IRN AP08956260 "Development of the composition and technology of original herbal medicines for the treatment and prevention of *Helicobacter pylori*-associated diseases"

Relevance

Helicobacter pylori is a special type of pathogenic bacteria that infect the stomach and duodenum, lead to inflammation and the development of gastritis, ulcers and subsequently to cancer. This bacterium is recognized by the International Agency for Research on Cancer (IARC) and the World Health Organization as a Class I cancer. A decrease in the effectiveness of standard antibiotic therapy for the elimination of *H. pylori* was recorded in more than 80% of patients due to the development of resistance to antibacterial drugs. Therefore, more and more research is devoted to the search for more effective and alternative therapeutic approaches, for example, phytomedicine.

For the first time in world practice, according to the results of the study, it was found that the dry ultrasonic extract of *Thymus serpyllum* L. has a pronounced antibacterial effect against *Helicobacter pylori* and is a promising substance for the creation of medicines for the treatment and prevention of *Helicobacter pylori*-associated diseases.

It was experimentally established that all experimental samples of pharmaceutical compositions, with a minimum concentration of substances "Thymus serpyllum extract dry" and "Thymus crebrifolius extract dry" have a pronounced antibacterial effect and inhibit the growth of the reference strain of *Helicobacter pylori* ATCC43504 by 100%, while showing a dose-dependent effect.

Therefore, the development of the optimal composition and technologies for obtaining liquid dosage forms in the form of syrups, suspensions and emulsions containing the substances "Thymus serpyllum extract dry" and "Thymus crebrifolius extract dry", the study of their antibacterial effect against control and clinical strains of *Helicobacter pylori* in an *in vitro* experiment and the study of pharmacological action in an animal experiment, the determination of an effective and therapeutic dose, treatment regimen, is an urgent task.

Objective: To develop the optimal composition and technology for obtaining original medicines based on domestic plant raw materials for the treatment and prevention of *Helicobacter pylori*-associated diseases.

Expected results

1. The optimal composition of liquid dosage forms in the form of syrups, suspensions and emulsions containing the substances "Thymus serpyllum extract dry" and "Thymus crebrifolius extract dry" in various concentrations will be developed. Experimental samples of syrups, suspensions and emulsions will be developed to study the antibacterial effect against clinical strains of *Helicobacter pylori*.

2. The antibacterial effect of experimental samples of syrups, suspensions and emulsions containing various concentrations of substances "Thymus serpyllum extract dry" and "Thymus crebrifolius extract dry" will be studied in relation to control and clinical strains of *Helicobacter pylori* in an *in vitro* experiment. Samples of liquid dosage forms will be taken that exhibit a relatively high antibacterial effect against test strains with a minimum concentration of substances "Thymus serpyllum extract dry" and "Thymus crebrifolius extract dry".

3. The antibacterial effect of original herbal medicines will be studied in an *in vivo* experiment against clinical strains of *Helicobacter pylori*, the effective dose, therapeutic dose and treatment regimen will be determined.

4. A technology for obtaining original herbal medicines for the treatment and prevention of *Helicobacter pylori*–associated diseases will be developed. Regulatory documents for original medicines will be developed in the form of ARD projects and laboratory regulations for obtaining.

Research group

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List of publications

1. Patent of the Republic of Kazakhstan No. 34162 for the invention dated 11.02.2020. Application of dry extract of Thymus crebrifolius Klok. (Thymus crebrifolius Klok.) as an antimicrobial agent of a wide spectrum of action //Akhmetalimova A.M., Bokayeva A.B., Ivasenko S.A., Akhmetova S.B., Loseva I.V.

2. Patent of the Republic of Kazakhstan No. 34245 for the invention dated 26.03.2020. A method for obtaining an ultrasonic extract from Thymus serpyllum (Thymus serpyllum L.), which has an antibacterial effect against *Helicobacter pylori* // Orazbayeva P.Z., Shakarimova K.K., Ivasenko S.A., Akhmetova S.B., Loseva I.V.

3. A positive decision on the issuance of a Eurasian patent dated 06.04.2020 under application No. 201800259/28. A method for obtaining an ultrasonic extract from Thymus serpyllum (Thymus serpyllum L.), which has an antibacterial effect against *Helicobacter pylori* // Orazbayeva P.Z., Shakarimova K.K., Ivasenko S.A., Akhmetova S.B., Loseva I.V.

4. Adekenova A.S., Sakenova P.Y., Ivasenko S.A., Khabarov I.A., Adekenov S.M., Berthod A. Gram-Scale Purification of Two Sesquiterpene Lactones from *Chartolepsis Intermedia* Boiss. // Chromatographia. **–** 2016. – V. 79. – P. 37-43.

5. Marchenko A.B., Ivasenko S.A., Laryushina Y.A., Turgunova L.G., Turmukhambetova A.A., Moraru D., Chesca A. Relationship between trimethylamine N-oxide and total cardiovascular risk in the population of Central Kazakhstan // Acta Medica Mediterranea. – 2018. – № 34. – P. 59-63.

6. Kultanov B.Z., Dosmagambetova R.S., Ivasenko S.A., Tatina Ye.S., Kelmyalene A.A., Assenova L.H. The Study of Cellular and Molecular Physiological Characteristics of Sperm in Men Living in the Aral Sea Region // Open Access Macedonian Journal of Medical Sciences. – 2016. – V. 4, № 1. – P. 5-8.

7. Lavrinenko A., Azizov I., Solomadin M., Kolesnichenko S. Molecular-genetic features of MSSA, isolated in Kazakhstan // International Journal of Infectious Diseases. – 2018. - V. 73. - P. 159.

8. Akhmaltdinova L., Lavrinenko A. Antifungal susceptibility testing by flow cytometry // International Journal of Infectious Diseases. – 2018. - V. 73. – P.173–174.

9. Lavrinenko A.V., Kolesnichenko S.I., Amanova D.E. Antibiotic resistance of *A. baumannii* in Kazakhstan // European Surgical Research. - 2019. - V. 60(2). - P. 75.

10. Akhmetalimova A.M., Ivasenko S.A., Marchenko A.B., Ishmuratova M.Yu., Poleszak E., Ludwiczuk A., Loseva I.V. The study of the chemical composition of *Thymus eremita* Klok. and *Thymus rasitatus* Klok. from the Karaganda region // News of the National Academy of Sciences of the Republic of Kazakhstan-Series Chemistry and Technology. - 2018. – V.5, № 431. - P. 20–25.

Achieved results

1. Experimental batches of the substance "Thymus serpyllum extract dry" in the amount of 500 g and the substance "Thymus crebrifolius extract dry" 500 g have been developed to develop the optimal composition and technology for obtaining liquid dosage forms based on them. The quality indicators of the obtained substances meet all the requirements of analytical regulatory documents.

2. The optimal composition of liquid dosage forms based on the substance "Thymus serpyllum extract dry" and the substance "Thymus crebrifolius extract dry" was developed for the first time. Experimental samples of syrups, suspensions and emulsions containing the substance "Thymus serpyllum extract dry" and the substance "Thymus crebrifolius Klok. extract dry" in concentrations of 20, 50, 100 and 150 mg/ml have been developed to study the antibacterial effect against clinical strains of *Helicobacter pylori*.

3. A collection of clinical strains of *Helicobacter pylori* was created for the first time. Eleven strains of *Helicobacter pylori* were isolated from biopsies of the mucous membrane of the upper gastrointestinal tract collected from 50 patients. According to the results of the study of the sensitivity of the obtained clinical strains of *Helicobacter pylori* to antibiotics, 18.2% of the strains are resistant to amoxicillin, to clarithromycin -18.2%, to metronidazole – 100%, to tetracycline – 27.3% and levofloxacin – 45.5%. Double antibiotic resistance of *Helicobacter pylori* was registered to levofloxacin and metronidazole in 18.2% of strains. Resistance of *Helicobacter pylori* to three antibiotics was established in 18.2% of strains and to four antibiotics in 18.2% of strains.

4. It was established for the first time that all experimental samples of syrups, suspensions and emulsions containing the substances "Thymus serpyllum extract dry" and "Thymus crebrifolius extract dry" in concentrations of 20 mg/ml, 50 mg/ml, 100 mg/ml and 150 mg/ml have antibacterial effect against control and clinical strains of *Helicobacter pylori,* regardless of their resistance to antibiotics. At the same time, all the studied samples exhibit a dose-dependent effect. A relatively higher antibacterial effect against the control and clinical strains of *Helicobacter pylori* is possessed by syrup TS №1, with a minimum concentration of the substance "Thymus serpyllum extract dry" (20 mg/ml) and syrup TC №1, with a minimum concentration of the substance "Thymus crebrifolius extract dry" (20 mg/ml).

5. It was established for the first time that syrup TS №1 and syrup TC №1, after treatment of animals for 7 days at a concentration of 125 mg/kg, has pronounced antibacterial activity *in vivo* against *Helicobacter pylori*, exceeding the effect of comparison drugs taken according to the standard scheme. Syrup TS №1 and syrup TC №1 are recommended as promising domestic medicines for the treatment and prevention of *Helicobacter pylori* - associated diseases.

6. For the first time, technologies have been developed for the production of original medicines "Thymus serpyllum syrup 100 ml" and "Thymus crebrifolius syrup 100 ml" for the treatment and prevention of *Helicobacter pylori* - associated diseases. The technologies for producing "Thymus serpyllum syrup 100 ml" and "Thymus crebrifolius syrup 100 ml" were introduced on the basis of the Research Center of the «MUK» NCJSC.

7. Laboratory regulations for the production of "Thymus serpyllum syrup 100 ml" (LR-005491-MK-02-21) and "Thymus crebrifolius syrup 100 ml" (LR-005491-MK-03-21) were developed and approved for the first time. On the basis of the Research Center of the «MUK» NCJSC, the production of experimental batches of medicines for pharmacological research has been organized. Drafts of analytical regulatory documents have been developed to control the production and quality assessment of "Thymus serpyllum syrup 100 ml" and "Thymus crebrifolius syrup 100 ml".

Information for potential users

The invention relates to the field of medicine; namely, to a method for obtaining herbal remedies that have a pronounced antibacterial effect against *Helicobacter pylori*, which can be used as a substance for creating medicines and preparations for the treatment and prevention of *Helicobacter pylori*-associated diseases.

Scientific publications within the framework of the project

1. Eurasian patent No. 036266 of 20.10.2020, A method for obtaining an ultrasonic extract from Thymus serpyllum (*Thymus serpyllum* L.), which has an antibacterial effect against *Helicobacter pylori* //Orazbayeva P.Z., Shakarimova K.K., Ivasenko S.A., Akhmetova S.B., Loseva I.V. (QCFES RK)

2. Ivasenko S.A., Shakarimova K.K., Bokayeva A.B., Marchenko A.B., Lavrinenko A.V., Kolesnichenko S.I. Study of the phenolic compounds of the dry extract of *Thymus crebrifolius* with use HPLC–UV and HPLC-ESI-MS/MS combined method // Bulletin of the University of Karaganda – Chemistry. – 2021. - V. 102, № 2. – P. 18-23. (QCFES RK, Web of Science, Scopus) https://doi.org/10.31489/2021Ch2/18-23.

3. Ivasenko S., Shakarimova K., Bokayeva A., Lavrinenko A., Kolesnichenko S., Rakhimova B., Orazbayeva P., Poleszak E., Korona-Glowniak I., Ishmuratova M. *In vitro* anti-*Helicobacter activity* of aqueous-alcoholic extracts of *Thymus serpyllum* L. // Pharmaceutics. – 2021. – submitted.

4. Ivasenko S., Bokayeva A., Shakarimova K., Lavrinenko A., Kolesnichenko S., Orazbayeva P., Poleszak E., Korona-Glowniak I., Ishmuratova M. *In vitro* anti-*Helicobacter activity* of aqueous-alcoholic extracts of *Thymus crebrifolius* Klok. // Pharmaceutics. – 2021. – submitted.