (older than 6 years): 15 drops 5–6 times per day.

Kids of preschool age (older than 1 year): 10 drops 5–6 times per day.

Treatment should be continued for 1 weeks after resolution of disease's acute symptoms (pharyngalgia).

*After resolution of ACUTE symptoms:*

Adults: 25 drops 3 times per day.

Kids of school age (older than 6 years): 15 drops 3 times per day.

Kids of preschool age (older than 1 year): 10 drops 3 times per day.

**Overdose**

No cases of overdose were observed until the present time. In case of overdose, gastrointestinal (GI) tract side effects could deteriorate. Treatment – symptomatic.

**Side effects**

GI tract: nausea, vomiting.

Allergic reactions are possible. Medicine use should be terminated if signs of allergic reaction emerge.

**Interaction with other drugs**

Combination with antibacterial medicines is possible and rational. Interactions with other medicines was not described.

**Special indications**

Ethanol content in the product is 16.0 to 19.5% (volume-based). Maximal single dose (25 drops) contains 0.21 g of ethanol; maximal daily dose (25 drops 6 times per day contains 1.26 g of ethanol.

In cases when symptoms of disease do not resolve or patients' condition deteriorates after 7 days of treatment, physicians' consultation is needed.

Medicine may become cloudy or precipitate during storage, which does not influence effectiveness.

Shake medicine before use!

Hold bottle straight when in use.

**Effects on ability to drive and performing other activities**

While using medicine, caution should be taken while driving and doing other activities with potential danger, that require increased concentration of attention and rapid psychomotor reactions (since preparation contains ethanol).

**Presentation**

Oral drops. Dark glass bottle of 50 or 100 ml with drop dispenser on the top, with screw cap and safety ring. Every bottle with prescribing information is placed within foldable carton.

**Storage conditions**

Store in dry, light-proof place at temperature below 25°C. Keep out of children's' reach!

**Terms of distribution**

Over the counter.

### 4.1.2 Comparator product

**Trade name:** Shalfej, orodispersible tablet ("Valeant" LLC)

**INN: –**

**Pharmaceutical form:** orodispersible tablet

**Composition:**

One tablet contains:

*Active ingredients:*

*Salvia L.* dry extract – 12.50 mg

*Salvia L.* essential oil – 2.40 mg

*Excipients:*

ascorbic acid, malic acid, sorbite, aspartame, magnesium stearate, colloidal silica, honey flavor, quinoline yellow (E-104), indigo carmine (E-132).

**Pharmacotherapeutic group**

Herbal antiseptic medicine.

**Anatomical-therapeutical-chemical classification code (ATC): –**

**Description**

Flat round tablets with beveled edge, blue-green color with brighter and darker granules, and specific smell. Both sides printed with “NP” and tree on the background.

**Pharmacological effect**

Combined medicine, containing complex of biologically active substances. Possesses anti-inflammatory, antimicrobial and expectorant effects. Has anastaltic effect.

**Therapeutic indications**

In complex therapy of inflammatory disease of upper respiratory tract (tonsillitis, laryngitis, pharyngitis) and oral cavity (stomatitis, gingivitis).

**Contraindications**

Increased individual sensitivity to medicine components, pregnancy and breastfeeding, child age younger than 5 years old, acute nephritis, fructose intolerance, phenylketonuria.

**Posology and method of administration**

Topical. Keep in mouth until completely dispersed, without chewing.

Adults and adolescents older than 15 years: 6 tablets per day every 2 hours.

Kids aged 10 to 15 years: 4 tablets per day every 3 hours.

**Overdose**

No cases of overdose were observed until the present time.

**Side effects**

Allergic reactions are possible. Medicine use should be terminated if signs of allergic reaction emerge.

**Interaction with other drugs**

Use of sage medications might affect action of medicines, acting through γ-aminobutiric acid receptors (e.g., benzodiazepines, barbiturates). Concomitant use with such medicines is not recommended. Sage preparations may interact with hypoglycemic and anticonvulsant drugs, enhance sedative effect of other medicines and alcohol. Medicine could impact iron and other macro- and/or micronutrients absorption.

**Special indications**

In cases when symptoms of disease do not resolve or patients' condition deteriorates after 5–7 days of treatment, physician should be contacted.

**Effects on ability to drive and performing other activities**

Use of medication does not impact ability to conduct potentially harmful activities that require increased attention and rapid psychomotor reactions (driving vehicles, work work with machinery).

**Presentation**

Orodispersible tablets.

Polyvinyl chloride/A1 blisters of 10 tablets, 1, 2, 3, 4, or 5 blisters in carton with prescribing information.

**Storage conditions**

Store in temperature ≤ 25°C.

Keep out of children's' reach!

**Terms of distribution**

Over the counter.

## 4.2 Summary of non-clinical studies

### 4.2.1 Roots of althea

Althea roots contain 10% of mucilage, that is formed by mixture of pentozans and hexozans, approximately equal amount of pectin substances, starch (up to 37%), sucrose (up to 10%), fatty oil (1.5–2%) [2]. Softening effect of *radix Althaeae* is derived from high polysaccharide hydrocolloids that form protective layer at oral and pharyngeal mucosa, desensitize irritated peripheral receptors of airways, therefore restoring mucin layer function that leads to decrease irritation and inflammation grade [3].

Yamada et al. (1985) demonstrated that even in the highest tested concentration (100 µg/ml) polysaccharide fraction extracted from althea roots had slight anticomplementary effect *in vitro* (grade of total hemolytic complement inhibition was < 50%) [4].

Aqueous extracts of althea roots stimulated phagocytosis and release of oxygen radicals and leukotrienes from human neutrophils *in vitro* [5,6]. Aqueous extracts of althea roots *in vitro* also promoted elution of cytokines, interleukin (IL)-6, and tumor necrosis factor-α (TNF-α) from human monocytes, therefore demonstrating anti-inflammatory and immune stimulating effect [5,6].

Purified polysaccharides (polysaccharide contents > 95%) extracted from *Althaea officinalis* roots were tested for adhesive properties using isolated pig cheek mucosa. In this experiment

polysaccharides had demonstrated moderate adhesion to epithelium in concentration-dependent manner [7].

Althea root 10% decoction and methanole extract expose significant bacteriostatic activity towards many pathogens, leading to parodonthium disease: *Porphyromonas gingivalis, Prevotella spp., Actinomyces odontolyticus, Veilonella parvula, Eikenella corrodens, Fusobacterium nucleatum, Peptostreptococcus spp., Capnocytophaga gingivalis.* Метаноловый экстракт был особенно активным в отношении *Porphyromonas gingivalis, Prevotella spp.* и *Actinomyces odontolyticus* (minimal inhibitory concentration [MIC] ≤ 3125 mg/l). MICs were higher for decoction (4096–8192 mg/l) [7].

Mascolo et al. (1987) studied anti-inflammatory action of herbal extracts, including 80% althea root ethanol extract, in rats with paw edema induced by carrageenan injection. Authors concluded that administration of 80% althea root ethanol extract at a dose of 100 mg/kg did not prevent carrageenan-induced paw edema development [8]. However, in other experiment anti-inflammatory action of ointment, containing 20% of althea root extract, has been demonstrated: after application of ointment on the rabbit ear with inflammation induced by ultraviolet or tetrahydrofurfuryl alcohol, significant anti-inflammatory effect, that was less than one promoted by 0.5 dexamethasone ointment, was observed. Synergy of action was observed with concomitant use of study preparations [7].

Nosál'ova et al. (1992) studied antitussive action of polysaccharides, extracted from *Althea officinalis*, and *Althea officinalis* extract, in non-anesthetized cats of both sexes. Polysaccharide fraction at a dose of 50 mg/kg was as effective as *syrupus Althaeae* at a dose of 1000 mg/kg, moreover, it overcame action of prenoxdiazine at a dose of 30 mg/kg. Antitussive effect of polysaccharide fraction was less pronounced than that of droprozine, but more significant than action of *Althea officinalis* extract [9]. Ability of ramnogalacturonan, polysaccharide isolated from althea roots, to suppress cough was studied in male guinea pigs, sensitized or not sensitized by ovalbumin. Polysaccharide was administered at a dose of 25 or 50 mg/kg. Antitussive effect of ramnogalacturonan at highest dose was equal to that of codeine at a dose of 10 mg/kg *per os*. Duration of ramnogalacturonan action was shorter in sensitized animals [7].

In mice, median lethal dose ($LD_{50}$) of althea root polysaccharide extracts after oral administration was > 5000 mg/kg [7]. Extract demonstrated antiproliferative effect against lung cancer cell line (A549) [10]. In Ames test with *Salmonella typhimurium* (strains TA 98, TA 100, TA 1535, TA 1537, and TA 100) althea root preparations did not exhibit mutagenic action independent of metabolic activation [7].

### 4.2.2 Chamomile flowers

Camomile flowers (*flos Chamomillae*) contain ether oil (0.4–1.5%) with chamazulene fraction of 1–15%). Other components include α-bisabolol and related sesquiterpenes (up to 50% of oil). Apigenin and other flavonoid glycosides compose up to 8% (dry weight) of preparation [6].

Cinco et al. (1983) have studied antimicrobial activity of chamomile aqueous ethanolic solution in various concentrations. Aqueous ethanolic solution of chamomile completely inhibited growth of *Staphylococcus aureus*, *Streptococcus mutans* и *Streptococcus salivarius* (MIC = 10 mg/ml).

Authors noted that chamomile extract was most active against gram-positive bacteria, especially streptococci. Researches emphasized moderate, but statistically significant antibacterial activity towards *Candida albicans* ($p < 0.05$). Moreover, extract inhibited growth of *Trichomonas vaginalis* at a concentration of 2.5 mg/ml, while at a concentration 10 mg/ml death and lysis of bacteria were observed [11].

Aggag at al. (1972) demonstrate that chamomile ether oil *in vitro* inhibited *Staphylococcus aureus* and *Bacillus subtilis*. Chamomile oil with volume percentage (v/v %) calculated as component volume of total volume, of 0.025 v/v % did note demonstrate antimicrobial activity. However, with 0.7 v/v % oil added to medium containing *Staphylococcus aureus* or *Bacillus subtilis*, pronounced bacteriostatic effect was observed. At the same time, this concentration of oil did not affect growth of *Escherichia coli* and *Pseudomonas aeruginosa*. Death of all *Staphylococcus aureus* or *Candida albicans* was observed with 0.7 v/v % oil, and with 0.6 v/v % oil for *Bacillus subtilis* [12].

Chamomile extracts inhibit cyclooxygenase and lipoxygenase activity *in vitro* [6,13], therefore suppressing synthesis of prostaglandins and leukotrienes, that are known to induce inflammation. Bisabolol and bisabolol oxide inhibit 5-lipoxygenase, however, bisabolol is more active [6,14].

Tubaro et al. (1983) studied anti-inflammatory effect of medicinal chamomile in male Swiss albino mice experiments. Local inflammation was induced by application of 15 µl of 2.5% croton oil emulsion at right ear inner side. Chamomile extract at the doses of 0.08, 0.25, 0.75 mg/animal inhibited edema by 1.1% (no statistically significant difference with control), 8.5% ($p < 0.05$), and 23.4% ($p < 0.05$), respectively. Efficacy of chamomile extract at a dose of 0.75 mg was comparable to that of benzydamide at a dose of 0.45 mg, which reduced edema by 26.6% [15]. Results of the study were concordant with data of later study by Loggia et al. (1990) [16].

Intraperitoneal administration of chamomile disk flowers infusion to 12 female Swiss-NOS mice did not exhibit acute toxicity in any of the doses studied (720 or 1440 mg/kg) [17]. Chamomile extract only slightly influenced normal cells viability, but caused statistically significant inhibition of various human cancer cell lines' viability [18]. No mutagenic properties were detected *Salmonella typhimurium* TA-97a, TA-98, TA-100, and TA-104 strains regardless of metabolic activation [19].

### 4.2.3 Horsetail herb

Asian and North-American species of horsetail contain lots of quercetin 3-O-β-D-glucopyranozide (isoquercetin) at its malonate ethers. It also contains apigenin- and lutheolin-5-O-glycosides at its malonate ethers. Flavonoids of European chemotype are generally composed of quercetin-3-O-sophorozide together with genquanine and derivates of kaempferol [6].

Woo et al. (1979) have studied horsetail herb extract's antimicrobial activity. Addition of 500 µcg solution at disc inhibited growth of *Bacillus subtilis* and *Streptococcus faecalis*, however, extract did not impact growth of *Streptococcus aureus*, *Escherichia coli*, and *Candida albicans* [20].

Newton et al. (2002) have also studied antimicrobial activity of horsetail herb. Authors found out that MIC of methanol extract against *Mycobacterium aurum* and *Mycobacterium smegmatis* exceeded 500 µg/ml [21].

Radulovic et al. (2006) demonstrated that 1:10 diluted oil had very pronounced antimicrobial activity in wide spectrum, including: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enteritidis, Aspergillus niger,* and *Candida albicans* [22]. However, in other experiments aqueous ethanolic and ethanolic extracts of horsetail did not exhibit antifungal effect [6,23,24]. Horsetail herb ethanol extract (95%) in various concentration had antifungal activity against *Fusarium culmorum, Fusarium solani, Penicillium notatum,* and *Scopulariopsis species* [6].

Myagmar et al. (2000) demonstrated that horsetail herb aqueous extract promoted radical neutralization in rat liver microsomes cell culture [25], that is in concordance with results of later study of Nagai et al. (2005) [26].

Oh et al. (2004) have shown that components isolated from methanol extract (phenol petrozine onitine and lutheoline), exhibited hepatoprotectory properties *in vitro* towards cytotoxic action of tacrine in human hepatocyte cell line Hep G2. Median effective concentration ($EC_{50}$) was 85.8 ± 9.35 µmol/l and 20.2 ± 1.4 µmol/l, respectively [27].

Do Monte et al. (2004) studied antinociceptive (formalin assay) and anti-inflammatory action (carrageenan-induced paw edema) action of horsetail stem extract in mice. Antinociceptive action was dose-dependent and was not alleviated by naloxone, on the contrary to morphine. Statistically significant decrease of paw licking time was observed after extract administration at a dose of 50 or 1000 mg/kg. Extract at a dose of 100 mg/kg after intraperitoneal injection decreased grade of edema by 30% 2 h after inflammation induction. At a dose of 50 mg/kg edema decreased by 25 and 29% two and four hours after induction, respectively [28].

After intraperitoneal administration of horsetail herb aqueous ethanolic extract at a dose of 200 or 400 mg/kg to rats, latency time of convulsion induced by pentylenetetrazol increased, severity of seizures decreased as well as proportion of animals who developed convulsions (25 and 50% of animals after administration of 200 or 400 mg/kg dose, respectively). Extract also prevented animal mortality that confirmed anticonvulsant activity [29].

In rats, intraperitoneal injection of ethanol (50%) extract of horsetail herb at a dose of 2 or 5 g/kg led to death of 12 and 37.5% animals, respectively. Injection of extract at a dose of ≤ 1 g/kg did not cause any deaths. Since $LD_{50}$ was > 5 g/kg horsetail herb extract was considered non-toxic [30].

Intraperitoneal administration of horsetail herb extract to mice at a dose of 500–1000 mg/kg led to slight increase in spontaneous activity and frequency of convulsions [20]. There were no signs of chronic toxicity in rats that were orally treated with 100 mg/kg extract for 14 days [30]. In human lymphocytes, mild clastogenic effect of horsetail herb ethanol extract was observed: number of micronuclear cells in samples not exposed to radiation, increased, while in radiated cells it decreased. Another experiment demonstrated that 80% ethanol extract enhanced micronucleus formation (by 21% compared to control) [30,31].

### 4.2.4 Walnut leaves

Walnut leaves (*Juglandis folia*) contain tannins, ether oil, hydrojuglon rapidly oxidizing to juglon with bactericide action, carotene, vitamins C, PP, and B, aldehydes, alkaloids, flavonoids, coumarins, anthocyans, quinones. From walnut leaves 26 terpenoids were isolated by distillation [32].

Dauronas et al. (2010) have studied antioxidant properties of walnut leaves. Authors demonstrated that dry walnut leaf extract decreased number of lipid peroxidation products in incubation medium by 27.6% at a concentration of 50 mg/l, and by 60.8% at a concentration of 200 mg/l (compared to control) [33]. Later Zhao et al. (2014) received similar results [34].

Mollica et al. (2017) *in vitro* confirmed ability of walnut leaf dry extract (25 µl of 2 mg/ml solution) to inhibit α-amylase and α-glycosidase – enzymes responsible for starch cleavage to glucose [35].

Walnut leaf ethanol extract *in vitro* inhibited growth of both gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*) bacteria, as well as fungi (*Candida albicans, Candida glabrata, Candida krusei*), however it did not exhibit activity against *Bacillus subtilis* [36].

Mollica et al. (2017) studied impact of walnut leaf powder on diabetes caused by streprozocin in rats. Nutraceutical preparation administration led to body weight increase and blood glucose levels normalization: glycemia levels in animals that received walnut leaves, was equal to intact rats and ones receiving metformin. Walnut leaf did not cause nephrotoxicity and had positive impact on lipid profile [35]. Nasiri et al. (2017) in similar study demonstrated that administration of walnut leaves to rats with diabetes had neuroprotective effect [37].

$LD_{50}$ in mice was 6500 mg/kg [33]. Triterpenes isolated from leaves and branches of *Juglans sinensis* had antiproliferative properties towards malignancies, caused by ability to induce apoptosis [38]. Desoxyribonucleic acid (DNA) fragmentation study in DNA-comet assay demonstrated significant genotoxicity of *Juglans regia* extracts at a concentration of 250 ppm. On the contrary, Ames test reveal antimutagenic potential of walnut extracts towards various mutagens (4-nitro-O-phenyldiamide, sodium azide, and S9-dependent mutagen 2-aminofluororenone). In presence of S9 walnut extract was mutagenic in TA2637 *Salmonella typhimurium*, but did not possess mutagenic activity against TA98 and TA100 strains. In assays without metabolic activation, no mutagenic properties of walnut were observed in any of abovementioned strains [36].

### 4.2.5 Milfoil herb

Milfoil herb (*herba Millefolii*) contains 0.2–1.0% of ether oil. Since plant is chemically polymorphous conglomerate of herbal type, while chemical composition depends on chromosome layout. Diploid and tetraploid plants contain proazulene sequiterpenes, that transform to colored azulenes, including chamazulene (up to 25%) and achillicine, under thermal exposure. Other main components in tetraploid plants include β-pinene (23%), α-pinene (5%), and caryophyllene (10–22%). Hexaploid plants do not contain azulene sequiterpenes and contain approximately 50% of

mono- and sequiterpenes, many of that are oxidated, including camphor (19%0, sabinene (18%), 1,8-cineole (10%), and β-pinene (9%) among others [6].

Studies *in vitro* demonstrated that 50% ethanol extract of milfoil inhibited growth of *Shigella dysenteriae*, but did not affect *Escherichia coli* or *Salmonella enteritidis.* Similar activity was observed with a concentration of 50 µl/plate [39]. Methanol extract of aerial milfoil parts *in vitro* inhibited growth of 18 clinical isolates of *Helicobacter pylori* with MIC = 50 µg/ml [40].

Rekka et al. (1996) proved that chamazulene is active as antioxidant [41].

Lyss et al. (20002) studied ani-inflammatory properties of milfoil herb parts. Santamaryn, sequiterpene lactone from medicinal source, at a concentration of 100 µmol/l had moderate inhibitory action on nuclear factor-κB (NF-κB) transcription, that regulated transcription of inflammation mediators, such as cytokines and chemokines [6,42].

Goldberg et al. (1969) have studied ani-inflammatory action of various milfoil extract fraction in female mice experiments. Intraperitoneal injection of aqueous milfoil capitula extract fraction at a dose of 40.0 mg/kg inhibited paw edema (maximal by 35%), induced by injection of 0.125 mg of yeast into hind paw. Authors also evaluated local anti-inflammatory properties of extract fractions. In rabbits with skin irritation induced by sodium lauryl sulfate application, maximum decrease of irritation was 50% for one of the fractions [43].

Mascolo et al. (1987) proved that gavage administration of 80% ethanol extract of aerial milfoil parts to rats at a dose of 100 mg/kg inhibited paw edema, induced by carrageenan, by 29% [8]. Yasukawa et al. (1998) have studied ability of different herbal extracts (including milfoil extract) to reduce inflammation caused by application of 1 nmol 12-O-tetradecanoylphorbol-13-acetate diluted in 20 µl of acetone, to external side of ICR mice right ear. Authors noted that topical administration of extract at a dose of 1.0 mg/kg/ear led to slight anti-inflammatory effect: inhibition grade was 36% [44].

After intraperitoneal administration of milfoil aerial parts aqueous extract to rats $LD_{50}$ was 1.5 g/kg. In gavage or subcutaneous administration of flower extract to mice $LD_{50}$ exceeded 1 g/kg [6,45]. Single administration of milfoil extract to mice at dose 100 mg/kg led to diuretic effect, while urine turned dark-brown [43]. No signs of chronic toxicity were observed in mice and rat experiments [46].

Aqueous-alcoholic extract of milfoil at a concentration of 75 or 100 µg/ml decreased proliferation of various human cancer cell lines [47]. Bali et al. (2015) demonstrated that methanol milfoil extract was cytotoxic towards human prostate cancer cell line and promoted apoptosis [48].

Results of early reproductive toxicity studies of milfoil medicinal substrates are contradictive. In one study addition of 25–50% w/w milfoil to rat food inhibited estrus [49]. However, oral administration of leaf extract to rats did not affect time of first mating, fertility, and number of fetuses [6,50]. Tincture of milfoil raw drug material did not exhibit mutagenic action in Ames test at a concentration of 160 µl per disc against *Salmonella typhimurium* TA98 and TA100. Metabolic activation did not interfere results of assay [6,45]. Mice were administered with intraperitoneal injections of milfoil herb ethanolic extract at a dose of 200 mg/kg/day for 20 days or with aqueous-alcoholic at a dose of 300 mg/kg/day for 30 days. Metaphasic counts were increased in germinal epithelium [51].

### 4.2.6 Oak bark

Oak bark (*Quercus cortex*) contains 10–20% of tannins – derivatives of gallic and ellagic acids, 13–14% pentozans, up to 6% of pectin substances, quercetin, catechin, mucus, starch, and flobalen [32].

Antimicrobial and antifungal activity of 27 different pure tannins was studied *in vitro* against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Candida albicans,* and *Cryptococcus neoformans*. Only slight or moderate activity was observed towards abovementioned pathogens, however, gallic acid successfully inhibited *Cryptococcus neoformans* (MIC = 250 μg/ml). Oak bark methanolic extract in ethyl acetate or water had pronounced bactericide activity against *Staphylococcus aureus* [52].

Tarnuev (2010) have studied influence of oak bark extract in experimental gastric damage by cinchophen in rats. Hyperemia and flattening of gastric mucosa terrain were less pronounced in animal treated with oak bar extract and quercetin, compared to that of control rats. Mean size of erosion in rats that received oak bark extract an quercetin was statistically significantly lower than in control animals ($p < 0.05$) [53].

$LD_{50}$ of digallic acid after gavage administration to rats was 2.26 ± 0.083 g/kg. In deceased rats, liver necrosis, nephritis and acute gastroenteritis were observed [52]. Chronic toxicity of oak bark or its' preparations was not studied, still, toxicity of tannins was evaluated in broiler roosters. In the course of experiment pancreatic weight increased in birds that received food with increase tannin content, effect was dose-dependent. Moreover, dipeptidase and α-glycosidase activities were decreased in intestinal mucosa [52].

Frequent exposure to wood dust increases frequency of neoplasia's [52]. On the other hand, administration of ellagic acid with food to A/J mice inhibited oncogenesis, induced by 4-(metylnitrosamino)-1-(3-pirydyl)-1-butanole, by 54%. In other experiment, rates of methylbenzylnitrosamine-induced esophageal cancer decreased in rats that received ellagic acid enriched diet [54]. Oak tannins exhibited genotoxic properties in experiment with human lung embryonic cell line MRC-5. Oak wood caused chromosome aberrations without metabolic activation, while with S9 fraction (i.e. in metabolic activation conditions) no sign of genotoxicity were observed [52]. DNA-comet assay in Chinese hamster cell line B14 revealed that digallic, ellagic, and gallic acids were genotoxic and cytotoxic [52].

### 4.2.7 *Traxacum officinale* herb

Main components of milk-govan are sequiterpenes, including bitter eudesmanolides, tetrahydrodentine B, and taraxacolide β-D-glucopyranoside, and 11,13-dihydrotaraxic acid β-D-glucopyranoside. Taraxacoside, that is a derivative of *p*-hydroxyphenyl acetate; triterpenes, taraxasterol, ψ-taraxasteerol, and taraxerol; and also, inulin (2–40%) [6].

Study *in vitro* demonstrated that 95% ethanolic extract of milk-govan aerial parts at concentration 1.0 mg/ml did not inhibit growth of *Bacillus globifer*, *Bacillus mycoides*, *Bacillus subtilis*,

*Escherichia coli, Fusarium solani, Klebsiella pneumoniae, Penicillium notatum, Proteus morganii, Pseudomonas aeruginosa, Salmonella gallinarum, Serratia marcescens, Staphylococcus aureus, Mycobacterium smegmatis*, and *Candida albicans* [55,56]. Absence of antibacterial effect against *Escherichia coli, Salmonella enteritidis, Salmonella typhosa, Shigella dysenteriae*, or *Shigella flexneri* was also noted with 50% ethanolic extract of whole herb at dose 50 µl/dish [39].

Kim et al. (2000) found out that exposure of rat primary astrocyte culture with whole milk-govan aqueous extract at concentration 100 or 1000 µg/ml led to statistically significant ($p < 0.05$) inhibition of TNF-α release induced by bacterial lipopolysaccharide and P substance. Exposition with extract at 100 or 1000 µg/ml also decreased IL-1 synthesis on astrocytes, that were stimulated with bacterial lipopolysaccharide and P substance. Authors also noted, that *radix cum herba Taraxaci* could reduce TNF-α synthesis by inhibition of IL-1 production, therefore exhibiting anti-inflammatory effect [57].

Yasukawa et al. (1998) found out that use of milk-govan dry leaf methanolic extract at dose 2.0 mg/ear in mice reduced ear inflammation induced by 12-O-tetradecanoylforbol-13-acetate, by 69% [44]. Gavage administration of 95% ethanolic extract of whole plant to mice at dose 1.0 g/kg led to inhibition of convulsions, induced by benzoquinone [6,58]. Administration of whole plant 95% ethanolic extract into abdominal cavity at a dose of 100 mg/kg inhibited carrageenan-induced paw edema by 42% and decreased pain, as was seen by hot plate test, and also reduced severity of convulsions caused by benzoquinone [6,58].

Mascolo et al. (1987) proved that gavage administration of milk-govan dry root 80% ethanolic extract to rats at a dose of 100 mg/kg decreased carrageenan-induced paw edema by 25%, while indomethacin use at a dose of 5 mg/kg reduced edema by 45% [8].

Muto et al. (1994) demonstrated that gavage administration of aqueous, but not methanolic, milk-govan whole plant extract at a dose of 2 g/kg caused protective effect against ethanol-induced experimental gastric ulcer. However, methanolic extract did not cause similar effect [6,59].

Gavage administration of milk-govan root or herb 95% ethanolic extract at a dose of 8.0 ml/kg during 30 days increased diuresis, but decreased body weight of rats. Body weight loss reached 30%. In higher doses impact on diuresis and body weight were significantly less pronounced [60].

$LD_{50}$ of milk-govan herb extract after intraperitoneal administration to mice was 28.8 mg/kg [60]. Milk-govan extract had pronounced antiproliferative action towards Erlich's adenocarcinoma and Lewis lung carcinoma, and reduced number of metastasis. In other experiment it was demonstrated that *Taraxacum officinale* leaf extract prevented breast cancer (MCF-7/AZ line) from invasion into type I collagen [61].

### 4.2.8 Tonsilgon® N

Studies *in vitro* were conducted in order to compare immune modulatory influence of Imupret® (trade name for Tonsilgon® N in Ukraine) on tonsil cells with that of other herbal medicines, such as Immunal®, Proteflazid®, and Echinacea compositum. Researchers studied influence of abovementioned herbal medicines in various dilutions on content of lymphocyte, expressing

cluster of differentiation (CD) glycoprotein CD56 (natural killers). Results of experiments demonstrated, that Imupret® and, in lesser extent, Proteflazid® promoted increase in tissue CD56+ lymphocytes expression, while Imupret® was active in wider range of dilutions. Increase in natural killers activity was also observed with Imupret® [62].

Melnikov et al. (2005) during *in vitro* experiment with palatine tonsil cells of chronic tonsillitis patients demonstrated that Tonsilgon® N stimulated phagocytosis [63].

Melnikov et al. (2006) studied immunomodulatory action of Tonsilgon® N in Wistar laboratory rats. Animals were immunized with sheep erythrocytes and received study medicine for next five days according to manufacturer's guide in doses calculated on weight basis. In the course of experiment authors proven that Tonsilgon® N significantly activated antibody synthesis in animals' spleen, while its immunomodulatory activity was comparable to Thymogen. Number of antibody-producing cells was much higher in animals, receiving Tonsilgon® N, than in animals from control group. Moreover, researchers noted that during evaluation of natural cytotoxic cells' activity its destructive capability increased under Tonsilgon® N or Thymogen exposure [64].

Kovalenko et al. (2008) also studied immunomodulatory action of Tonsilgon® N herbal preparation *in vivo*. Male CBA mice and first-generation hybrids (F1), obtained after mating CBA and C57BL/6 mice with secondary immune deficiency, caused by cyclophosphamide intraperitoneal injection at a dose of 200 mg/kg, were used in the study. After 24 hours post-injection mice from study groups were administered with 3 doses of Tonsilgon® N, suspended in 1% starch solution, at a dose of 7 or 70 mg/kg. In CBA mice from control group, cyclophosphamide-induced immune deficiency was evident by statistically significant ($p < 0.001$) inhibition of humoral immune response after sheep erythrocyte injection by 28.1%. compared to intact mice. After triple oral administration of Tonsilgon® N at a dose of 7 or 70 mg/kg led to significant ($p < 0.001$) increase in antibody synthesis up to levels comparable with ones in intact animals [65].

## 4.3 Summary of clinical studies

Tonsilgon® N (Bionirica, Germany), oral drops, is indicated for treatment of acute and chronic diseases of upper respiratory tract (tonsillitis, pharyngitis, laryngitis) and prevention of VRIs complications, and as an addition to antibiotic treatment of bacterial infections. Various studies were conducted that proved efficacy of preparation for approved indications [32,66–71]. Smirnova (2001) compared efficacy of two herbal preparations use – Sinupret® and Tonsilgon® N for prevention and treatment of acute respiratory disease in sickly children aged 2 to 14 years. In children aged < 12 years old Tonsilgon® N was prescribed in liquid form (10–20 drops orally 3 times per day depending on age) with tea, juice, or water. For kids > 12 years of age Tonsilgon® N was prescribed in pills (1 pill 3 times/day), not chewing, with small amount of water. Sinupret® was also prescribed either in liquid or firm preparation depending on age. Therapy with Tonsilgon® N led to decrease in inflammation and edema, pharyngalgia, and improvement of tonsil tissue condition. The course of respiratory infection was improved and prevention of complications, i.e., eustachitis and/or otitis. Authors observed statistically significant ($p < 0.05$) decrease in yearly number of acute respiratory disease (ARD) episodes by 1.3-fold. In sickly children that received Tonsilgon® N phagocytosis activity increased, improvement in local immunity function was

observed as increase of $_{S}$IgA and lysozyme in saliva. At the same time, increase in immunoglobulins (Ig) A and M serum concentrations was detected. Moreover, Tonsilgon® N use, and Sinupret® as well, promoted increase of antiviral immunity in sickly children mostly by increase in IFN-α and, especially, IFN-γ [70].

Vavilova et al. (2017) presented results of non-interventional prospective study of Tonsilgon® N (drops and pills) efficacy and tolerance in treatment of various relapsing upper airways infections in children. Children, that were treated with Tonsilgon® N drops, received 10 drops 5–6 times per day (for kids aged 2–5 years) or 15 dops 5–6 times per day (for kids aged 6–11 years). When children were prescribed with Tonsilgon® N pills, following treatment scheme was applied: 1 pill 5–6 per day (for kids aged 6–11 years). Study treatment duration was 14 days. During 14-day treatment with Tonsilgon® N significant decrease in severity or complete resolution was observed for objective symptoms of upper respiratory tract infections: at visit 2 (approximately 15 days of study) hyperemia of mucosa was not observed in 93% of patients, tonsil edema – in 98% of kids (n = 516). The most frequent diagnoses in this group were tonsillitis and pharyngitis and were subjected for separate analysis. In most of patients with pharyngitis (n = 151) objective symptoms of mucosal hyperemia and tonsil edema resolved by visit 2. In 90.7% of kids (n = 137) hyperemia of mucosa completely resolved by visit 2, tonsil edema was not observed in 98.7% of kids (n = 149) by that time. Similar dynamic was observed in tonsillitis patients (n = 106): at visit 1 hyperemia of mucosa was noted in 106 cases (mostly of moderate severeness), in 91 kids (85.8%) this symptom was completely resolved by visit 2. Tonsil edema, that was registered in 106 patients at visit 1, resolved after 14 days in 103 (97.2%) kids. Adverse drug reaction (ADR) (limb urticaria) was registered in one child. Researchers found out history of allergy to *Chamomilla officinalis* in this patient. Treatment was terminated, and additional measures were not required [67].

Drynov et al. (2001) conducted clinical study in order to evaluate efficacy of Tonsilgon® N use in treatment of children aged 3 to 5 years with chronic tonsillitis. Patients received Tonsilgon® N for 6 months at 15–25 drops (dependent on age) three times per day. After one year post Tonsilgon® N treatment start in 20 (62.5%) out of 32 patients' therapy was evaluated as highly effective, in 9 (28.1%) – as effective, in 3 (9.3%) kids – as moderately effective. On average, efficacy score in overall patient population reached 2.53 ± 0.17 points. Authors did not note any cases of disease deterioration [71].

Abdulkerimov et al. (2018) evaluated efficacy of Tonsilgon® N oral drops in treatment of patients with subcompensated type of chronic tonsillitis concomitant to standard pharmacotherapy. In open randomized study 60 patients aged 18 to 55 years were enrolled. Patients from group 1 were prescribed with Tosilgon® N by 25 drops *per os* 3 times per day for 30 days together with tonsil lacunar rinse using Tonsillor device (10 procedures). Patients from group 2 were only treated with tonsil lacunar rinse using Tonsillor device (10 procedures). In comparative analysis of clinical efficacy of treatment with Tonsilgon® N and standard conservative therapy and physical cleansing of palatal tonsil lacunas, in the first case positive dynamic and effect from treatment at day 60 was noted in 86% of patients, in second case – only in 53%. Tolerability of treatment was good in all patients [66].

According to data of Fedorova (2014), Imupret® (Tonsilgon® N trade name in Ukraine) had pronounced preventive effect during seasonal increase in acute VRIs. Pilot study enrolled 365 pupils aged 6 to 11 years. Kids from experimental group received Imupret® 1 tablet 2 times per

day for 25 days. Kids in control groups either received influenza vaccine (control group 1) or did not receive Imupret® or influenza vaccine (control group 2). Frequency of acute VRIs in experimental group and control groups 1 and 2 was 16, 19, and 58%, respectively, while in kids with developed acute VRI duration of disease was 4.6, 5.3, and 7 days, respectively [32].

Climova et al. (2014) evaluated efficacy of Tonsilgon® N use in complex treatments of children aged 3 to 11 years old that were admitted to ear, nose and throat disease department with diagnosis of chronic adenoiditis exacerbation. All patients received systemic antibacterial treatment. Patients from main group additionally received Tonsilgon® N oral drops. At treatment day 5 nasal discharge (purulent, mucopurulent, or mucous) were not observed in 15 (48.85%) children from main group and in 10 (35.07%) of kids from control group ($p = 0.04$), at day 7 – in 62.5% of children from main group and in 53.33% of children from control group ($p = 0.04$). At day 9 no nasal discharge was observed in 29 (90.63%) of child's that received herbal preparation, at the same time in kids that only received standard treatment, this proportion was only 76.67% (25 kids) ($p = 0.01$). Night nasal breathing was improved by treatment day 5, intergroup difference was statistically significant ($p < 0.05$) favoring patients from main group at treatment day 7 [69].

Garaschenko et al. (2005) studied efficacy of treatment with Tonsilgon® N for seasonal prevention of acute VRIs and its' complications in organized children's groups. Tonsilgon® N was administered to 50 kids from 4$^{th}$ grade classes. Every class was divided into three groups: students from one group (16, 16, and 18 kids, respectively) received Tonsilgon® N (1 pill 2 times per day), children from group two were prescribed with other schemes of prevention (influenza vaccine, homeopathy etc.) or were not subjective for prevention ($n = 160$). During epidemics amongst kids that received Tonsilgon® N only 16% got sick, while in kids who did not receive prevention, 58% got sick. In most (87.5%) of children, who were treated with Tonsilgon® N, course of the disease was mild, while mild course of acute VRI was noted only in 51.7% of kids from no-prevention group. Approximately half of children (48.3%) who did not receive prevention, suffered severe influenza or acute VRI, mean number of days off school due to disease was 6 in whole group [68].

## 4.4 Potential risk and benefit of the study drugs for the study participant

Patients with acute viral tonsillopharyngitis (mild to moderate) or exacerbation of chronic tonsillopharyngitis.

Listings of known AEs that occur during use of study product Tonsilgon N , was placed in Section 4.1.1, for comparator product (Shalfej) – in Section 4.1.2.

In order to decrease frequency of AEs thorough evaluation of eligibility to inclusion criteria (Section 9.1) and non-inclusion (Section 9.2) before use of investigational/comparator product was planned in present study. In order to avoid possible impact of products on embryo study will only enroll women with negative pregnancy test who consent to comply with permitted effective methods of contraception during study participation (Section 10.3).

Blood sample collection for blood count may be associated with discomfort for patient, related to pain during injection, and possible bruising in venipuncture points. Significantly rarer in venipuncture spot infectious complication or systemic infection may develop. Vertigo and/or weakness may be observed during and soon after blood sample collection.

During pharyngoscopy patient may experience pressure at the base of the tongue and mild discomfort, during scarification sample collection from the surface of tonsils and throat – mild pain and dry heaving. Moreover, mechanical damage to oropharyngeal mucosa (scarification sample collection for local immunity assessment) and tonsil during inflammation may promote secondary bacterial infection and complicated presentation of tonsillopharyngitis.

Other study procedures, conducted in present protocol, including physical examination, are routine for general clinical practice. Frequency of those procedures does not create any discomfort for patient.

Based on collected experience, use of study product Tonsilgon N and comparator product Shalfej, orodispersible tablet (“Valeant” LLC), is effective for treatment of upper respiratory tract disease (tonsillitis, pharyngitis, laryngitis), that present as more rapid resolution of respective symptoms caused by local anti-inflammatory and antiseptic effects.

At the same time, it is known that use of study product Tonsilgon N may be associated with side effects: GI tract related AEs (nausea, vomiting) and allergic reactions. Use of comparator product Shalfej, orodispersible tablet (“Valeant” LLC) may also lead to allergic reactions. Allergic reactions may present threat for life of patients participating in the study.

Prior to signing Informed consent patients will be informed about possible risk and discomfort related to participation in the study, a well as need to inform Investigator about health issues in case it occurs. Investigator will thoroughly track patients’ condition in the course of all study. If required (e.g., in case of AE of SAE development), Investigator will make all necessary diagnostic test and/or prescribe required therapy.

Therefore, risks for patients in the currents study may be considered acceptable and minimal, while enrollment of patients into study will be rational from ethical point of view and safe.

Clinical study will be conducted in accordance with clinical study protocol and principles of good clinical practice (GCP) (directive of Russian Federation Ministry of Health [MH] from 01.04.2016 N 200n “On approval of good clinical practice principles”; National standard of Russian Federation GOSTR 52379-2005 “Good clinical Practice”; Principles of good clinical practice of Eurasian Economic Union [EAEU] [approved by Decision Eurasian economic commission Council from 03.11.2016 № 79]). Treatment of patients will be conducted by qualified medical staff taking into account up-to-date clinical guidelines for treatment of patients with acute viral tonsillopharyngitis or exacerbation of chronic tonsillopharyngitis. Diagnostic procedures and treatment will be free of charge for patients in current study. Therefore, potential benefit from study participation overcomes the risks related to investigational or comparator product use, as well as risk related to study procedures.

## 4.5 Brief description of dosage regimen and method of administration

According to laws and regulations, study will be performed with samples of studied medicines provided by Sponsor.

Investigational product (Tonsilgon® N, oral drops) and comparator product (Shalfej, orodispersible tablets) are medicinal products authorized in Russian Federation and approved for treatment of upper respiratory tract diseases. Dosing regimen and duration of treatment with those medicines planned for currents study are in accordance with guidance in latest actual instructions on use of products approved by Russian Federation MH.

Patients (n = 30) who will be assigned to treatment with investigation product (Tonsilgon® N, oral drops) will take medicine orally 30 minutes before or after the meal by 25 drops 6 times per day from day 1 till day 7 (inclusive). Medicine should be kept in mouth for 30 seconds before swallowing.

Patients (n = 30) assigned to group of treatment with comparator product (Shalfej, orodispersible tablets) will be obliged to take medicine 6 times per day, 6 tablets with 2-hour intervals from day 1 to till day 7 (inclusive). Tablets should be kept in mouth until fully dispersed. Medication should be taken 30 minutes before of after the meal.

## 4.6 Description of study population

Study will enroll adult patients (aged 18 to 55 years) during first 24 hours after symptom onset of acute viral tonsillopharyngitis or exacerbation of chronic tonsillopharyngitis.

Eligible patients who met all inclusion criteria and did not meet any of non-inclusion criteria will be randomized to groups for treatment with investigation or comparator product. Treatment duration will be 7 days.

Overall, it is planned to enroll 70 participants. For investigation product and comparator product groups, 30 patients each will be assigned. For control group, 10 healthy volunteers will be enrolled.

## 4.7 Justification of the clinical study

Issue of infectious diseases with its high contagiousness, rapid distribution impaction significant proportion of population, epidemic tension and need for use of modern anti-epidemic measures is one of most relevant problems worldwide. Respiratory tract diseases are leading in overall morbidity structure, while its' major part are acute VRIs and its complications of viral and mixed viral/bacterial nature: tracheitis, bronchitis, pneumonia. High acute VRI morbidity with development of severe, frequently lethal complications presents as serious socio-medical and economic issue with excessive healthcare organizations workload, demand for quarantine measures provision, economic damage caused by populations' temporary disability [32].

Frequent ARDs are leading in childhood morbidity structure, especially during first years of life. Frequency of appointments for different forms of ARDs depends on various factors and, by different authors' data, is 20 to 81% of child population. Acute forms of respiratory disease lead to structural changes in respiratory tract mucosa, breakdown of adaptive protection mechanisms, and development of early chronic disease of upper and lower respiratory tract, as well as other somatic diseases.

On average, every sickly child, depending on age, suffers from 3–4 to 5–6 ARD episodes annually. Among children visiting preschool institutions, sickly children are significant proportion (58.9%).

It is noteworthy that for 26.9% of cases not only frequent or relapsing diseases, but also persistent (from 7 to 14–18 days) respiratory tract infections. In such kids ARD presentation is severe and complicated by adenoiditis (85%), sinusitis (26%), tracheobronchitis (14%), and bronchitis (12.5%). These unique properties of ARD in sickly children are related to immaturity and impairment of immune system, and inhibition of non-specific resistance of growing body, as well [70,72–76].

Taking into account respiratory disease pathogenesis and different grades of immune impairment, major role in prevention and recovery in sickly children is taken by immune system rehabilitation using various immune modulators that affect different parts of immune system [70,75–77].

Transformation of infection/inflammatory process to chronic state with development of chronic infectious, infectious/allergic, or autoimmune disease also represents significant issue. More and more scientific data indicate involvement of immune system into development of various chronic diseases, tools for treatment and prevention of which are limited. In this context it is especially relevant to consider immune system as major part modulating chronic disease risk. With this background, increased interest for use of natural immune system modulators, developed from active substances of medicinal herbs, in order to prevent and treat various chronic diseases [32].

Many immune modulators are known by now: thymus preparations (Tactivin), preparations of microbial nature (Bronchomunal), synthetic peptides (Thymogen, Licopid, Polyoxidonium), interferon preparations (Leukinferon, Viferon), but immune modulators of herbal natures, Tonsilgon® N in particular, are of special interest [70,78]. One of main mechanisms of action for herbal medications' with complex impact on pathological processes is ability to change mode of immunological reactions – promote immune stimulating or immune modulatory effect that may be a key to immune rehabilitation [32]. Tonsilgon® N (Bionirica, Germany) – is a classic herbal medication for treatment and prevention of inflammatory disease of upper respiratory tract. Product has anti-inflammatory, antiseptic, immune modulating, antibacterial, and obductive properties. This multitude of properties is caused by herbal components of preparation: althea root, chamomile flowers, horsetail herb, walnut leaves, milfoil herb, oak bark, and milk-govan herb. Product is manufactured in forms of pills and drops, is well tolerated, can be combined with antibiotics and may be prescribed for children older than 1 year a [69]. Further study of immune modulating properties, as well as efficacy and safety variables of Tonsilgon® N will expand spectrum of knowledge on this product from "risk/benefit" point of view in patients with acute and chronic respiratory tract disease.

## 4.8 Regulatory framework of the clinical study

Current document is the protocol of clinical study, that is planned to be conducted in accordance with principles of Declaration of Helsinki of World Medical Association (WMA) (approved at 18 WMA Assembly in Helsinki in June 1964, last edition approved at 64 Assembly in Fortaleza in October 2013), three-sided agreement on GCP, approved by international conference on harmonization E6(R2) (International Conference on Harmonization, ICH E6(R2) from 09.11.2016 г.) and regulated by current regulations of EAEU and laws of Russian Federation:

- Rules of registration and expertise of drugs for medical use (approved by Decision of Eurasian economic Commission from 3 November 2016 № 78);
- Rules of good clinical practice of EAEU (approved by Decision of Eurasian economic Commission from 3 November 2016 № 79);
- Federal law from 12.04.2010 № 61-FZ “On drug circulation” (in actual edition);
- Federal law from 27.07.2006 № 152-FZ “On personal data” (in actual edition);
- Order No. 200н of the Ministry of Health of the Russian Federation On approval of the rules of Good Clinical Practice dated April 01, 2016
- National Standard of the Russian Federation GOST R 52379-2005 Good Clinical Practice
- Resolution No. 714 of the Government of the Russian Federation On approval of the standard rules of compulsory life and health insurance for patients participating in clinical studies of a medicinal product dated September 13, 2010 (current version)
- Order No. 986н of the Ministry of Health of the Russian Federation On approval of the Regulations on the Ethics Council dated November 29, 2012
- Order No. 878 of the Ministry of Health of the Russian Federation On the composition of the Ethics Council dated October 30, 2017
- Order No. 202н of the Ministry of Health of the Russian Federation On approval of the Procedure for posting information about the composition of the Ethics Council, its work plans and current activities on the official website of the Ministry of Health of the Russian Federation in the information and telecommunications network Internet dated May 7, 2018.

# 5 Purpose and objective of clinical study

1. To evaluate influence of Tonsilgon N drops intake on throat local immunity parameters (secretory immunoglobulin A [$_{S}$IgA], interferon (IFN)-α, tumor necrosis factor (TNF), lysozyme, lactoferrin, (IL)-1, -6, -8, -10, and -17);
2. To evaluate clinical efficacy of tonsillopharyngitis treatment with Tonsilgon N drops (Bionirica SE) compared to Shalfej, orodispersible tablets (“Valeant” LLC)
3. To evaluate seriousness and probability of adverse events (AEs) and serious adverse events (SAEs) in study groups. Pregnancy cases data will be collected in separate.

# 6 Description of general plan (design) of clinical study

## 6.1 Primary and secondary endpoints

***Primary efficacy endpoinst:***

**Primary endpoint:**

Dynamic of tonsillopharyngitis severity by change of TSS score at day 4 compared to day 0 ($TSS_{d4} - TSS_{d0}$).

**Secondary endpoints:**

- Proportion of patients achieved clinically significant improvement (TSS score ≤ 5) at study day 4;
- Severity of pain ~~and~~ or discomfort in throat by TSS subscale – proportion of patients with every grade of severity at baseline and day 4;
- Time until complete resolution of every symptom evaluated by patients' diary;
- Proportion (%) of patients completely recovered by day 4 (disease outcome by objective evaluation of Study Doctor);
- Dynamic of every tonsillopharyngitis symptom severity by 4-point scale (0 to 3) at visit 2 compared to visit 1;
- Dynamic of every tonsillopharyngitis symptom severity by 4-point scale (0 to 3) at visit 3 compared to visit 1;
- Dynamic of local immunity markers (sIgA, TNF, IFN~~secretory immunoglobulin A, tumor necrosis factor, interferon~~-α, lysozyme, lactoferrin, ~~interleukin~~IL-1, -6, -8, -10, and-17) in mucosa of tonsils and back pharynx at visit 2 compared to visit 1;
- Dynamic of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 3 compared to visit 1;
- Dynamic of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 4 compared to visit 1;
- Proportion of patients (%), in which contents of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 2 was equivalent to values in healthy persons (if deviation was detected at visit 1);
- Proportion of patients (%), in which contents of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 3 was equivalent to values in healthy persons (if deviation was detected at visit 1);
- Proportion of patients (%), in which contents of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and-17~~secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17~~) in mucosa of tonsils and back pharynx at visit 4 was equivalent to values in healthy persons (if deviation was detected at visit 1).

***Primary safety endpoints:***

- Frequency of AEs and SAEs ~~adverse events and serious adverse events~~ registration in treatment groups;



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- Frequency of AEs and SAEs ~~adverse events and serious adverse events,~~ related to use of study drugs~~treatments~~;
- Evaluation of vital signs;
- Physical examination.

Description of AEs ~~adverse events~~ will be presented according to following scheme:

- Description of AE~~adverse event~~;
- Severity of presentation;
- Duration;
- Relation to study drugs~~treatment~~;
- Outcome.

## 6.2 Study design

### 6.2.1 Study type

Stud is planned as interventional, open-label, randomized, single-center comparative clinical trial in parallel groups.

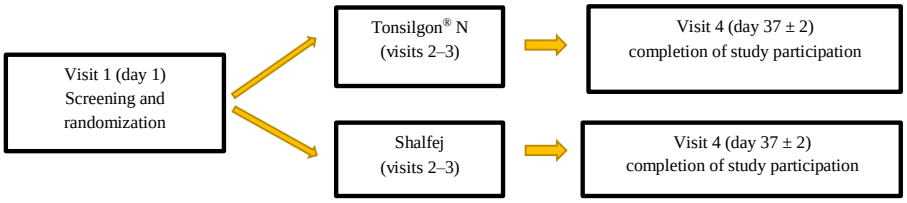
Randomization – measure aimed to minimize subjectivity by random assignment of patient. Randomization will prevent subjective, including unwilling, investigator's selection of patients to one of groups, that will significantly differ from population, enrolled to second group.

Open type of study is related to substantial difference in appearance a method of administration of investigation product (oral drops) and comparator product (orodispersible tablets), that excludes complete blinding of study doctor and patient.

In present study efficacy of tonsillopharyngitis treatment with Tonsilgon N will be evaluated as well as its' impact on selected parameters of oropharyngeal local immunity. To assess efficacy of treatment routine clinical methods will be used: pharyngoscopy, physical examination, patient survey, and dynamic evaluation of tonsillopharyngitis symptoms by patient. To assess influence of Tonsilgon N on local oropharyngeal immunity invasive diagnostic method (scarification) of upper mucosa layer of tonsils and pharynx back scarification will be used with following assay for secretory immunoglobulin A, interferon-α, tumor necrosis factor, lysozyme, lactoferrin, interleukins-1, -6, -8, -10, and -17. To evaluate normal values of indicated parameters in tonsil and back pharynx mucosa samples of 10 healthy volunteers will be examined.

As a primary endpoint, dynamic of Tonsillopharyngitis Severity Score (TSS) points will be used. Questionnaire is used in clinical practice to assess dynamic in symptom pronunciation during infectious-inflammatory process in throat [1]. TSS scale version of contract research organization Appletree AG was adapted for purposes of current study (see Section 6.2.4.7).

### 6.2.2 Study flow chart




*Note: healthy volunteers are not included in the scheme, since they will be evaluated one time.*

## 6.2.3 Tabulated schedule of study procedures

### I. Schedule of study procedures for patients:

| Study procedures | Treatment period | | | Follow-up period | Unscheduled visit[1] | Premature discontinuation visit |
| --- | --- | --- | --- | --- | --- | --- |
| | Visit 1 Day 1 | Visit 2 Day 4 | Visit 3 Day 8±1 | Visit 4 Day 37±2 | | |
| Signing of the informed consent form | X | | | | | |
| Demographic, main disease medical history, past and concomitant diseases, prior therapy data collection | X | | | | | |
| Patient complaints collection | X | X | X | X | X | X |
| Concomitant therapy registration | X | X | X | X | X | X |
| Vital signs evaluation (blood pressure, heart rate, respiratory rate, body temperature) | X | X | X | X | X | X |
| Physical examination | X | X | X | X | X | X |
| Pharyngoscopy | X | X | X | X | X | X |
| McIsaac scale assessment | X | | | | | |
| Streptatest | X | | | | | |
| Blood count[2] | X | | X | | X[3] | X[3] |
| Pregnancy test for females with preserved reproductive potential | X | | | | | |
| Inclusion/non-inclusion criteria evaluation | X | | | | | |
| Randomization | X | | | | | |
| Scarification sample collection of upper mucosa layer of tonsils and back pharynx[4] | X | X | X | X | | X |
| Dispensing of study drug/comparator drug | X | | | | | |
| Return of unused drugs and used blisters (for comparator drug) | | | X | | | X[5] |
| Dispensing of patients' Diary | X | | | | | |
| Evaluation of patients' Diary | | X | X | X | X | X |
| Return of patients' Diary to investigator | | | | X | | X |
| Compliance assessment by data from patients' diary | | X | X | | | X[5] |
| AE/SAE registration | X[6] | X | X | X | X | X |
| Exclusion criteria evaluation | | X | X | | X[5] | |

[1] During unscheduled visit, any necessary additional procedures may be performed at investigators' judgement.
[2] Hemoglobin, hematocrit, erythrocytes, thrombocytes, leukocytes with formula, erythrocyte sedimentation rate.
[3] If clinical indications are presents (by investigators' judgement)
[4] To evaluate sIgA, IFN-α, TNF-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17.
[5] If visit performed prior to visit 3.
[6] AEs related to study procedures during the visit are evaluated.

**II. Healthy volunteer examination will be performed single time:**

| Study procedures | Visit 1 Day 1 |
|---|---|
| Signing of the informed consent form | **X** |
| Subject complaints collection | **X** |
| Medical history collection | **X** |
| Demographic (sex, age, race) | **X** |
| Vital signs evaluation (blood pressure, heart rate, respiratory rate, body temperature) | **X** |
| Concomitant therapy registration | **X** |
| Physical examination | **X** |
| Pharyngoscopy | **X** |
| Blood count | **X** |
| Inclusion/non-inclusion criteria evaluation | **X** |
| Scarification sample collection of upper mucosa layer of tonsils and back pharynx[7] | **X** |

[7] To evaluate sIgA, IFN-α, TNF-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17.

## 6.2.4 Study procedures

#### *6.2.4.1 Medical history, demographics, anthropometrics*

During medical history collection attention should be paid to prior diseases, concomitant chronic diseases, genetics, chronic intoxications (smoking status, alcohol consumption, narcotics), allergological history, prior surgeries and traumas, continuous and intermittent medicinal therapy. Method of contraception permitted by protocol and used by participant across whole study and following month should be emphasized.

Sex, age, race, and ethnicity should be noted in source documents.

#### *6.2.4.2 Physical examination*

Physical examination will be conducted according to general principles of internal disease management: consequently, general examination, skin and mucosa's examination, including pharyngoscopy, lymph nodes palpation, musculoskeletal system evaluation, palpation, percussion and auscultation of main organ systems (cardiovascular, respiratory, digestive, urinary).

#### *6.2.4.3 Pharyngoscopy*

Pharyngoscopy will be carried out at all visits according to clinical center standards. Patient will be asked to widely open mouth, tongue is lowered by the spatula if necessary, for better view. Study doctor will examine back of pharynx, palate, palatoglossal (anterior) and palatopharyngeal (posterior) palatal arched, palatal tonsils.

#### *6.2.4.4 Laboratory testing*

Blood samples for the tests will be obtained in the morning, after the overnight fasting (8-10 hours after the last meal), from the cubital vein, using aseptic/antiseptic technique. Approximately 5 ml of blood will be drawn for blood count. Blood count will be performed at Visits 1 and 3, and as indicated – at Unscheduled and Premature discontinuation Visits.

Blood count will include assessment of following parameters: hemoglobin, hematocrit, erythrocyte count, thrombocyte count, leukocyte count with formula, erythrocyte sedimentation rate.

A urine pregnancy test with a test strip is performed in all women of childbearing potential at screening.

Samples obtained by scarification probe of upper layer of palatal tonsils and back of pharynx mucosa will be assayed for sIgA, IFN-α, TNF-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17.

#### *6.2.4.5 Assessment of vital signs*

The evaluation of the vital signs (heart rate, pulse rate, blood pressure, body temperature) is performed before the physical examination, at rest (after 15 minutes of rest, at least one hour after smoking and two hours after meal). The pulse rate is determined on the radial artery (or on the carotid artery if the pulsation on the radial artery is weak) over one minute, with the patient sitting. The respiratory rate is determined over one minute at rest, with the patient lying, by counting the respiratory movements of the chest or abdominal wall, without attracting the patient's attention.

Blood pressure measurements will be performed on the brachial artery, with the patient sitting, by the Korotkoff method, using a certified sphygmomanometer or tonometer with a cuff that will have a length and width matched to the length and circumference of the patient's upper arm.

#### *6.2.4.6 Evaluation of streptococcal pharyngitis probability by McIsaac scale*

Evaluation of streptococcal pharyngitis probability by McIsaac scale will be performed at screening.

| **Criterion** | | **Score (point)** |
| --- | --- | --- |
| Body temperature > 38°C | | 1 |
| Absence of cough | | 1 |
| Tender anterior cervical adenopathy | | 1 |
| Tonsillar swelling and exudate | | 1 |
| Age (years) | 3–14 | 1 |
| | 15–44 | 0 |
| | ≥ 45 | –1 |

Total score for each criterion calculated. Only patients with McIsaac scale score ≤ 1 (0 or 1) may enter the study.

#### *6.2.4.7 Filling-out TSS questionnaire*

Questionnaire (point scale) TSS for physician is used in clinical practice and clinical studies to assess dynamic in symptom pronunciation during infectious-inflammatory process in throat (Bereznoy VV, Riley DS, Wassmer G, Heger M. Efficacy of extract of Pelargonium sidoides in children with acute non-group A beta-hemolytic streptococcus tonsillopharyngitis: a randomized, double-blind, placebo-controlled trial [1]). One of such studies with TSS use was conducted in 2005 in Germany by contract research organization (CRO) Appletree AG, whose version was adapted for purposes of current protocol.

TSS (Tonsillopharyngitis Severity Score) is a questionnaire for evaluation of presence and severity of following symptoms: throat pain, difficulty to swallow, salivation, mucosa hyperemia, hyperthermia by 4-point scale:

- 0 = no symptom;
- 1 = mild symptom;
- 2 = moderate symptom;
- 3 = significant symptom.

Body temperature measured in armpit will be classified as following:

- 0 points < 37.5 °C;
- 1 point from 37.5 to < 38.5 °C;
- 2 points from 38.5 to < 39.5 °C;
- 3 points ≥ 39.5 °C.

TSS will be filled out by study doctor based on patients' complaints and physical examination. Study doctor will summarize every symptom score to count TSS total score. Sum of TSS points

will be evaluated at screening to assess inclusion criteria and baseline tonsillopharyngitis severity, and at visits 2 and 3 as well (Day 4 and Day 8 ± 1) to assess efficacy of treatment in both study groups.

#### *6.2.4.8 Streptatest*

Smear from tonsils, oropharynx and all inflamed/exudative areas will be performed using special swab and tongue forceps. While smearing, attention should be paid to prevent saliva contact with swab. Test should be caried out immediately after sample collection with Streptatest® test strip. Positive result for streptococcus group A: in control and test zones two purple stripes are visible. Absence of strips in control and test zones means that analysis was performed incorrect and shall be repeated.

#### *6.2.4.9 Scarification sample collection of upper mucosa layer of tonsils and back pharynx*

### 6.2.5 Visit procedures

#### *6.2.5.1 Visit 1 (day 1)*

- Signing the informed consent form to participate in the study
- Demographic, main disease medical history, past and concomitant diseases, prior therapy data collection
- Patient complaints collection
- Concomitant therapy registration
- Vital signs evaluation (blood pressure, heart rate, respiratory rate, body temperature)
- Physical examination
- Pharyngoscopy
- McIsaac scale assessment
- Streptatest
- Blood count
- Pregnancy test for females with preserved reproductive potential
- Inclusion/non-inclusion criteria evaluation
- Randomization
- Scarification sample collection of upper mucosa layer of tonsils and back pharynx[1]
- Dispensing of study drug/comparator drug
- Dispensing of patients' Diary

7 Registration of AEs/SAEs

#### *7.1.1.1 Visit 2*

- Patients' complaints collection
- Concomitant therapy registration
- Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature)
- Physical examination
- Pharyngoscopy
- Scarification sample collection of upper mucosa layer of tonsils and back pharynx
- Evaluation of patients' Diary
- Compliance assessment by data from patients' diary
- Registration of AEs/SAEs

8 Exclusion criteria evaluation

#### *8.1.1.1 Visit 3 (day 8 ± 1)*

- Patients' complaints collection
- Concomitant therapy registration
- Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature)

[1] For evaluation of secretory immunoglobulin A, interferon-α, tumor necrosis factor, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and -17.

- Physical examination
- Pharyngoscopy
- Blood count
- Scarification sample collection of upper mucosa layer of tonsils and back pharynx
- Return of unused drugs and used blisters (for comparator drug)
- Evaluation of patients' Diary
- Compliance assessment by data from patients' diary
- Registration of AEs/SAEs
- Exclusion criteria evaluation

#### *8.1.1.2 Visit 4 (day 37 ± 2)*

- Patients' complaints collection
- Concomitant therapy registration
- Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature)
- Physical examination
- Pharyngoscopy
- Scarification sample collection of upper mucosa layer of tonsils and back pharynx
- Return of patients' Diary
- Evaluation of patients' Diary
- Registration of AEs/SAEs

#### *8.1.1.3 Unscheduled visit*

- Patients' complaints collection
- Concomitant therapy registration
- Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature)
- Physical examination
- Pharyngoscopy
- Blood count[2]
- Evaluation of patients' Diary
- Registration of AEs/SAEs
- Evaluation of exclusion criteria

#### *8.1.1.4 Premature discontinuation visit*

- Patients' complaints collection
- Concomitant therapy registration
- Evaluation of vital signs (blood pressure, heart rate, respiratory rate, body temperature)
- Physical examination

[2] In case of clinical indications (by Study Doctor's opinion)

- Pharyngoscopy
- Scarification sample collection of upper mucosa layer of tonsils and back pharynx
- Return of unused drugs and used blisters (for comparator drug)[3]
- Return of patients' Diary
- Evaluation of patients' Diary
- Compliance assessment by data from patients' diary
- Registration of AEs/SAEs ~~adverse events/serious adverse events~~ ~~Оценка Дневника пациента~~
- 

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## 8.2 Measures taken to minimize subjectivity

The study will be open-label, interventional, randomized, single-centered, comparative clinical trial in parallel groups. Randomization (random allocation of patients into study groups) will be performed using table, pre-generated by block method (with variable block size), envelope method.

## 8.3 Study drug administration regimen

Patients who will be allocated to treatment with investigation product (Tonsilgon® N, oral drops) will take medicine orally 30 minutes before or after the meal by 25 drops 6 times per day from day 1 till day 7 (inclusive). Medicine should be kept in mouth for 30 seconds before swallowing.

Patients assigned to group of treatment with comparator product (Shalfej, orodispersible tablets) will be obliged to take medicine 6 times per day, 6 tablets with 2-hour intervals from day 1 to till day 7 (inclusive). Tablets should be kept in mouth until fully dispersed. Medication should be taken 30 minutes before or after the meal.

## 8.4 Dosage form, packaging, and labeling of the study drugs

This study is post authorization one, all medications are authorized and will be used on approved indications. Medicinal products will of industrial series in manufacturers' packaging will be used in the current study. In addition to manufacture label, containing information on name, pharmaceutical form, method of administration, composition, series number, shelf life, and manufacturer, every package will be marked by sticker with following information: note "clinical trial use only", clinical trial protocol number, name and contacts of sponsor, full name of investigator, number of study center.

## 8.5 Duration of patients' participation in the study

Maximum duration of participation in the present study will be 39 days.

## 8.6 Rules of termination of some parts of the clinical study and/or the clinical study as a whole

The study can be stopped for the following reasons:

1. At the initiative of the sponsor:

[3] If visit is conducted prior to visit 3.

a. Obtaining new toxicological or pharmacological data, or SAE data, which necessitate reconsideration of the previous benefit-risk estimate of participation in the study;
b. The frequency of AEs and/or their severity do not allow continuation of the study;
c. Other reasons, including administrative ones;

2. At the initiative of the investigator: for example, the frequency of AEs and/or their severity unacceptably increase the risk of patients' participation in the study.
3. By decision of regulatory authorities.

If the study is stopped prematurely, the sponsor is obliged to notify the staff of the study sites, as well as the regulatory authorities, specifying the reason for the premature termination of the study.

The rules for terminating the study and/or treatment for each study participant are listed in Section 9.3.

## 8.7 Study drug accountability procedures

The study sites will be provided with a investigation and comparator products amount sufficient for conducting the study with the number of patients planned for screening.

The responsible employee of the study site keeps a study drug log specifying the receipt of the drugs at the study site, dispensation of the drugs to study participants, and the return of unused drugs. The storage conditions are indicated as well. The study drug can be used only for the purposes of this clinical study.

Employees of the sponsor or regulatory authorities can check the study drug accounting log, as well as the availability of the drug, during the monitoring/audit of the study site. The drug should be stored in compliance with the storage conditions in an area accessed only by the study site employees responsible for the distribution of the drug.

## 8.8 Storage of randomization codes and unblinding procedures

At the randomization visit, the patient will be assigned a randomization code at the time of randomization. The patient's randomization code is indicated in the documents intended for use outside the study site (electronic Case Report Form [eCRF], SAE reports, etc.). The assigned randomization code of the patient is entered into the eCRF and the relevant forms. The patient's randomization code is not changed during the course of the study.

Randomization plan will be stored for control access. Study staff is responsible for compliance of randomization to applied rules.

Randomization plan will be evident for study doctor, principal investigator, Sponsor, and regulatory authorities (if SAE observed).

## 8.9 A list of data reported in the Case Report Form (without prior recording in written or electronic form) and considered as source data

All data obtained in the framework of this clinical study will be pre-recorded in writing and/or electronically (whichever is applicable in the health center) in the source medical documentation,

and subsequently entered into the eCRF. No data will be recorded directly in the eCRF without prior registration in the source documentation.

# 9 Inclusion, non-inclusion, and exclusion criteria

## 9.1 Inclusion criteria

Study will enroll patients fulfilling all following criteria:

1. Males and females aged 18 to 55 (inclusive);
2. Diagnosis at inclusion – “mild acute tonsillopharyngitis” or “chronic tonsillopharyngitis exacerbation”;
3. Body temperature measured in armpit ≤ 37.5°C;
4. Tonsillopharyngitis severity by TSS ≥ 8 points;
5. Time from first symptoms’ onset until visit to physician – not more than 24 hours;
6. Signed informed consent form;
7. For females with childbearing potential and males – consent to use effective method of contraception across all study and following 1 month after its completion.

Healthy volunteers’ inclusion criteria:

1. Males and females aged 18 to 55 (inclusive);
2. Absence of acute disease signs;
3. Absence of chronic disease by medical history data;
4. Volunteer did not receive any medicines during 30 days prior to visit;
5. Absence of deviations from normal in physical examination and by vital signs assessment;
6. Absence of deviations from normal values in blood count.

## 9.2 Non-inclusion criteria

Patients applying to at least one of the following criteria will not be enrolled:

1. Any signs and symptoms of bacterial (streptococcal) tonsillopharyngitis (McIsaac scale score > 1);
2. Positive result of express-test Streptatest;
3. Consumption of antibiotics during < 48 hours prior to inclusion;
4. Patients earlier received tonsillectomy or tonsillotomy;
5. Use of local treatments for oropharyngeal disease (aerosols, gargle solutions, tablets/orodispersible tablets/lozenges) during 24 hours prior to inclusion and/or impossibility to discontinue use of any local treatments, except used in study, during study course;
6. Use of systemic, inhaled or nasal glucocorticosteroids during 30 days prior to study start, injectable corticosteroids – during 3 months prior to study start and/or plans to use glucocorticosteroids (except topical dermal ones) during the course of study;
7. Impossibility to withdraw for study period any medicinal preparations that could influence result of current study, e.g., antiviral medicines, or preparations incompatible with study treatments (see Section “Prohibited concomitant therapies”);
8. Pharyngitis granulosa;
9. Signs of fungal oropharyngeal infection (white caseous plaques easy removable by pallet);
10. Clinical signs of diphtheria;

11. Presence of signs of sinusitis, otitis, eustachitis, laryngitis, tracheitis, bronchitis (since indicated conditions could demand indication of medicines, that could possibly affect evaluation of study results; it is acceptable to include patients with rhinitis with use of therapies permitted by the Protocol);
12. Vaccination of patient conducted in 30 days prior to inclusion;
13. Assumed low patients' compliance with treatment or inability to undergo procedures and follow restrictions according to study protocol (e.g., as a result of psychiatric disorders);
14. Clinically meaningful deviations of blood count, including any of the following signs: leukocytosis > $9x10^9$/L, neutrophilia > 78%, band neutrophil content > 6% or presence of younger neutrophil forms, erythrocyte sedimentation rate > 30 mm/h;
15. Liver diseases;
16. History of craniocerebral injury;
17. Brain diseases;
18. Any cardiovascular, kidney, liver, gastrointestinal (GI), endocrine, and nervous system diseases or any other diseases/conditions that, by Study Doctor's opinion, could lead to unsafety of patients participation in the study;
19. Any concomitant diseases that require use of medications influencing immune system (immune system modulators, stimulators, suppressors) or antibiotics;
20. Need to use medications that act through γ-aminobutyric acid receptors (e.g., barbiturates and benzodiazepines);
21. Pregnant, lactating women or women planning pregnancy during next two months;
22. Women of reproductive age, that did not confirm use of highly effective contraception methods (combined oral contraceptives, double barrier method);
23. Misuse of alcohol, or use of other psychoactive substances
24. Known hypersensitivity for any component of Tonsilgon N (chamomile, althea, oak bark, taraxacum, horsetail, walnut, milfoil, plants of *Compositae* family) or salvia and other related herbs (*Asteraceae* family).

## 9.3 Exclusion criteria

1. Negative dynamic of disease with signs of secondary bacterial infection (onset of any McIsaac scale criteria absent at inclusion or other signs that are considered as bacterial infection signs by the Study Doctor);
2. Need for surgical treatment;
3. Study Doctor decided to exclude patient for his/her good;
4. Withdrawal of informed consent (unwilling to continue participation in the study);
5. Serious deviation from the study protocol;
6. Individual intolerance of study drugs;
7. Development of AE or SAE event that requires diagnostics and/or treatment that could significantly alter current study procedures;
8. Patient does not follow the rules of study participation;
9. Erroneous inclusion (e.g., patient was enrolled with violation of inclusion/non-inclusion criteria of Protocol);
10. Presence of non-inclusion criteria during study conduct;

11. Patient receives/needs to receive additional treatment, that could influence study result or affect patients' safety (see Section «Prohibited concomitant medications");
12. Other conditions or events requiring discontinuation of patient from the study, by Study Doctors' opinion.

Unveiling of reasons for exclusion from study in enrolled volunteers leads to subject's withdrawal at the moment when such causes were discovered. Every healthy volunteers' premature discontinuation case should be documented with obligatory indication of reason for premature discontinuation/discontinuation from study, and Sponsor should be notified within 24 hours.
Volunteers may participate in the study only once. Volunteers that discontinued study, cannot participate it repeatedly. If volunteer have decided to refuse study participation or did not complete it for some reason, then his/her randomization ID will not be used again.

# 10 Information about medicinal products used in the clinical study

## 10.1 Study drug and reference drug

**Study drug:** Tonsilgon® N, oral drops, 100 ml.
**Comparator drug**: Shalfej, orodispersible tablets.

## 10.2 Compliance assessment

Compliance control will be carried out by records in patients' Diary, as well as control of unused product and utilized blisters (for comparator).

## 10.3 Allowed concomitant therapies

It is allowed to use any medicinal products other than those listed in Section 10.4.

Across all study and 30 days after completion female participants of childbearing potential, and male participants whose partners are of childbearing potential, should use effective contraception methods.

Effective contraception methods are:

- Complete abstention;
- Oral contraceptives (combined progestogen-containing, progestogen-only);
- Injectable progestogen;
- Levonorgestrel implants;
- Estrogen-containing vaginal ring;
- Contraceptive transdermal plasters;
- Intrauterine device (IUD) or intrauterine spiral (IUS) that complies to efficacy criteria, indicated in guidance for use;
- Male-partner is sterile (vasectomy with documented azoospermia) before female enrollment, if male-partner is the only partner of patient. For this term "documented" is related to examination carried out by investigator/patients' responsible person or medical

history overview for study inclusion eligibility, that was received orally from patient or during medical records overview;

- Double barrier method: condom or occlusion cap (diaphragm or cervical/arched caps) plus spermicide (foam/gel/cream/suppository).

Female is incapable of conception after documented surgical sterilization, and in natural menopause lasting at least 2 years.

Male participants should use adequate contraception method with their partner after informed consent signature until completion of study and for 30 days after.

## 10.4Prohibited concomitant therapies

Use of any medication indicated for treatment of conditions other than current disease (acute tonsillopharyngitis or exacerbation of chronic tonsillopharyngitis) and not indicated in non-inclusion criteria, as well as in the list below:

1. Hypoglycemic medications;
2. Anticonvulsant medications;
3. Sedative medications, including barbiturate and benzodiazepines;
4. Medications, affecting γ-aminobutiric acid receptors;
5. Glucocorticosteroids in any dosage form, except ones for dermal topical use;
6. Immune system modulators, stimulators;
7. Immune system suppressors;
8. Antivirals;
9. Antibiotic medications with systemic exposure or local effect at tonsils and pharynx;
10. Antifungal medications with systemic exposure or local effect on tonsils and pharynx;
11. Any medications for topical therapy (aerosols, sprays, inhaled medicines, gargles, tablets/orodispersible tablets/lozenges/troches) for pharyngeal or nasal application.

# 11 List of efficacy endpoints

**Primary endpoint:**

Dynamic of tonsillopharyngitis severity by change of TSS score at day 4 compared to day 0 ($TSS_{d4}$ – $TSS_{d0}$).

**Secondary endpoints:**

- Proportion of patients achieved clinically significant improvement (TSS score ≤ 5) at study day 4;
- Severity of pain ~~and~~ or discomfort in throat by TSS subscale – proportion of patients with every grade of severity at baseline and day 4;
- Time until complete resolution of every symptom evaluated by patients' diary;
- Proportion (%) of patients completely recovered by day 4 (disease outcome by objective evaluation of Study Doctor);
- Dynamic of every tonsillopharyngitis symptom severity by 4-point scale (0 to 3) at visit 2 compared to visit 1;
- Dynamic of every tonsillopharyngitis symptom severity by 4-point scale (0 to 3) at visit 3 compared to visit 1;
- Dynamic of local immunity markers (~~sIgA, TNF, IFN-α, salivary immunoglobulin A, mucosal factor interferon-α~~, lysozyme, lactoferrin, ~~interleukin~~ IL-1, -6, -8, -10, and -17) in mucosa of tonsils and back pharynx at visit 2 compared to visit 1;
- Dynamic of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17 ~~salivary immunoglobulin A, mucosal factor interferon-α~~) in mucosa of tonsils and back pharynx at visit 3 compared to visit 1;
- Dynamic of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17 ~~salivary immunoglobulin A, mucosal factor interferon-α~~) in mucosa of tonsils and back pharynx at visit 4 compared to visit 1;
- Proportion of patients (%), in which contents of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17 ~~salivary immunoglobulin A, mucosal factor interferon-α~~) in mucosa of tonsils and back pharynx at visit 2 was equivalent to values in healthy persons (if deviation was detected at visit 1);
- Proportion of patients (%), in which contents of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17 ~~salivary immunoglobulin A, mucosal factor interferon-α~~) in mucosa of tonsils and back pharynx at visit 3 was equivalent to values in healthy persons (if deviation was detected at visit 1);
- Proportion of patients (%), in which contents of local immunity markers (sIgA, TNF, IFN-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17 ~~salivary immunoglobulin A, mucosal factor interferon-α~~) in mucosa of tonsils and back pharynx at visit 4 was equivalent to values in healthy persons (if deviation was detected at visit 1).

# 12 Evaluation of safety endpoints

## 12.1 List of safety endpoints

Following safety endpoints will be evaluated:

- Frequency of AEs and SAEs ~~adverse events and serious adverse events~~ registration in treatment groups;
- Frequency of AEs and SAEs ~~adverse events and serious adverse events,~~ related to use of study drugs ~~treatments~~;
- Evaluation of vital signs;
- Physical examination.

Description of AEs ~~adverse events~~ will be presented according to following scheme:

- Description of AE~~adverse event~~;
- Severity of presentation;
- Duration;
- Relation to study drugs~~treatment~~;
- Outcome.

AEs will be coded based on the Medical Dictionary for Regulatory Activities (MedDRA)

## 12.2 Methods and timeframes for the assessment, recording, and analysis of safety endpoints

The methods used for the evaluation of safety endpoints are detailed in Section 6.2.4. The safety assessments will be conducted throughout the study (see the scheduled procedures by visits in Section 6.2.3).

## 12.3 Requirements for the reports, registration, and reporting of adverse events and intercurrent diseases

### 12.3.1 Definition of adverse events and serious adverse events

Adverse event: Any untoward medical event occurring in a participant of a clinical study after the administration of a medicinal product, which can have no causal relationship with the administration of the drug. Thus, an AE can be any untoward symptom (including a laboratory abnormality), complaint, or disease, temporally associated with the use of a medicinal product, regardless of the causal relationship with the medicinal product.

A SAE is any unfavorable medical event that:

- resulted in death;
- is life-threatening;
- results in disability or incapacity;
- is a congenital anomaly or birth defect;
- requires hospitalization or prolongation of existing hospitalization;
- is medically significant: the decision whether expedited reporting is appropriate in other situations (e.g., in the case of medically significant events which are not directly life-threatening for the study participant and do not lead to death or hospitalization but place the patient at risk or require a medical intervention to prevent any of the above conditions) is made based on medical and scientific judgment. Such cases should usually be considered SAEs.

When referring to AEs, the term “severity” is used to describe the intensity of a specific event and is not synonymous to the term “seriousness”. The reports adding important information on

specificity, increase in incidence or severity of known, previously documented serious adverse events (side effects) are AE reports.

Information on currently known side effects of this study drug is presented in the Investigator's Brochure. It will be included in the Patient Information Leaflet and must be discussed with the study subjects.

### 12.3.2 AE severity

The term “severity” is used to describe the intensity of a specific event. “Severity” must be distinguished from “seriousness”. Throughout the study, the Investigator will identify AEs and classify them as follows:

- mild AE: transient or mild discomfort that does not affect the daily activities of the study subject; no medical intervention/therapy is required;
- moderate AE: mild to moderate activity limitation; help from another person may be required; no or minimal medical intervention/therapy is required;
- severe AE: extensive activity limitation, usually requiring help from another person; medical intervention/therapy is required, possibly hospitalization; any SAE;

### 12.3.3 Causal relationship to study drug

WHO causality scale will be used for causal relationship assessment:

| | |
| --- | --- |
| Certain | Event or laboratory test abnormality, with plausible time relationship to drug intake. Cannot be explained by disease or other drugs. Response to withdrawal plausible (pharmacologically, pathologically). Event definitive pharmacologically or phenomenologically (i.e., an objective and specific medical disorder or a recognized pharmacological phenomenon). Rechallenge satisfactory, if necessary. |
| Probable/Likely | Event or laboratory test abnormality, with reasonable time relationship to drug intake. Unlikely to be attributed to disease or other drugs. Response to withdrawal clinically reasonable. Rechallenge not required. |
| Possible | Event or laboratory test abnormality, with reasonable time relationship to drug intake. Could also be explained by disease or other drugs. Information on drug withdrawal may be lacking or unclear. |
| Unlikely | Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible). Disease or other drugs provide plausible explanations. |
| Conditional/Unclassified | Event or laboratory test abnormality. More data for proper assessment needed. Additional data under examination. |
| Unassessable/Unclassifiable | Report suggesting an adverse reaction. Cannot be judged because information is insufficient or contradictory. Data cannot be supplemented or verified. |

### 12.3.4 Adverse event recording

Any AEs (both non-serious and serious), occurring from the time of signing the specific informed consent form for this study until the end of the study must be recorded in the source medical documents and entered into the dedicated eCRF pages.

To detect AEs, study subjects must be asked leading questions at each study visit. AEs may also be detected when the subjects report them on their own initiative during visits or in the periods between visits and when detected during examination, based on the results of laboratory tests and other assessments. All AEs must be treated as appropriate.

The measures taken for the treatment of AEs must be recorded and classified into one of the following categories: “no actions taken”, “administration of medication (concomitant therapy)”, or “other treatment”. The actions taken in relation to the study drug must also be documented and classified into one of the following categories: “no changes”, “discontinuation”, “dose reduction”, “dose escalation”, “temporary withdrawal”, “unknown actions”, and “not applicable”. Concomitant therapy, other treatment methods and changes in the use of the study drug must also be recorded.

Pre-existing medical conditions/diseases prior to the beginning of the administration of the study drugs are recorded as concomitant diseases but may also be registered as AEs, only if they worsen after the subject enrollment in the study. Abnormal laboratory findings or investigation results are considered AEs only if they cause clinical signs and symptoms, are clinically relevant, and require treatment.

AEs must be assessed at each visit (or more frequently, if indications are present) for any changes in severity, suspected relationship to the study drug, interventions carried out for its treatment, and outcome.

***AEs occurring between the signing of the informed consent and the first dosing of the study drug***

In the period from the signing of the informed consent and the first dosing of the study drug, only SAEs are planned to be registered.

***AEs occurring after the first dosing of the study drug***

The investigator must monitor all the AEs and SAEs occurring from the administration of the first dose of the study drug, during the study, and up to the last study visit of the subject, at which the final outcome of the event is assessed and entered into the eCRF.

***Ongoing SAEs at the time of the last visit***

If an AE is ongoing at the time of the last visit, the Investigator must continue to follow-up the patient until the AE resolves or stabilizes/enters a permanent state.

The Investigator must submit SAE follow-up reports as shown in Section 12.3.5 SAE Reporting below.

***SAEs occurring after the last visit***

At the last visit, the Investigator must instruct each subject to report any new AEs (outside the follow-up period of the protocol) occurring within 30 days from the end of participation in the study. The Investigator must report SAEs as shown in Section 12.3.5 SAE reporting below.

***Clinically relevant laboratory abnormalities at the time of the last visit***

The Investigator must document clinically relevant laboratory abnormalities identified at the last visit as AEs, even if a relationship with the use of the specific study drug cannot be established.

### 12.3.5 Serious adverse event reporting

It is extremely important that the Investigator immediately (i.e., within 24 hours from the moment of becoming aware of a SAE) reports any serious AE, even if the Investigator does not consider it to be a treatment-related SAE.

SAEs should be reported by sending a scanned copy of the completed Serious Adverse Event Reporting Form as initial or follow-up report. Form should be sent by fax or email to sponsor representative with copy to manager responsible for the study, using contact information from table below.

The Investigator must also submit all the updates/new information using a new SAE Reporting Form to follow up on the previously reported SAE. Subsequent information must specify whether the event has resolved or is ongoing, whether a diagnosis has been established, whether treatment has been administered, and if yes, which treatment, and whether the subject continues to participate in the study or was discontinued.

Recurrent episodes, complications, or progression of the initial SAE must be reported as additional information to the initial SAE report, regardless of how much time has passed from the initial SAE.

Any new SAE considered to be completely unrelated to a previously reported one must be reported as a new and separate event.

The Investigator must provide the Sponsor with answers to any questions regarding the SAE reports within 24 hours.

The Investigator must keep in the Investigator's File for this study the notifications on information delivery to all recipients.

Contact information for SAE reporting::

| | |
| --- | --- |
| Full name: | |
| Position: | |
| Organization name: | |
| Address: | |
| Phone: | |
| E-mail: | |

**Reporting to health authorities**

The Sponsor shall submit all the reports to the concerned regulatory authorities within the established timeframes.

**Training of Investigators**

By signing the study protocol, the Principal Investigator confirms that he/she has received training and was informed of the Sponsor's requirements and his/her obligations on reporting SAEs/AEs and pregnancy cases, as specified in the study protocol.

## 12.4Pregnancy

The Investigator must immediately (i.e., within 24 hours after becoming aware of a pregnancy case) inform the Sponsor and the manager responsible for the study, as specified in Section 12.3.5, of any cases of pregnancy occurring during the clinical study in its subjects or partners of its subjects. Pregnancy cases are registered only from the time of the first dosing.

The Investigator must immediately discontinue the pregnant subject from further participation in the study and follow-up the pregnancy to determine its outcome, including spontaneous or elective abortion, detailed information on delivery and any birth defects, congenital malformations, or complications in the mother and/or the newborn.

Pregnancy cases must be recorded on the Clinical Trial Pregnancy Reporting Form. Additional information about the pregnancy must be recorded on the same form and must include the potential relationship of any pregnancy outcome with the use of the study drug.

Any SAEs occurring during pregnancy must be reported using the SAE Reporting Form.

## 12.5Method and duration of subject follow-up after an adverse event

An identified AE must be followed-up until its resolution or until it is considered to be permanent. The outcome of the event must be recorded and classified into one of the following categories: recovered without sequelae, recovered with sequelae, recovering, unchanged/not resolved, death, unknown outcome.

# 13 Description of statistical methods

## 13.1 Description of analysis methods

The statistical analysis will be conducted using specialized software, which will be chosen when the statistical analysis plan is finalized.

Quantitative data will be presented as the number of observations, arithmetic mean, 95% confidence interval (CI) for the mean (unless otherwise specified), standard deviation, median, interquartile range (25 and 75 centiles), minimum, and maximum.

Ordinal, categorical, and qualitative data will be presented as absolute frequencies (number of observations), relative frequencies (propotions), and 95% CIs (unless otherwise specified).

The risk variables (relative risk, odds ratio, and hazard ratio for survival) will be presented as point estimates and 95% CIs.

If other no noted in statistical analysis plan, statistical tests will be performed with two-side 5% significance level.

Previous and concomitant diseases, as well as adverse events will be coded using the current version of the MedDRA classifier (Russian version, 22.0 or higher). Prior and concomitant medications will be described with ATC classifier.

This section briefly describes the planned analysis. Full analysis will be detailed in the statistical analysis plan.

### 13.1.1 Demographic and other baseline characteristics (comparability of groups for the analysis)

Demographic variables, baseline and follow-up data such as medical history, concomitant disease (classified by MedDRA) and concomitant medications will be described by groups in ITT population + healthy volunteers' population (for baseline variables). In addition to all three groups characterization, combined information will be presented on treatment group (without division for treatment methods).

For all applicable baseline parameters (including sIgA, IFN-α, TNF-α, lysozyme, lactoferrin, IL-1, -6, -8, -10, and -17) comparison with healthy subject group and overall treatment group will be performed. For quantitative variables t-test and Mann-Whitney test will be used (depending on the results of distribution assessment, that will be carried out by Shapiro-Wilk test), for quantitative variables – Fisher's exact test.

### 13.1.2 Primary efficacy endpoint analysis

Dynamic of tonsillopharyngitis severity by change of TSS score at day 4 compared to day 0 ($TSS_{d4}$ – $TSS_{d0}$) was chosen as primary efficacy endpoint in the present study. There will be no dynamic evaluation for healthy volunteers, therefore, no data for such comparison will be collected.

For intergroup comparison by variable ($TSS_{d4}$ – $TSS_{d0}$) covariate analysis (ANCOVA) will be used, baseline TSS will serve as covariate, while study group will be factor. If covariate analysis will reveal statistically significant difference, two-side 95% confidence interval will be calculated by least square means method. Intergroup differences will be considered significant if both 95% confidence interval borders will exceed 0.

Primary efficacy endpoint analysis will be carried out in ITT population with additional analysis in PP sample.

### 13.1.3 Secondary endpoint analysis

Following efficacy endpoints will be presented as quantitative variables:

- Dynamic of local immunity markers: secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17 in mucosa of tonsils and back pharynx at visit 3 compared to visit 1;
- Dynamic of local immunity markers: secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17 in mucosa of tonsils and back pharynx at visit 4 compared to visit 1;
- Severity of pain and or discomfort in throat by TSS subscale – proportion of patients with every grade of severity at baseline and day 4;

For these variables t-test and Mann-Whitney test will be used (depending on the results of distribution assessment, that will be carried out by Shapiro-Wilk test), for quantitative variables – Fisher's exact test.

Following efficacy endpoints will be presented as qualitative variables:

- Proportion of patients achieved clinically significant improvement (TSS score ≤ 5) at day 4;
- Proportion (%) of patients completely recovered by day 4 (disease outcome by objective evaluation of Study Doctor);
- Dynamic of every tonsillopharyngitis symptom severity by 4-point scale (0 to 3) at visit 2 compared to visit 1;
- Dynamic of every tonsillopharyngitis symptom severity by 4-point scale (0 to 3) at visit 3 compared to visit 1;
- Dynamic of local immunity markers: secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17 in mucosa of tonsils and back pharynx at visit 2 compared to visit 1;
- Proportion of patients (%), in which contents of local immunity markers (secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17) in mucosa of tonsils and back pharynx at visit 2 was equivalent to values in healthy persons (if deviation was detected at visit 1);

- Proportion of patients (%), in which contents of local immunity markers (secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17) in mucosa of tonsils and back pharynx at visit 3 was equivalent to values in healthy persons (if deviation was detected at visit 1);
- Proportion of patients (%), in which contents of local immunity markers (secretory immunoglobulin A, tumor necrosis factor, interferon-α, lysozyme, lactoferrin, interleukin-1, -6, -8, -10, and-17) in mucosa of tonsils and back pharynx at visit 4 was equivalent to values in healthy persons (if deviation was detected at visit 1).

Comparison between groups will be made with Fisher's criterion.

To evaluate time required for every symptom resolution detected by patients' Diary, Kaplan-Mayer curves will be constructed for every group, comparison will be done with log-rank test. Secondary efficacy endpoint analysis will be carried out in ITT population with additional analysis in PP sample.

### 13.1.4 Safety endpoint analysis

Safety assessments will include the following:

- Frequency of AEs and/or SAEs in treatment groups, including:
- Rate of treatment-related AEs/SAEs;
- Vital signs dynamic
- Results of physical examination,

For quantitative variables t-test and Mann-Whitney test will be used (depending on the results of distribution assessment, that will be carried out by Shapiro-Wilk test), for quantitative variables – Fisher's exact test.

Safety endpoints will be analyzed in safety population.

## 13.2 Interim analysis

No interim analysis is planned for this study.

## 13.3 Planned number of clinical study subjects and sample size justification

Sample size calculation was based on results of study NCT03095521, published at clinicaltrials.gov website (https://clinicaltrials.gov/ct2/show/study/NCT03095521). In this study comparison between Angal lozenges and Anti-Angin lozenges (both containing chlorhexidine and lidocaine) was performed. Change in total TSS score by day 4 was 6 point on average, while standard deviation for score change was 3 points. Based on the assumption that placebo effect at current disease is around 50% of medicine effect (i.e., 3 points), effect size is approximately equal to one.

In the present study it is expected that effect size will be less and at least 0,4, taking into account herbal nature of study medicine and comparator.

Method based on covariate analysis (ANCOVA) with correction for baseline TSS score was used for sample size calculation. All calculations were made in G*Power software version 3.1

**F tests –** ANCOVA: Fixed effects, main effects and interactions

| | | |
| --- | --- | --- |
| **Analysis:** | A priori: Compute required sample size | |
| **Input:** | Effect size f | = 0.4 |
| | α err prob | = 0.05 |
| | Power (1−β err prob) | = 0.8 |
| | Numerator df | = 2 |
| | Number of groups | = 3 |
| | Number of covariates | = 1 |
| **Output:** | Noncentrality parameter λ | = 10.2400000 |
| | Critical F | = 3.1504113 |
| | Denominator df | = 60 |
| | Total sample size | = 64 |
| | Actual power | = 0.8044215 |

Therefore, total size of three groups should be at least 64 complete cases in order to observe expected effect towards primary endpoint. Considering possibility of 10% patients' withdrawal during the study 70 patients should be enrolled: 10 patients will compose control group, 60 – treatment group, divided in two subgroups (30 patients each).

## 13.4The significance level used for the study

All types of statistical analyses in current study will be performed with two-side significance level of 5% ($p < 0.05$ is considered statistically significant).

## 13.5Clinical study termination criteria

Study may be terminated if conditions, described in Section 8.6 of current protocol.

## 13.6Management of missing, non-evaluable, and uncertain data

During the monitoring visits to the study site, clinical research associates (monitors) authorized by the Sponsor will review the eCRFs of study subjects for the absence of required data. If information contained in the source documents is missing from the eCRF, queries and instructions on the necessary corrections will be submitted to the Investigator.

During the review of the database of study results, the statistician designated by the Sponsor and the Data Control and Processing Managers will check for uncertain, missing or non-evaluable data, which may also result in the submission of queries to the Investigators.

If possible, the Investigators will correct the errors identified in the eCRFs and will inform the Principal Investigator and the Sponsor designees. If the identified data errors cannot be corrected after the subjects completed their participation in the study, a sensitivity analysis of the resulting values to the presence of uncertain data will be conducted as part of the statistical analysis. Information about missing, uncertain, and non-evaluable data will be presented in the Final Clinical Study Report.

Analysis of all endpoints and other variables will be conducted with present data only without imputation, what is caused by short duration of treatment.

## 13.7Reporting of any deviations from the initial statistical plan

All deviations from the initial statistical plan must be described and justified in a protocol amendment and/or the Final Clinical Study Report (in the latter case, the statistical analysis plan developed before the beginning of the final statistical analysis must include the list of these deviations and the reasons for deviating from the statistical plan as per protocol).

## 13.8Selection of clinical study subjects for analysis

### 13.8.1 Population selected by assigned treatment (ITT)

ITT, also known as full analysis set (FAS), sample includes all randomized patients exposed to the study drug. This is the main dataset for primary and secondary endpoint.

### 13.8.2 Per-protocol population (PP)

The primary and secondary efficacy endpoints will be additionally analyzed using the per-protocol population of the study (PP). Subjects will be excluded from the PP population in the following cases:

- If they meet the exclusion criteria.
- If they use prohibited concomitant therapies.
- In case of other significant protocol deviations deemed as considerably affecting the primary efficacy evaluation for the subject.

### 13.8.3 Safety population

The dataset for safety evaluation is identical to the full analysis set (FAS) and includes all the subjects exposed to at least one dose of the study drug, reference drug, or recommended standard therapy, without exceptions. However, unlike the intention-to-treat population, subject data is analyzed based on actual treatment received (if it is different from the treatment to which the

subject was assigned at randomization). All types of safety analysis will be based on the safety analysis population.

### 13.8.4 Healthy volunteers' population

Data from all volunteers that have undergone signal diagnostic procedure as per protocol.

All decisions about planned data analyses should be taken before database lock.

# 14 Description of quality control and quality assurance measures

## 14.1Study monitoring and quality control

The monitoring of the clinical study according to the monitoring plan and standard operation procedures (SOP) before the beginning, during, and at the end of the study ensures the successful conduct of the study and guarantees the collection of accurate date, timely detection of potential errors, documentation of the clinical study process, protection of the rights of study subjects, and compliance with Good Clinical Practice guidelines (ICH GCP), international legislative requirements and the legislation of the Russian Federation.
The monitoring of the study sites within this study is planned to be conducted remotely. Routine remote study monitoring includes:

- confirmation that the obtaining of the informed consent, screening and subject enrollment are conducted and documented as appropriate;
- verification of data entered in the eCRF and the source documents of the study subjects;
- confirmation that adverse reactions occurring during the study are recorded and timely reported;
- confirmation that the study staff follows the requirements for diagnostic and treatment procedures specified in the study protocol;
- confirmation that supply, storage, dispensing, and disposal of the study drug and materials are documented;
- confirmation that the employees of the study site and external laboratory have the competencies required for the study;
- confirmation that the diagnostic and laboratory equipment meets the requirements for safe and appropriate use within the study;
- confirmation that the Investigator communicates with the Local Ethics Committee on safety issues and Sponsor-approved protocol amendments.

The quality control of the study results is ensured by the Sponsor’s employees/designated person maintaining the electronic database of the study, who identify non-compliances, erroneous data, and missing data during the cross-review of all eCRFs. If any questions or the need for clarification arise, a special form (clarification query) is submitted to the Investigator by e-mail/fax, and the query must be answered in written within 7 days of its delivery.

In compliance with regulatory requirements, the Sponsor or competent authorities have the right to verify (audit) the logistics of the study and study documentation. The Investigator must ensure direct access to all source documents and all necessary information to persons authorized to conduct audits or inspections.

## 14.2 Protocol amendments

The signatures of the Investigators on the Protocol Signature Page represent a written confirmation of the consent to conduct the study in accordance with this Protocol. When conducting the clinical study, the study materials may be changed and supplemented. Such changes and additions are considered amendments.

A protocol amendment is a written description of changes or a formal explanation of the text of the clinical study protocol. Amendments may be either substantial or non-substantial. Prior to implementation, any protocol amendments must be approved in compliance with internal SOPs of the Sponsor and subsequently approved by regulatory authorities and IEC and signed by the Investigator.

The Decree No. 775 of the Ministry of Health of the Russian Federation "On the Approval of the Procedure for Reviewing a Report on the Need for Amendments to the Protocol of a Clinical Study of a Medicinal Product for Human Use", dated August 31, 2010, provides the list of substantial/non-substantial amendments and the procedure for the submission of relevant materials for expert examination.

Amendments to the materials of the clinical study are considered substantial if they may affect the objectives, organization, methodology, statistical methods for the processing of the clinical study results, and measures for ensuring the safety of the study subjects.

Amendments to the materials of the clinical study are considered non-substantial if they do not affect the objectives, organization, methodology, statistical methods for the processing of the clinical study results, and measures for ensuring the safety of the study subjects.

If any amendments must be made to this protocol, the study Sponsor submits to the Ministry of Health of the Russian Federation a report on the need to amend the clinical study protocol. After reviewing the provided updated materials, the Ministry of Health of the Russian Federation makes a decision to approve or reject the proposed amendments. Protocol amendments must be kept together with the initial version of the protocol. The number and date of amendment must be indicated on the title page of the protocol.

## 14.3 Protocol deviations

A protocol deviation is the failure to comply with the approved study protocol.

A major protocol deviation is a deviation which, according to the Investigator or the Investigator's designee, may lead to the discontinuation of the subject from the study or the exclusion of subject data from the clinical and/or statistical part of the study. Deviations which are not classified as major are considered minor protocol deviations.

The study site personnel and/or the study monitor (if present at the study site) must inform the Sponsor about major protocol deviations as soon as possible. The Sponsor may suggest to re-classify the protocol deviation (minor to major or vice versa) based on the conducted review. In this case, the Sponsor's classification prevails and must be presented to the study site personnel together with a written justification.

The Sponsor must be informed of minor protocol deviations.

Notifications and reports on protocol deviations are submitted to competent authorities and the corresponding ethics committee in compliance with applicable requirements/guidelines/law.

**Documentation of protocol deviations**

The Investigator or his/her designee must document and explain any deviation from the approved study protocol. In exceptional cases, the Sponsor may be informed of protocol deviations orally

(if an immediate action/notification is required), which must be followed by a written notification (e.g., by e-mail; in a message on the course of a study period). All protocol deviations must be described in the Final Clinical Study Report.

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# 15 The description of the ethical conduct of the clinical study

## 15.1 General provisions

This study will be conducted in accordance with the World Medical Association's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, adopted in 1964, with its subsequent modifications and additions; National Standard of the Russian Federation GOST R52379-2005 "Good Clinical Practice" dated September 25, 2005; Federal Law No. 61-FZ "On Circulation of Medicines" dated April 12, 2010 (current revision), and ICH guidelines, including E6 Good Clinical Practice (ICH E6 GCP).

## 15.2 Informed consent process

Prior to inclusion in the study, the patients are provided written information and oral explanation of the goals, objectives, and methods of the study, as well as the expected benefits and possible risks associated with participation in the study. In addition, the patients must be notified of the voluntary nature of their participation in the study and their right to stop participating at any time, and of the fact that this decision would not affect the quality of medical care provided to them. The patients do not have to explain the reasons for withdrawal from the study, however, the Investigator should try to find out what these reasons are, without violating the patient's rights. The informed consent should be obtained prior to any study-related procedures.

The data obtained during the study shall be processed while respecting the confidentiality of subject data. The subjects must be informed of the planned computer data processing and the conditions for publishing such data (e.g., presentation at medical conferences, in journal papers, and other public sources), presented only in an aggregate form, which do not allow the identification of the subjects.

The subjects must be informed that authorized representatives of health authorities and the Sponsor will have access to their confidential medical information for monitoring, inspection, and audit purposes. In such cases, strict confidentiality and non-disclosure of any subject identifying information must be ensured.

The informed consent form (Patient Information Leaflet) must be completed in two copies, and personally signed and dated by the subject and Investigator. The Investigator must keep one copy of the signed informed consent form in the Investigator's File and hand over the second copy to the subject.

## 15.3Confidentiality and identification of study subjects

The confidentiality of identifying information of the subjects will be ensured whilst respecting the privacy and personal data protection rights in compliance with regulatory requirements. Any identifiable patient records will be kept confidential and can be disclosed only to the extent permitted by law. If study results are published, personal subject data will remain confidential.

## 15.4Enrollment of subjects from vulnerable and special populations

The inclusion/non-inclusion criteria do not provide for the participation of subjects from vulnerable populations in this study.

Groups of special interest in the current study are women with childbearing potential that according to inclusion criteria may only be enrolled into study, if they agree to use effective contraception that was noted in patient information sheet and confirmed by informed consent form signature.

# 16 Description of data management and record keeping

All study-related records and documents at the study site, including those kept in the Investigator's File (including eCRFs, Informed Consent Forms, logs, patient logs, etc.), and the source documents of the subjects must be stored for 15 years after the study is completed. The study Sponsor must ensure the integrity and availability of all the clinical study materials throughout the entire life cycle of the study drug. The archived data may be stored as scanned copies in paper, optical, or electronic format. The Principal Investigator must immediately inform the Sponsor about cases of inadvertent damage/destruction of the clinical study materials and their transfer to another storage place. Deliberate destruction of archived study materials is possible only with the written approval of the study Sponsor.

All the information received, including information about AEs/SAEs will be included in source documents and then entered in the eCRF. No information that is not reflected in source documents will be entered in the eCRF.

As the planned patient visits are carried out and the Investigator completes the eCRFs, the Sponsor-designated monitors will compare the eCRF against source documents. If the eCRFs are completed appropriately and accurately in agreement with the data in the source documents, the monitor confirms in the eCRF that the data in the source documents and eCRF were reviewed against each other by checking the reconciliation checkbox. If at the stage of eCRF data review the Data Control Manager and/or the biostatistician have questions regarding data, all clarifications and changes added to the eCRF will be documented by generating electronic queries for the clarification of eCRF data. Answers to such queries provided by the Investigator in the eCRF are reviewed by the monitor, including for relevance of the amended data to the content of the query (if applicable), and if the answer is considered to be sufficient, the query will be closed. Otherwise, the query will be reopened with additional clarifying information for the Investigator.

The Investigator must provide information confirming the possibility to enroll subjects in a timely manner while meeting the criteria specified in the protocol.

The study must be conducted in accordance with the protocol and the Sponsor’s applicable standard operation procedures. If changes need to be made to the protocol, the procedure described in Section 14.2 of this protocol must be followed.

It is mandatory for the Investigators to complete the source medical records and eCRFs for all study subjects.

The Investigator is responsible for entering complete and accurate information into the eCRFs. All the data entered into the eCRFs must be reflected in the source documents of the subject in printed format or written by the Investigator or another authorized employee of the study site.

All relevant details on the participation of the subject in the study are recorded in the eCRF in agreement with source documents. The eCRF must include data on the completion of participation in the study by the subject. The eCRF must be completed within up to 7 days after the subject’s visit to the study site.

The eCRF must be completed in accordance with the instructions for eCRF completion. Any errors must be corrected by entering the new value into the eCRF, while the old value is saved in the

audit trail. Explanations must be provided in the eCRF for any missing data by checking a special checkbox confirming that data is missing. If necessary, the Investigator may include in the eCRF comments to this checkbox to explain the causes of data missing. eCRFs must be authenticated by the Investigator's signature. Such signatures certify that the information contained in the eCRFs is accurate.

For subjects who are prematurely discontinued from the study, the Study Completion section of the eCRF is filled in, where it is mandatory to explain in details the reason for discontinuation.

All information about the study and any collected data are strictly confidential. The Investigator may provide study-related information to persons who are not directly involved in the conduct of the study only with the Sponsor's permission.

The final report consisting of the statistical and clinical reports will be drafted after the database lock and after the statistical processing of the study results is completed.

The final report is signed by the Principal Investigator at the study site, who confirms the results and conclusions of the study, authenticating the report by applying the stamp of the respective institution.

## 16.1Direct access to source data/documents

Source data represent all the information contained in the original records and certified copies related to clinical data, observations, and other measures taken during the study, required for study reconstruction and assessment. The Investigator provides the Ethics Committee and regulatory authorities the possibility to conduct monitoring, audit(s) and expert review of the study and grants them direct access to source data/records.

Source data must be kept in good condition for the entire period provided for by local and international law and by written agreements with the Sponsor company. The Investigator indicates in the source records of each subject that he/she participates in this study including at least the following information: the individual identification code, personal data of the subject (full name, address), dosing dates, vital signs, any AEs, study completion date, and the primary reasons for treatment discontinuation (if applicable).

The Investigator is obligated to grant the Sponsor's clinical research associate, auditor designated by competent authorities, representatives of the insurance companies and ethics committees direct access to source data and documents.

# 17 Funding and insurance

## 17.1 Funding

Sponsor-investigator will und current study. Contracts between sponsor and investigation venter will be made.

## 17.2 Insurance

The Principal Investigator of the study site is responsible for patient safety during this study. If a subject experiences an AE, the Principal Investigator and his/her employees will provide medical care to the subject and take all possible treatment measures.

If the patient agrees to participate, his/her participation in this study will be insured by the company in accordance with the Federal Law No. 61-FZ “On Circulation of Medicines” dated April 12, 2010, Resolution No. 714 of the Government of the Russian Federation “On Approval of the Standard Rules of Compulsory Life and Health Insurance for Patients Participating in Clinical Studies of a Medicinal Product” dated September 13, 2010, and Resolution No. 393 of the Government of the Russian Federation “On Amendments to the Standard Rules for Compulsory Life and Health Insurance for Patients Participating in Clinical Studies of a Medicinal Product” dated May 18, 2011.

In accordance with Resolutions No. 714 of September 13, 2010 and No. 393 of May 18, 2011 “On Approval of the Standard Rules of Compulsory Life and Health Insurance for Patients Participating in Clinical Studies of a Medicinal Product”, the insurance claim amount based on agreement is as follows:

a) in case of death of the insured person: 2 million rubles. The specified insurance claim amount is divided among the beneficiaries according to their number, in equal parts;

b) in case of damage to the health of the insured person, resulting in:

confirmed group I disability: 1.5 million rubles;

confirmed group II disability: 1 million rubles;

confirmed group III disability: 500 thousand rubles;

c) in case of damage to the health of the insured person, not resulting in confirmed disability: maximum 300 thousand rubles.

Each patient will be handed over the original copy of the insurance policy and an information card describing the insurance conditions provided for by the policy, as well as the actions to be taken in case of injury. To ensure anonymity, the personal data of each subject will be replaced in the insurance policy with the Individual Identification Code assigned during the study according to the pattern established for the Russian Federation. In case of damage to a patient’s health occurring in relation with the clinical study, the insurance company undertakes to reimburse all costs for the medical examination and treatment necessary as a result of direct exposure to the study drug, reference drug, and/or medical procedures used according to the study protocol.

For more information, patients may contact the insurance company:

The study does not provide for any additional types of voluntary insurance or any other treatment and/or compensation opportunities in case of a subject's death or injury within the study.

The Sponsor is not responsible for any losses, damage and/or injury that may be caused to the patient if such damage and/or injury result from:

- the use of a prohibited medication during the study;
- subject's deviation from the study protocol or requirements, and/or any other instructions or recommendation provided by the Investigator;
- action or inaction of a third party failing to respond adequately to an AE or reaction to the study drug.

# 18 Publications

The information contained in this document is the property of the Sponsor and may be disclosed to third parties only with the Sponsor's written approval. Access to this information is provided only to the Investigators and the employees of the study site involved in the conduct of the study, IEC members, and representatives of health authorities authorized to monitor the clinical study. Subject that may participate in this study are provided with information about the study in the amount necessary to make a decision whether to consent to participate.
The study Sponsor has exclusive rights to the results of this study. No data of this study may be presented or published without prior written permission from the Sponsor

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