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PROSPECTS OF GALLERIA MELLONELLA LARVAE USAGE IN PREVENTION AND COMPREHENSIVE TREATMENT OF HUMAN TUBERCULOSIS

Natalia Vladimirovna Valitova[1*], Svetlana Fedorovna Kolosova[2], Mikhail Sergeevich Danilov[3], Sergey Sergeevich Lutai[4], Sergey Adamovich Weinberger[5]

[1]Candidate of Veterinary Science, Leading Research Worker, Senior Lecture,Academic Department of Water and Forest Resources,East Kazakhstan State Technical University named after D. SerikbayevUst-Kamenogorsk, Republic of Kazakhstan.

[2]Candidate of Agriculture, Chief Research Scientist,Academic Department of Water and Forest Resources,East Kazakhstan State Technical University named after D.Serikbayev Ust-Kamenogorsk, Republic of Kazakhstan.

[3]Candidate of Veterinary Science, Leading Research Worker, Assistant Professor,Academic Department of Water and Forest Resources,East Kazakhstan State Technical University named after D. Serikbayev,Ust-Kamenogorsk, Republic of Kazakhstan

[4]Master of Agriculture, Teacher,Academic Department of Water and Forest Resources,East Kazakhstan State Technical University named after D. Serikbayev,Ust-Kamenogorsk, Republic of Kazakhstan

[5]Master of Technical Sciences, Head of Laboratory, Scientific and Production Center for Building Technologies, East Kazakhstan State Technical University named after D. Serikbayev,Ust-Kamenogorsk, Republic of Kazakhstan

Abstract:

Objective: *The study of mycobacterium susceptibility to the combined application of anti-tuberculosis drugs and "Alcohol Balsam" based on beekeeping products.*

Methods: *The anti-tuberculosis activity of ATD (anti-tuberculosis drugs) and dietary supplements "Alcohol Balsam" combination was studied in in vitro experiment in 2016. The results of swabs microscopy were assessed by the amount of bacteria within microsopic view and intensity of the "curve". The growth of MBT (mycobacterium tuberculosis) surface culture in test tubes was determined visually according to the number of colonies on the surface of the breeding ground. The research scientists tested 11, 12, 13 and 14 days' drug effects on mycobacterium culture.*

Findings: *In case of the main anti-tuberculosis drugs and "Alcohol Balsam" combined application, the growth of drug-sensitive and multidrug-resistant strains of tuberculosis bacterium is suppressed by 100% in earlier phases compared to the anti-tuberculosis drug application alone.*

Conclusions: *Various dilutions of 'Alcohol balsam' based on beekeeping products being added to the breeding ground of the first and second line anti-tuberculosis drugs increase their drugs bactericidal effects. Moreover, in earlier phases they suppress the growth of drug-sensitive and multidrug-resistant strains of tuberculosis mycobacterium in 100 % of cases.*

Thus, the efficacy and expediency of Galleria mellonella larvae based drugs application for human tuberculosis prevention and comprehensive treatment have been proved.

Keywords: *Treatment and prevention of tuberculosis; beekeeping products; Galleria mellonella larvae; Galleria mellonella; Mycobacterium tuberculosis.*

Corresponding Author:

Natalia Vladimirovna Valitova,

Candidate of Veterinary Science, Leading Research Worker, Senior Lecture,
Academic Department of Water and Forest Resources,
East Kazakhstan State Technical University named after D. SerikbayevUst-Kamenogorsk
Kazakhstan.

E-Mail: valitova.ekstu@yandex.ru

QR code

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INTRODUCTION:

One of the most serious issues that medicine has dealt with for a considerable course of time is the treatment and prevention of human tuberculosis. Today, when modern science has achieved significant results in various fields, tuberculosis (TB) is still a great problem [40, 1].

Despite the considerable progress in tuberculosis reduction in the world, for several decades Kazakhstan has been on the list of countries with the highest disease level, including multidrug-resistant and extensively drug-resistant tuberculosis. It is necessary to point out that tuberculosis is mostly common among the working age population which includes people aged 18 to 44 years old, more than half of the patients are less than 34 years old [11].

This situation results from the high resistance of mycobacterium tuberculosis to drugs, with the assortment of anti-tuberculosis drugs (ATD) remaining unchanged for considerable time; and being practically not replenished. The current anti-tuberculosis drugs affect the human organism very negatively, reduce the immune system and contribute to the malfunction of all organs and systems. [16].

It is possible to increase the effectiveness of treating tuberculosis patients in various locations by the combined use of chemotherapeutic and other biologically active drugs and/or addition of raw materials with the purpose of reducing endogenous intoxication, treating undesirable side effects caused by TB drugs [16], normalizing metabolism and accelerating recovery processes [2,].

Drugs that contribute to the immune system normalization and have a wide range of therapeutic effects should be searched for among the new classes of biologically active raw materials. Having stated that, it is necessary to point out that the research scientists of this article develop drugs based on the greater wax moth larvae, propolis and honey.

Galleria mellonella is a pest of honey bees [22]. Feeding with honeycombs of honey and bee pollen, Galleria mellonella larvae (GML) contains all the biologically active substances that beekeeping products are made of. According to a number of clinical studies, GML extract has a therapeutic effect in diseases of various aetiology and localization, including respiratory diseases such as acute respiratory infections, pneumonia, bronchitis, and, most importantly, in case of TB [22].

Tuberculosis causative agent protective shell, that makes the disease highly resistant to many ATD, contains waxlike substances, which are the main element of GML nutrition. G.mellonella larvae absorbs up to 50% of products that wax is made of. They can digest the myristyl ether of palmitic acid, which is the reason why they have such specific lipases in contrast to other insects [19, 21].

When studying the properties of G.Mellonella extract for the purpose of anti-tuberculosis activity factor identification, it was found that the lipolytic and antibacterial properties are provided by the protein component of the extract, including lipases, capable of destroying certain vital lipids for mycobacterium and other species-specific lipid layer molecules of the cell wall [15].

GML drugs tuberculostatic effect was defined. Experimental guinea pigs were intraperitoneally administered with a single dose of 100,000,000 bacteria incubated at 37 °C for 18 hours in unprocessed lipase and/or esterase preparations isolated from GML and showed significant regress of the tuberculosis process. Within seven weeks after inoculation, no visible pathology was observed, while control animals died in seven weeks. However, the preparations did not show any bactericidal activity, and experimental guinea pigs showed the onset of tuberculosis process at the eighth week after the infection [25].

Based on the aforementioned information, it is concluded that even after a short exposure of GML preparations, MBT became avirulent and more vulnerable to ATD.

The relevance of the research is that the combined use of ATD and preparations based on GML and propolis increases the effectiveness of ATD, decreases negative effect on the human body, reduces treatment time and cost and therefore can be successfully used to solve one of the most important problems of medicine - tuberculosis treatment.

The main goal of the research work is to find out the mycobacterium drug susceptibility to the combined use of ATD and alcohol balsam that is, define the minimum inhibitory concentration of balsam (MIC) causing partial (+) or complete (0) growth suppression of the standard MBT dose at earlier times.

MATERIALS AND METHODS:

Within the framework of previous scientific research conducted in 2015-2017, "Alcohol balsam" and "Honey balsam" preparations were developed on the base of non-traditional beekeeping products with the aim of prevention and comprehensive treatment of human tuberculosis.

The research scientists tested physical and chemical parameters of preparations, and evaluated the safety and quality of products in the Academy of Nutrition of the Republic of Kazakhstan (Almaty). Company Standards have been developed and approved for the products [20], applications for inventions have been filled.

In 2016 there was an in vitro experiment to study the combined antitubercolousis activity of the "Alcohol Balsam" based on beekeeping products (AB), with the ethyl alcohol contain not exceeding

20%, and ADT towards the museum (H37RV) and multidrug-resistant (MDR) strain of Mycobacterium tuberculosis.

The experiment was carried out based in reference laboratory of the National Center for Tuberculosis Problems (city of Almaty) in accordance with the Methodological Guidelines for the antituberculosis activity of the pharmacological substances, the bacteriostatic and bactericidal activity of the new compounds in vitro experiments studying [17].

The following items were used in the experiment of mycobacterium drug susceptibility:

1. test preparation "Alcohol Balsam" based on beekeeping products (AB) in dilutions: 100; 20; 10; 5; 2.5; 1.25; 0.625 µg/ml of active substance. Before serial dilution, the test preparation for its sterility was passed through an ultra-thin bacterial filter Millex CA with a pore size of 0.22µm, diameter of 33mm;

2. first series ATD - rifampicin (R) 40 µg/ml, isoniazid (H) 1 µg/ml;

3. reserve series ATD - amikacin (Am) 20 µg/ml, moxifloxacin (Mfl) 20 µg/ml.

The bactericidal and bacteriostatic activity of the preparations was determined in vitro on the museum strain H37Rv and multidrug-resistant wild strain (MDR) (resistant to rifampicin and isoniazid) isolated from the patients.

4 groups were involved in the experiment. Each group contained test tubes with Shkolnikov breeding ground, various preparations and different periods of drugs exposure.

1 test group - AB + 0.2 ml of H37RV strain + H + R

2 test group - AB + 0.2 ml MDR strain + Mfx + Am

3 group - control - 0.2 ml of H37RV strain + H + R

4 group - control - 0.2 ml MDR strain + Mfx + Am

Schkolnikov breeding ground without preparations was used as control in all the experiments.

Each group contains 8 test tubes, one of which (No. 1) is the control one (without preparations), and the other 7 test tubes are with preparations in the indicated dilutions (Table 1).

5 durations of drug exposure to MBT were studied. Test tubes were incubated for 10; 11; 12; 13; 14 days at 37°C. On expiration of the incubation period, the precipitates on a liquid medium were washed with physiological saline. Swabs were made of the resulting suspension and stained according to Ziehl-Neelsen, then the test tubes for 0.2ml were inoculated with a dense egg medium of Levenshtein-Jensen (in duplicate).

The result of swabs microscopy was assessed by the amount of MBT visible in the microscopic field of view and by the intensity of the "curve" according to the following scheme:

no MB – within 100 microscopic fields of view -

1-9 MB – within 100 microscopic fields of view ±

10-100 MB – within 100 microscopic fields of view +

1-9 MB – within 1 microscopic field of view ++

10 and more within1 microscopic field of view , "curve" +++

The results of culture studies were assessed visually according to the number of colonies on the surface of the culture medium after 2.5 months of cultivation in a thermostat at 37°C according to the scheme

no colonies on the swarm -
a single colony, up to 20 +
colonies from 20 to 100 ++
more than 100 colonies +++

Table 1: Experiment scheme to determine the bactericidal activity of the combined use of ATD and Alcohol Balsam.

Group/ strain MBT	No of test tube and preparation							
	1	2	3	4	5	6	7	8
1 / H37RV	-	СБ 100 µg/ml + H+R	СБ 20 µg/ml + H+R	СБ 10 µg/ml + H+R	СБ 5 µg/ml + H+R	СБ 2,5 µg/ml + H+R	СБ 1,25 µg/ml + H+R	СБ 0,625 µg/ml + H+R
2 / MDR	-	СБ 100 µg/ml + Mfx+ Am	СБ 20 µg/ml + Mfx+ Am	СБ 10 µg/ml + Mfx+ Am	СБ 5 µg/ml + Mfx+ Am	СБ 2,5 µg/ml + Mfx+ Am	СБ 1,25 µg/ml + Mfx+ Am	СБ 0,625 µg/ml + Mfx+ Am
3 / H37RV	-	H+R	H+R	H+R	H+R	H+R	H+R	H+R
4 / MDR	-	Mfx+ Am	Mfx+ Am	Mfx+ Am	Mfx+ Am	Mfx+ Am	Mfx+ Am	Mfx+ Am

Literature Review

Despite all the achievements of modern medicine, tuberculosis is still one of the most serious diseases and the global problem of public health.

According to WHO (World Health Organization), 10.4 million new cases of the disease were registered worldwide in 2015 [42], 1.2 million (11%) of all the new patients were HIV-infected, about 480 000 new cases of tuberculosis with MDR. There were 1.8 million registered deaths caused by TB, 0.4 million of which were HIV-infected people [41,36].

In Kazakhstan, for the period of 2000-2015, the record of TB cases declined by 6% [35], MDR-TB cases among new tuberculosis patients by 2% [41], mortality by 72.8% [16].

Despite the obvious tendency, the level of morbidity and mortality caused by TB is still high [4, p. 283]. Kazakhstan is on the list of the top five countries with the largest number of new TB cases in 2015: Russia (115,000 - 0.08%), Ukraine (41,000), Uzbekistan (24,000), Kazakhstan (16,000 - 0.09%) and Romania (16,000) [35]. The level of new TB cases in places of detention in the Republic of Kazakhstan exceeded 1,000 cases per 100,000 arrested people (915 per 100,000 population were registered in prisons of European Region WHO) [35].

At the same time, the analysis of the epidemiological situation of TB in the world showed a tendency of MDR-TB morbidity increase [5]. According to WHO data, over the last five years, Kazakhstan, Russia, Estonia, Uzbekistan, Ukraine, Kyrgyzstan and Belarus have entered the list of countries with the highest number of MDR tuberculosis patients among the newly diagnosed (19.3-34.1%) [40, 25, 32]. TB is widespread and exceeds the WHO recommended incidence threshold in these countries up to 10 times [1, p. 459; 47, p. 7].

Infiltrative (79.3%) and focal TB (10.9%), exudative pleurisy (6.6%) are most common cases in the Republic of Kazakhstan. Also up to 40% cases of bacterial excretion were found in the patients examined [11]. Mostly, these are people of high-risk groups (alcoholics, smokers, HIV-infected, migrants, prisoners and those discharged from prison) [16].

Concerning gender distribution, the TB cases among children younger than 14 years old make up 45% in boys and 55% in girls. Among patients older than 15 years old, the incidence of men is 1.5 times higher than that of women. The most frequent cases are spread among women who are 25-34 years old (119 cases per 100,000), men who are 35-44 years old (180 cases per 100,000) [40, p. 175], which is also confirmed by Kazakhstani scientists [11].

One TB patient treatment requires more than 1 million KZT every year. Despite the availability of free ATD, due to temporary work incapacity, productivity (up to 37%) and incomes (by 10%) of TB patients are reduced, moreover, expenses caused by the need of purchasing additional drugs and adequate nutrition increase up to 33% [39].

For more than three decades, the standard 6-18-month R-CHOEP is used for TB treatment in the world practice. However, existing treatment regimens are based on the drugs that have been used for more than 40 years [24]. Often, treatment is complicated by the emergence of MBT drugs resistant that retain the ability to generate and lead to disease recurrence [38, 42].

Recording and controlling of MDR-TB complexity is due to the inadequacy of systematic follow-up examinations, lack of clear data on the geographical distribution of MDR forms by the regions, and also the limited participation of health workers, which significantly reduces the detection of outbreak hotbeds [16,44].

The development, introduction and industrial production of new drugs with high bacteriostatic and bactericidal MBT activity is a part of the tuberculosis infection control WHO programme.

Currently, research works are conducted in several areas: testing of known drugs by composing new combinations and treatment regimens based on them [34], development of new drugs ATD [9], including drugs made of natural raw materials [14], and additional physiotherapy procedures. Also, another area of research is the usage of drugs [23] designed to eliminate intoxication, improve the immune system, reduce side effects caused by chemotherapy and shorten treatment duration.

Among the approved antibacterial drugs used from 1981 to 2010, more than a half (about 65%) were natural antimicrobials (including rifampicin and aminoglycoside PTP) or made of natural raw materials [24].

For the first time since the introduction of rifampicin in the late 1960s, two drugs were approved in 2013 and 2014: bedaquiline and delamanid for MDR tuberculosis treatment. However, the data on toxicity and risk of unexplained mortality and other side effects limit its use for MDR-TB treatment [31].

TB is one of the diseases the course and outcome of which is largely determined by the immune status of the patients. Pathophysiological disorders caused by TB development negatively affect the course of the disease and its therapy. The development of tuberculosis immunity involves a variety of specific and nonspecific factors of body defence. This justifies the need to use various preparations in combination with etiotropic drugs activating the body defence systems and restoring disturbed homeostasis. Such a complex treatment can increase the effectiveness of therapy several times [18].

Therefore, modern phthisiology uses a variety of technologies which are mainly focused on TB patients treatment optimizing by improving their quality of life (adequate nutrition, sanatorium treatment), increasing the body natural resistance and obtaining the most positive effect in the shortest possible time [2]. A variety of preparations is available in addition to the main ATD treatment in the therapy regimen. These preparations mainly have immunomodulating [6], anti-inflammatory, antioxidant [3], hepatoprotective and other properties.

Any experimental therapy is aimed at treatment period reduction without increasing the rate of relapses, the increase of the body resistance and the reduction of treatment cost due to a shorter period of therapy.

In the struggle against tuberculosis, one of the indispensable factors is the search for new ATD that actively and directly affect TB causative agent without causing side effects, have a wide range of therapeutic effects and contribute to the normalization of the immune system.

However, the complexity of TB effective treatment is due to the biological properties of the pathogen. It is known that the protective shell of MBT contains waxlike substances, which cause its high resistance to many ATD.

Therefore, another area being researched by scientists, including the authors of this article, is the development of drugs reducing the resistance of MBT by destroying and/or changing the structure of the protective shell.

Scientists from the University of Queensland and the University of California in San Francisco have found a promising new method for TB-causing bacteria suppression. The research team headed by Professor Paul Ortiz de Montellano in the United States of America studied the effect of compounds on M.tuberculosis associated with cholesterol. It is known that cholesterol affects the TB virulence and infectivity. Two analogues of cholesterol with truncated, fluorinated side chains were synthesized stopping the growth of M.tuberculosis culture, since it cannot be used as a source of energy [33].

A fundamentally new class of the initial biologically active feedstock concerning the reduction of MBT resistance is represented by the GML.

Galleria mellonella parasitizes a bee family. This is the only insect whose larvae can digest the wax - a part of tuberculosis causative agent shell [22].

GML is known all over the world as an object of research.

The first scientist who started to study Galleria mellonella at the end of the 19th century, in search of a remedy for TB treatment was I.I. Mechnikov. Later Moscow doctor S.A. Mukhin developed effective combinations of the extract with the other biologically active components and created a complex preparation "Vita" on its basis, capable of repairing tuberculous caverns in the lungs [12].

N.A. Spiridonov developed and patented a method for large-scale production of GML active extract and studied the chemical composition of the extract, identified some active components [19].

Currently, GML is used for the production of different therapeutic effect drugs in the CIS (Commonwealth of Independent States) countries [13].

GML is primarily used abroad as a model and an alternative host for studying of various pathogens virulence factor and the immune response of larvae infected by bacteria since the GML system is functionally analogous to the mammalian immune system and possesses both humoral and cellular immunity [44].

Russian scientist S.I. Metalnikov made similar experiments with the TB causative agent in the early 20th century. He injected various doses of MBT into the larvae body cavity. Infected larvae did not die, but continued to grow, turning into an adult insect. During the study of GML blood and internal organs, it was found out that the phagocytes of larvae rapidly captured and digested MBT. And even after a large amount of MBT administration, they were destroyed in 2-3 days, and the infected larvae were perfectly healthy [10]. This made it possible to assume that the GML drugs have the same properties.

GML has a broad spectrum of effects. The lysozyme and anionic peptide in their hemolymph cause the fungistatic and fungicidal activity of preparations made of G.mellonella larvae. During the experiments, lysozyme inhibited the growth of Candida albicans fungus, significantly reducing its metabolic activity and causing changes in the topography and properties of the cell surface as compared to the control cells. A synergistic effect of peptide and lysozyme on C. Albicans was also found, which determined the important role in the early stages of fungus immune response [48].

In the development of new antiinfectives in the pharmaceutical industry, Antimicrobial proteins and peptides (AMP), which are also the humoral immune factor providing natural immunity, carry an important role. Therefore, antibacterial and scientists consider antifungal peptides and insect proteins as an alternative to antibiotics. G. mellonella serves as a good model for biochemical studies. Considering the size of the insect, it is easy to obtain hemolymph and other tissues as a source of many immunodeficient polypeptides [27]. Thus, purified antimicrobial peptide - gloverin was isolated from GML, and it showed specific antimicrobial activity against two different strains of Escherichia coli [49].

A resinous substance of a greenish-brown or brown colour with a pleasant aroma of poplar buds, honey, wax and vanilla, propolis, which is also a part of

the alcohol balsam, is used by bees to maintain aseptic conditions in the hive.

Propolis is a complex mechanical mixture of some groups of substances: resin - 50 - 85%, essential oils - 1.5 - 4.5%, wax - 12 - 40%, tannins - 4 - 10.5%, pollen 5 - 11%, mechanical impurities - 5 - 15%. It also contains vitamins A, C and E [7].

Propolis has antifungal, antiinflammatory, immunostimulating, regenerative, antioxidant [4,46], anesthetic and antitoxic effects. Antimicrobial effect of propolis is due to flavonoids and benzoic acid [29], which inhibit the growth of many microorganisms [37, p. 437; 43, p. 6].

Due to its properties, propolis solution is applied in case of the upper respiratory tract disease, dentistry, gastrointestinal diseases, arthritis, radiation therapy [28], etc.

The advantage of propolis, compared to the other medicinal products, is its harmless effect on the body and its possible application as an independent therapeutic agent and also in combinations with other drugs.

Based on the aforementioned, preparations or biologically active supplements containing extract of GML and propolis are promising in the fight against tuberculosis.

RESULTS:

The result of swabs microscopy was assessed by the number of mycobacterium within microscopic view and the intensity of "obliquity formation" under microscopy in accordance with the above scheme.

The expansion of the surface culture of H37RV and MDR strain in test tubes was determined visually according to the number of colonies on the surface of the breeding ground. The results of the experiment are presented in Table 2.

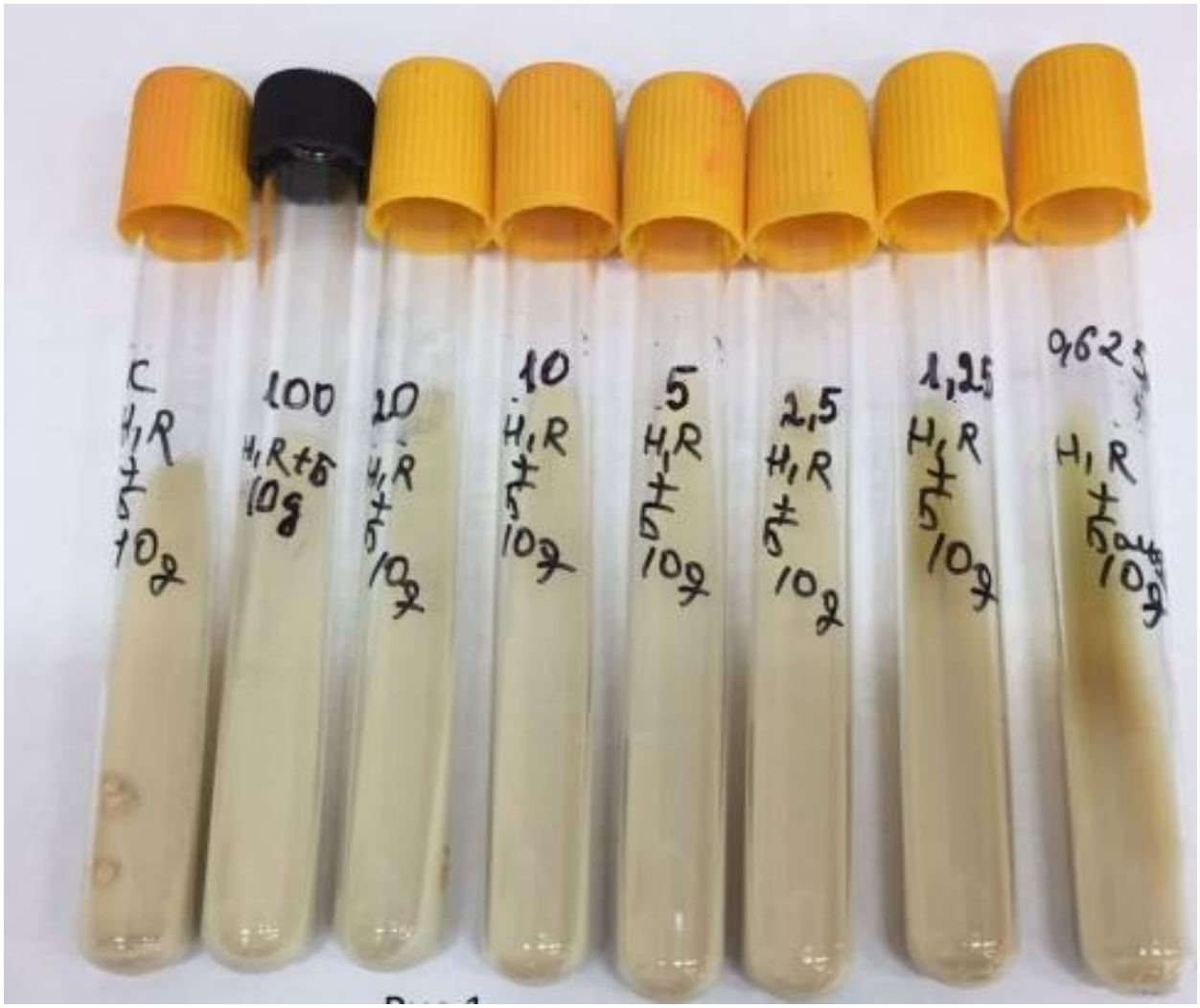
Table 2: Results of the bactericidal activity of the combined ATD and Alcohol Balsam application.

Group	Test tube No	Version	Results									
			microscopy (number of MBT within view, intensity of "obliquity formation")					cultural studies (number of colonies on the swarm)				
			period of incubation, day					period of incubation, day				
			10	11	12	13	14	10	11	12	13	14
1 group	1	Control (no preparation)	++	++	+++	+++	+++	++	+++	+++	+++	+++
	2	H37RV+H+R + AB 100 μg/ml	0	0	0	0	0	0	0	0	0	0
	3	H37RV+H+R + AB 20 μg/ml	0	0	0	0	0	0	0	0	0	0
	4	H37RV+H+R + AB 10 μg/ml	0	0	0	0	0	0	0	0	0	0
	5	H37RV+H+R + AB 5 μg/ml	0	0	0	0	0	0	0	0	0	0
	6	H37RV+H+R + AB 2.5 μg/ml	0	0	0	0	0	0	0	0	0	0
	7	H37RV+H+R + AB 1,25 μg/ml	0	0	0	0	0	0	0	0	0	0
	8	H37RV+H+R + AB 0,625 μg/ml	0	0	0	0	0	0	0	0	0	0
2 group	1	Control (no preparation)	++	++	++	++	++	++	++	+++	++	++
	2	MDR+Mfx+Am + AB 100 μg/ml	0	0	0	0	0	0	0	0	0	0
	3	MDR+Mfx+Am + AB 20 μg/ml	0	0	0	0	0	0	0	0	0	0
	4	MDR+Mfx+Am + AB 10 μg/ml	0	0	0	0	0	0	0	0	0	0
	5	MDR+Mfx+Am + AB 5 μg/ml	0	0	0	0	0	0	0	0	0	0
	6	MDR+Mfx+Am + AB 2,5 μg/ml	0	0	0	0	0	0	0	0	0	0
	7	MDR+Mfx+Am + AB 1,25 μg/ml	0	0	0	0	0	0	0	0	0	0
	8	MDR+Mfx+Am + AB 0,625 μg/ml	0	0	0	0	0	0	0	0	0	0
3 group	1	Control (no preparation)	+++	+++	+++	++	+++	+++	+++	+++	+	++
	2-8	H37RV + H + R	±	0	0	0	0	+	0	0	0	0
4 group	1	Control (no preparation)	++	++	+++	+++	++	++	+++	+++	+	++
	2-8	MDR+Mfx+Am	+	±	0	0	0	+++	++	0	0	0

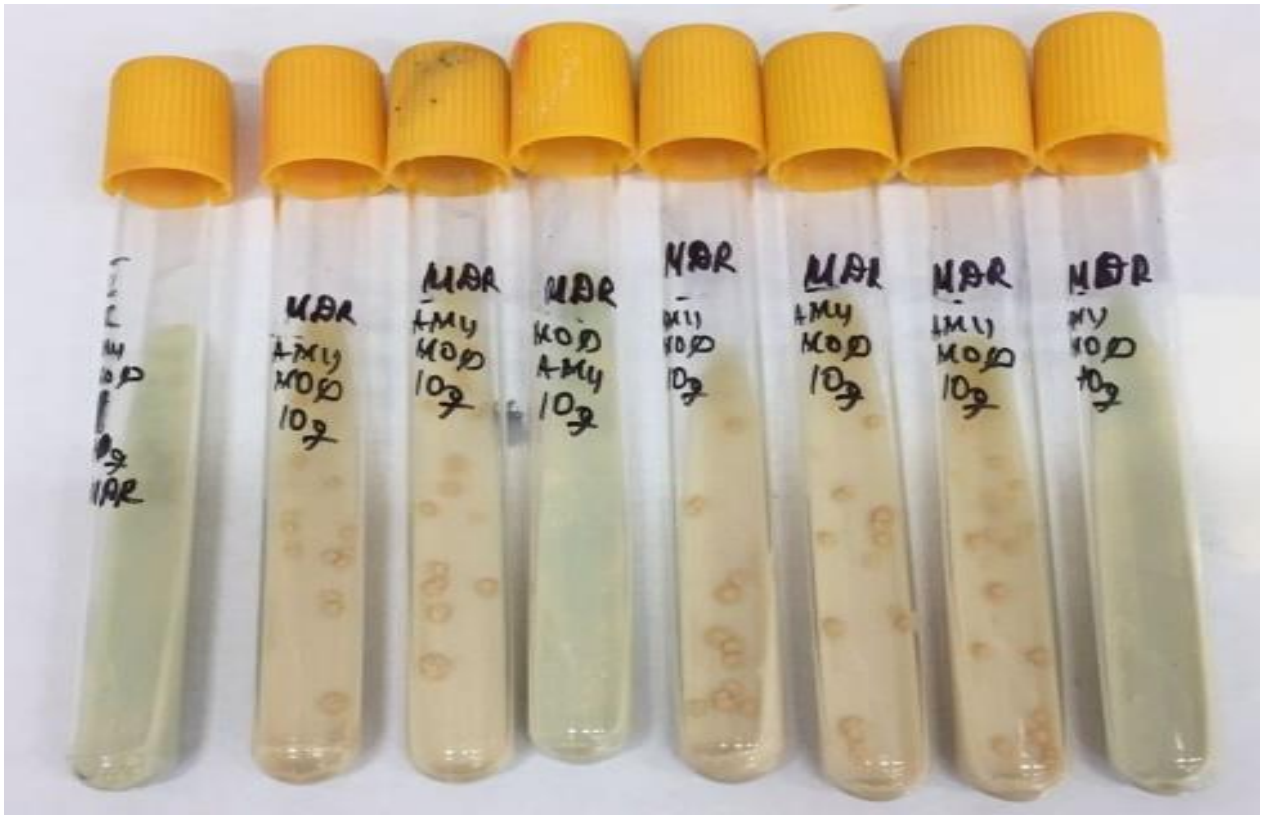
Swabs microscopy showed that when cultivating MTB for 10, 11, 12, 13 and 14 days, in case of ATD and AB availability, MBT was not detected in any dilution. During the control (in the third and the fourth groups), using only ATD, in case of 10-day and 11-day incubation periods, MBT in (+, ±) was detected, while ATD effect on MBT for 12, 13 and 14 MBT within microscopic view was not detected. There were 10 or more MBT or "obliquity formations" within one view when swab microscopy was under control.

When studying the results of the cultural method, in control tubes the research scientists recorded the growth of MBT colonies of characteristic ivory colour with the formation of a structure - like braids or plaits. This is typical of microcolonies of M. tuberculosis in early periods.

MBT growth was not detected in the first experimental group with the susceptible strain H37RV in any test tube into which AB and ATD were added (Picture 1).



Picture 1. Growth of H37RV strain surface culture in test tubes with ATD of the first series and Alcohol Balsam (B) with 10-day effect on the MBT culture



Picture 2. The growth of MDR strain surface culture in test tubes with preparations of the reserve series with 10-day effect on the MBT culture

Similar results were obtained in the second group - multidrug-resistant strains were supplemented with ATD and AB of the second series in the test tubes.
Somewhat differing results were obtained when MBT was supplemented with only ATD of the first and second series (third and fourth groups) (Picture 2). These groups also inhibited the growth of the strains studied, but later: in case of susceptible strains, this process was observed on day 11, in case of multidrug resistant strains - on day 12.

DISCUSSIONS:

Tuberculosis infection is impossible to control successfully without fundamentally new approaches to the process of diagnosis and treatment.
Therapy should be based on drugs that have high bacteriostatic and bactericidal activity to MBT, a wide range of therapeutic effects and normalize the immune system.
However, ATD available in clinics has been used for a long time; the range is almost not replenished. Those drugs are extremely toxic to the body and cause various undesirable side effects. Known MBT drug-resistant substances formation can cause disease recurrence.
This is the reason behind the search for new ATD actively and directly affecting the TB causative agent without any side effects.
The first step of the problem solution is the microbiological research which discovers the bacteriostatic and bactericidal activity of new drugs, their effectiveness, therapeutic dose and terms of treatment, as well as the comparative analysis of the antituberculous activity of new drugs as compared to the effectiveness of the traditional chemotherapeutic drugs.
The study of new drug combinations activity in in vitro experiments is carried out primarily with the use of laboratory test strains of human type H37Rv M. tuberculosis. In case of evident antibacterial activity of substances, the susceptibility of the strains isolated from pathological material of TB patients shall be studied. Strains susceptible to the main ATD and strains with different spectra and degree of resistance to them (monostable and MDR strains) shall be studied.
The main factor of MBT pathogenicity is toxic glycolipid (cord-factor), located on the surface and in the section of a cell wall. According to the chemical nature, it is a polymer consisting of a single molecule of trehalose disaccharide and associated equivalent ratios of mycoli and mycollic high-molecular fatty acids, as well as phyto-cortyldimycerosates. The cord factor has a toxic effect on the tissues and insulates MBT against phagocytosis, blocking the oxidative phosphorylation in the mitochondria of macrophages. M. tuberculosis without cord-factor is non-pathogenic or slightly pathogenic for humans and guinea pigs [8].

MBT virulence shall be identified by the biological samples method and the cord-factor detention by means of cytochemical reactions based on the property of virulent MBT (with a cord-factor) to use dyes firmly (neutral red or Nile blue) and retain the colour when alkali is added. The solution and non-virulence MBT change their colour [8, p. 549].
Staining the clinic isolate of M. tuberculosis attenuated in laboratory conditions and MBT non-pathogenic for various guinea pigs isolated from the environment with a neutral red colour has a negative reaction. Lipid analysis showed that MBT has lost the ability to synthesize phyto-cortyldimycerosates and other branched-chain cell lipids, which are the main factors of M. tuberculosis virulence, especially at an early stage of infection. Thus, the negative reaction during staining is a marker of virulence, indicating important disorders of the M.tuberculosis membrane and its drugs resistance decrease [30].
When staining according to the Ziehl-Neelsen method, MBT also retains the original red colour, which allows differentiating it from some nonpathogenic MBT [8].
Virulent strain of M.tuberculosis treated with lipidolytic enzymes of G. melonella larvae becomes less acid-fast when Tsilu-Nielsen stained, and neutral red staining has a negative reaction, which indicates the influence of GML preparations on the MBT shell. At the same time, the progress of MBT stops or slows down, because they temporarily lose their virulence, as shown by biological tests on guinea pigs [45].
The research scientists of the article offered to test "Alcohol Balsam" based on bee-keeping products.
According to the results of the studies in vitro experiments (on the breeding ground) it was proved that AB at any concentration enhances the bactericidal action of traditional ATD and achieves 100% cessation of culture growth of drug-susceptible and multiresistant MBT strains at an earlier time than in case of ATD application alone.
According to the results of the current research and the above literature data, the following conclusions were made: the effect of synergism caused by the combined application of ATD and AB was found out. AB reduces the resistance of pathogen, changing the composition and structure of the protective layer of the MBT shell. MBT becomes vulnerable after GML treatment and dies faster under ATD effect, which justifies the results of the studies.
Thus, the effectiveness and expediency of drugs based on GM larvae application for the prevention and combined therapy of human tuberculosis have been proved.
It is not possible to make a comparative analysis of the experimental results obtained with analogous results since such studies were conducted for the first time.

CONCLUSIONS:

In vitro experimental study of "alcohol balsam" on the basis of beekeeping products and the museum and multidrug resistant M. tuberculosis strain yielded the following results:

1. 100% cessation of cultures growth of MBT drug-susceptible and multidrug-resistant strains has been achieved both with the combined use of the main ATD and "Alcohol Balsam" with the use of anti-tuberculosis drugs only.

2. In case of the combined use of the main ATD and "Alcohol Balsam", cessation of cultures growth of mycobacterium tuberculosis drug-susceptible and multidrug-resistant strains was achieved at an earlier time than with the use of ATD only.

The application of ATD in combination with AB provides a higher overall antituberculosis effect than each of them does separately.

Thus, the study showed that various dilutions of 'Alcohol balsam' based on beekeeping products being added to the nutrient medium of ATD increase their drugs bactericidal effects. Moreover, in earlier phases they suppress the growth of drug-sensitive and multidrug-resistant strains of tuberculosis mycobacterium in 100 % of cases. The ATD application alone also had a good result, but at a later period of exposure.

Therefore, the preparations based on unconventional beekeeping products, including the GM larvae, can be used in prevention and treatment of human tuberculosis.

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