International Journal of Medical Sciences And Clinical Research (ISSN – 2771-2265)
VOLUME 02 ISSUE 12 PAGES: 09-20
SJIF IMPACT FACTOR (2021: 5. 694) (2022: 5. 893)
OCLC – 1121105677 METADATA IF – 5.654
Publisher: Oscar Publishing Services

Journal Website: https://theusajournals.com/index.php/ijmscr

Research Article

PRE-CLINICAL STUDIES OF THE ANTITUBERCULOUS DRUG BIOMAYRIN

Submission Date: December 12, 2022, Accepted Date: December 17, 2022,
Published Date: December 22, 2022
Crossref doi: https://doi.org/10.37547/ijmscr/Volume02Issue12-03

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ABSTRACT

By chemical modification of polygalacturonic acid with anti-TB drugs isoniazid, ethambutol and rifampicin, the macromolecular system Biomayrin was obtained. Pharmaco-toxicological studies have ascertained that Biomayrin is less toxic than low-molecular counterparts and has pronounced anti-tuberculosis activity, and with a two-fold lower dose when calculating the active substance, it has identical activity with the combined anti-tuberculosis drug Mayrin. Pharmacokinetic studies have shown that with the introduction of Biomayrin, the therapeutic concentration of the active ingredients (isoniazid, ethambutol and rifampicin) in the blood lasts longer than that of their low-molecular counterparts. It should also be noted that the chemical binding of isoniazid to the polysaccharide matrix slows down its metabolism to therapeutically inactive acetylisoniazid.

KEYWORDS

Polygalacturonic acid, isoniazid, ethambutol, rifampicin, mayrin, polymer- carrier, tuberculosis, prolongation.

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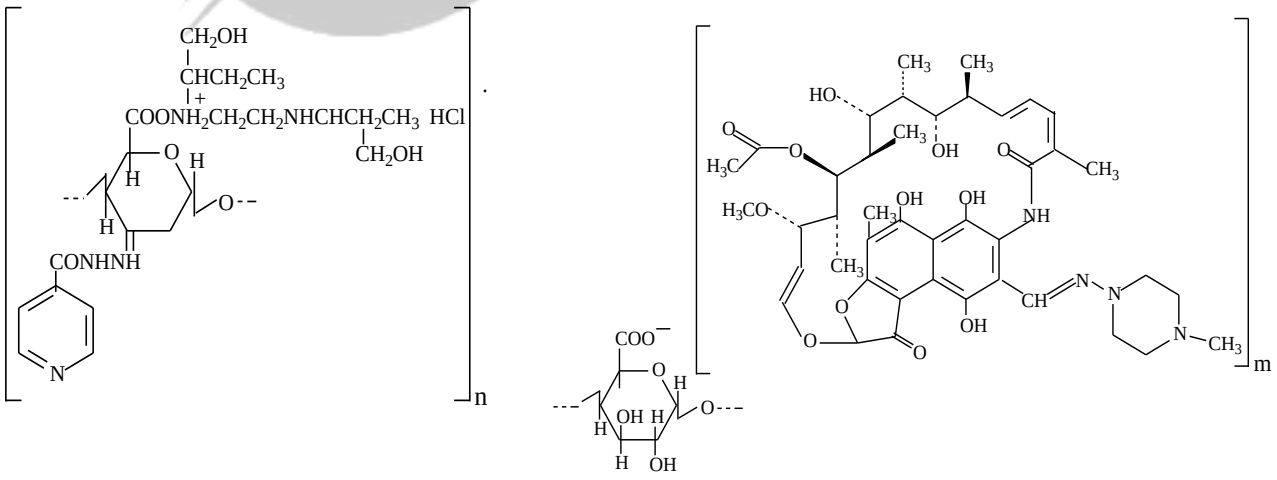
INTRODUCTION

In connection with the specificity of tuberculosis treatment - complex therapy (simultaneously with several anti-tuberculosis drugs), the duration of treatment, the need to assign multiple doses of large doses of medications and the occurrence of toxic-allergic complications due to this - it is very important to find a way to reduce the dosage of anti-TB drugs [1,2] . One of the ways to improve the conditions of drug therapy is the creation and use of polymeric anti-tuberculosis drugs of prolonged action. Such prolonged anti-TB drugs will not only increase the duration of action of tuberculostatics, but also reduce their side effects, reduce the overall consumption of the drug. Such macromolecular drug systems can be obtained by incorporating anti-tuberculosis drugs into the macromolecules of the polymer carrier.

In this connection, we have synthesized the macromolecular system by chemical binding of anti-tuberculosis drugs: isoniazid, ethambutol and rifampicin to a modified polygalacturonic acid macromolecule (PGA) with a molecular mass of 16 kDa, isolated from citrus pectin by demetoxylation. The macromolecular system is called Biomayrin. In the macromolecule of Biomayrin, isoniazid is chemically bound to the macromolecule PGA through the azomethine bond, ethambutol and rifampicin via the ionic bond [3].

The chemical structure of Biomayrin can be represented as polygalacturonic acid complexes with 4-pyridinecarboxylic acid hydrozone and [S-(R*, R*)] - 2,2 '- (1,2-ethanediyl diimino) bis (1-butanol) and polygalacturonic acid complex with 3 - [[(4-methyl-1-piperazinyl) imino] methyl] rifamycin:



$$(C_6H_6O_5)_n(C_6H_5N_3O)_m(C_{10}H_{24}N_2O_2)_s+(C_6H_7)_n(C_{43}H_{58}N_4O_{12})_h$$

n=20±3, m=13±3, s=10±3, h=5±2

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Biomayrin

This article presents the results of preclinical studies of Biomayrin.

METHOD

Objects of study: Anti-TB drugs:

Biomayrin: (isoniazid - 15 ± 3%, ethambutol - 15 ± 3%, rifampicin - 17 ± 3%).

Rifampicin - Becton, Dickinson and Company Sparks.

Isoniazid - Becton, Dickinson and Company Sparks.

Ethambutol - Becton, Dickinson and Company Sparks.

Preparation of Biomayrin solutions:

Pure Substance: Biomayrin in Levenstein-Jensen Nutrient medium

2. Final concentration = 4000 mg/l

dissolve 59.17 mg of Biomayrin substance in 5 ml of ethanol, then add 10 ml of sterile distilled water (heating of the solutions is possible);

2. Final concentration = 400 mg/l

11 ml of Solution I + 90 ml of sterile distilled water;

3. Final concentration = 200 mg/l

21 ml of Solution II + 20 ml of sterile distilled water;

4. Final concentration = 50 mg/l

10 ml of Solution III + 30 ml of sterile distilled water.

Table 1

The final concentration of the drug in the medium

The final concentration of the drug in the medium				
The volume of medium (mg/l)	40.0	20.0	10.0	5.0
Distilled water (ml)	450	45	45	45
Solution I, ml	\	\	2.5	\
Solution II, ml	50	\	\	\
Solution III, ml	\	5.0	2.5	\
Solution IV, в ml	\	\	\	5.0

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The study was conducted using the method of absolute concentrations with the content of anti-TB drugs on a nutrient medium Levenstein-Jensen in a dose according to McFarland №1, the turbidity standard is 10^6-10^8 bacteria/ml. To determine the susceptibility to anti-tuberculosis drugs, a virulent Reference M. Tuberculosis H37Rv strain was selected. To obtain reliable results, two dilutions of the bacterial suspension were prepared from each culture of the H37Rv strain:

Table 2

The composition of the statutory solution

Statutory solution	2 ml
K_1	0.9 ml NaCl = 0.1 statutory solution or 1.8 ml of NaCl = 0.2 ml of the statutory solution
K_2	0.9 ml NaCl = 0.1 statutory solution

Then, two dilutions from each H37Rv strain were inoculated into nutrient media containing anti-tuberculosis drugs. Inoculated culture media were incubated in a thermostat at 37°C for 28 days [4].

Bacteriostatic activity of Biomayrin in vivo was performed on 20 ordinary guinea pigs weighing 350-400 grams and 16 rabbits of the Chinchilla breed weighing 2.5-3.0 kg with the virulent strains of Mycobacterium tuberculosis "Humanis NQ 2520" [5].

Determination of acute toxicity. Acute toxicity and the clinical picture of intoxication were determined on white outbred mice (n=25) weighing 18-20 g of both sexes. The drugs were administered in 1 and 3% concentrations, depending on the dose once on an empty stomach intraperitoneally. The effect of the drugs was assessed by the change in behavioral reactions, the general condition and death of animals for 4 hours and within 14 days after drug administration. The results of the experiments were processed by the method of variation statistics. The LD_{50} calculations were made according to the method of Litchfield and Wilcoxon [6].

Pharmacokinetics. Pharmacokinetic studies were performed on outbred white male rats with an average weight of 200±20 g. In studies based on the composition of the active ingredients, the drug Mayrin was chosen as a reference drug. Combined anti-TB drug "Mayrin" where the main active ingredients are isoniazid (75 mg), ethambutol (300 mg) and rifampicin (150 mg), but in some combinations there is pyrazinamide, which is produced under the names ACT-4, Zukoks, Zukoks E, Zukoks Plus, Isozid comp 300 mg-H, Isocomb, Isoprodian, Kombitub, Mayrin-P, Prothiocomb, Lomicomb, etc. [7].

The concentration of isoniazid, its metabolite acetylizoniazid and rifampicin was determined by high

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performance liquid chromatography on a Zorbax Eclipse XDB C_{18} column (3x100mm). Ethambutol was determined spectrophotometrically [8]. Calculations of pharmacokinetic parameters were performed using the Borgia program [9].

RESULTS AND DISCUSSION

The results of drug susceptibility were assessed on the 28th day after sowing until pronounced growth in the control tube. Grown strains in the control tube are evaluated as sensitive if less than 20 colonies have grown in vitro with abundant growth. Strains are assessed as resistant if more than 20 colonies have grown in vitro.

As can be seen from table 3, the strain H37Rv showed sensitivity in all concentrations tested to Biomayrin.

Table 3

The sensitivity of the MBT (strain H37Rv) to the drug Biomayrin

	The final concentration of drugs	H37Rv	
		K_1-10^3	K_2-10^4
Biomayrin	40.0 µg	negatively	negatively
	20.0 µg	negatively	negatively
	10.0 µg	negatively	negatively
	5.0 µg	negatively	negatively

Further, to compare the anti-tuberculosis activity of Biomayrin with isoniazid, ethambutol and rifampicin anti-tuberculosis drugs, parallel to the solid nutrient medium (Levenstein-Jensen), diagnostic materials obtained from patients with a drug-sensitive form of tuberculosis were inoculated into a modified MGIT medium (Middlebrook 7H9) by adding BioMayrin. Similarly as in solid nutrient medium, rifampicin, isoniazid and ethambutol were added to individual MGIT tubes. The prepared inoculums were incubated in an automated test system BACTEC MGIT 960. Interpretation of the mycobacterial colonies growth was carried out using the built-in scanning algorithm inside the incubator for 12 days.

The results of determination of drug susceptibility to Biomayrin, rifampicin, ethambutol and isoniazid are presented in table 4.

Table 4

The sensitivity of the MBT to Biomairin rifampicin, ethambutol and isoniazid

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Tested material	H37Rv (classical strain)	16294 (patient)	16947 (patient)
The final concentration of drugs	40,0 µg		
Biomayrin	S	S	S
Rif	S	S	S
Etm	S	S	S
H	S	S	S

S- (sensitive) sensitive form

R- (resistant) stable form

As can be seen from table 4, all the studied drugs were assessed as sensitive.

The sensitivity of drug-resistant strains obtained from patients to the studied drugs was also investigated. The concentration of drugs on liquid nutrient media MGIT Middlebrook 7H9 was carried out in accordance with the solid nutrient medium Levenstein-Jensen. Dilutions of mycobacterial suspensions were prepared in ratios according to McFarland № 0.5.

In order to obtain reliable results, diagnostic materials obtained from patients with drug-resistant forms were sown on solid nutrient medium (Levenstein-Jensen), growth of M. tuberculosis in the media was observed for 21 days. The results of tests for drug susceptibility of resistant strains to the drug Biomayrin are shown in Table 5.

Table 5

The sensitivity of the MBT to Biomayrin rifampicin, ethambutol and isoniazid

Biomayrin	The final concentration of drugs	11748 (patient)		11891 (patient)	
		K_1-10^3	K_2-10^4	K_1-10^3	K_2-10^4
	40,0 µg	10 CFU	negatively	3 CFU	negatively
	20,0 µg	8 CFU	1 CFU	2+	2+

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	10,0 µg	20 CFU	2+	3+	3+
	5,0 µg	20 CFU	2+	3+	3+
Rif	40,0 µg	R	R	R	R
Etm	40,0 µg	R	R	R	R
H	40,0 µg	R	R	R	R

S- (sensitive) sensitive form

R- (resistant) stable form

As can be seen from table 5, resistant MBT strains showed sensitivity only to Biomayrin among the studied drugs at a concentration of 40 µg at a dilution of K_2-10^4, and in other cases showed resistance.

The comparative bacteriostatic activity of Biomayrin with isoniazid and Mayrin anti-TB drugs (isoniazid - 75 mg, ethambutol - 300 mg; rifampicin - 150 mg) was studied in vivo. The study was carried out on guinea pigs and rabbits of the "Chinchilla" breed with virulent Mycobacterium tuberculosis strains "Humanis NQ 2520". The preparations were administered orally, after 21 days of infection of animals with the Mycobacterium tuberculosis strain "Humanis NQ 2520". Isoniazid and Mayrin in a dose of 10 mg/kg, and Biomayrin based on the content of active substances.

The activity of the drugs was assessed by the index of damage to the internal organs, which was determined by the method of R. Vojtek (damage to the spleen):

IS =MS · 100%/MB,

where: IS - spleen index
MS - the mass of the spleen
MB – the mass of the body.

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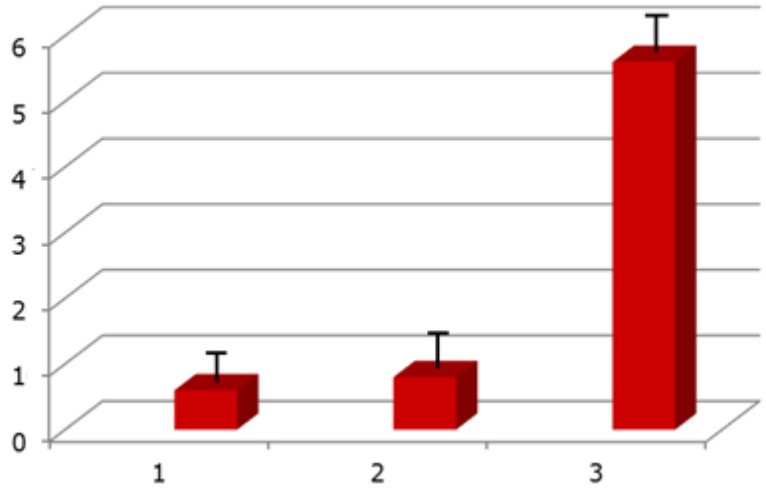
The results of the study are presented in Figure 1.



where: 1. Biomayrin; 2. Mayrin; 3. Control

Fig.1. Spleen lesion index.

As can be seen from Figure 1, Biomayrin and Mayrin show pronounced anti-tuberculosis activity. In particular, the lesion index of the spleen in control animals is equal to 5.4%, with the introduction of Biomayrin 0.32% and Mayrin equal to 0.24%. Pathologicoanatomic studies of experimental animals after the study showed that there were no significant changes in the internal organs of animals taking Biomayrin. And in animals taking Isoniazid and Mayrin, there were some changes in the liver.

Toxicological studies of Biomayrin were compared with low molecular weight analogues of isoniazid, ethambutol and rifampicin. The results of the experiments were processed by the method of variation statistics. Calculations LD_{50} were carried out by the method of Litchfield and Wilcoxon. On the basis of the conducted studies, the LD_{50} of the studied drugs were determined, which are presented in Table 6.

Table 6

Composition and LD_{50} of the studied anti-TB drugs

Composition	Content of isoniazid		Content of ethambutol		Content of rifampicin		LD_{50} mg/kg
	%	mole %	%	mole %	%	mole %	

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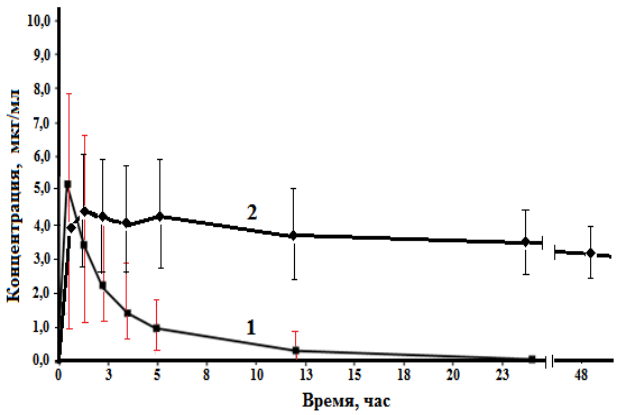
Isoniazid	100	100	-	-	-	-	224 (160÷300)
Ethambutol	-	-	100	100	-	-	1290 (1100÷1500)
Rifampicin	-	-	-	-	100	100	1570 (1350÷1800)
Biomayrin	15±3	13±3	15±3	10±3	17±3	5±2	≥ 5000

As can be seen from table 6, the acute toxicity of Biomayrin is much lower than that of low-molecular analogs; in particular, LD_{50} for Biomayrin was more than 5000 mg/kg, whereas for isoniazid it was 224 mg/kg, ethambutol 1290 mg/kg and rifampicin 1570 mg/kg.

The pharmacokinetics of Biomayrin was studied in comparison with Mayrin. Studies have shown that after a single oral administration of Mayrin at a dose of 7.5 mg/kg, the drug is quickly absorbed, reaching a maximum concentration (5.18 ± 0.54) in the blood after 1 hour, and in the lungs after 3 hours, and remains in bacteriostatic range within 12 hours (Fig. 2 a-1). The drug penetrates into all physiological fluids - cerebrospinal fluid, pleural, ascitic, milk in nursing mothers, and the level of its concentration in these fluids is similar to its level in plasma.

With the introduction of Biomayrin in a dose of 15 mg/kg, isoniazid appears in the blood of rats after 60 minutes at a concentration (4.68 ± 0.57) and then the concentration of isoniazid remains in the range of 3.0-6.0 mcg/ml for 48 hours Then there is a gradual decrease in the concentration of the drug in the blood up to 72 hours (Fig. 2 a-2).



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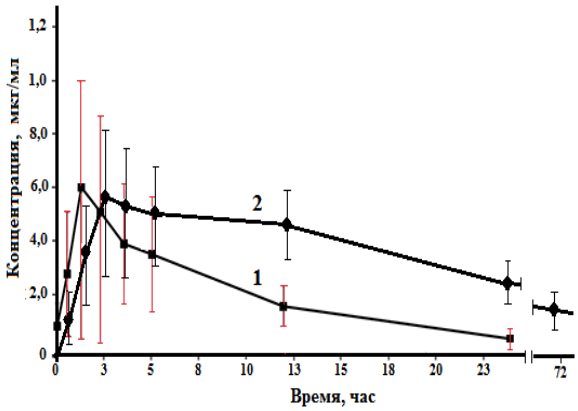

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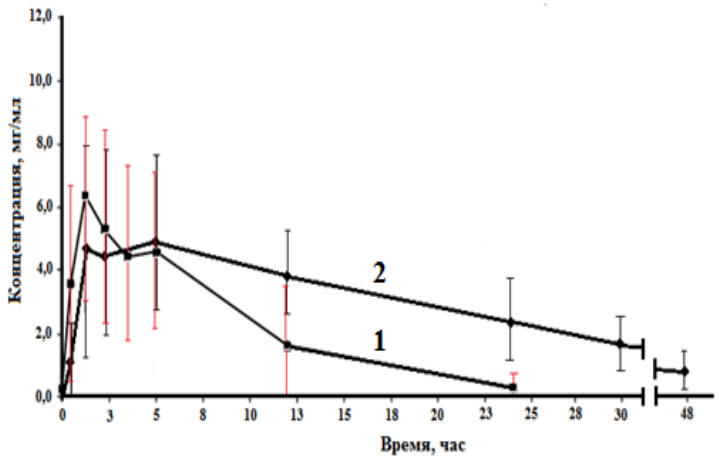

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Fig. 2.

Dynamics of changes in the concentration of a) isoniazid; b) ethambutol; c) rifampicin in serum after oral administration of Mayrin (1) and Biomayrin (2).

The maximum concentration of ethambutol in serum (6 μg/ml) is reached 2 hours after the administration of Mayrin and after 24 hours its concentration is less than 1 μg/ml (Fig. 2. b-2).

With the introduction of Biomayrin, the maximum concentration of ethambutol (5.83 μg/ml) in the blood is observed 3 hours after administration. In the future, the level of ethambutol in the blood gradually decreases to 72 hours (Fig.2 b-2).

The maximum concentration of rifampicin in the blood plasma after the administration of Mayrin is reached in 2-2.5 hours (6.3 μg/ml), it is found in therapeutic concentrations in the pleural exudate, sputum, contents of cavities, bone tissue, the greatest concentration is created in the liver and kidneys. At the therapeutic level, the concentration of the drug is maintained for 8-12 hours (Fig. 2 b-1).

Based on the data obtained on the elimination of drugs with urine from the body of rats, calculations of pharmacokinetic parameters for Biomayrin in the Borgia program were carried out. The results are presented in table 7.

Table 7

Pharmacokinetic parameters of Biomayrin

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Pharmacokinetic parameters	Isoniazid	Ethambutol	R-cin	Biomayrin		
				Isoniazid	Ethambutol	R-cin
The period of semi-absorption T1/2 (Ka), min	108.281	468.243	92.4	175.221	959.834	58.7
Half-life T1/(Kel), min	693.000	1420.08	752.2	742.765	2887.50	1626.7
Absorption constant Ka	0.00640	0.00148	0.007	0,00396	0.00072	0.01
Elimination rate constant Kel	0.00100	0.00049	0.0009	0.00093	0.00024	0.0004
The half-period of equilibrium concentration T1/2 Css max, min	0.290	0.366	0.25	0.341	0.366	0.105
Equilibrium concentration Css max, µg/ml	0.426	0.001	0.013	0.117	0.000	0.001
Volume of distribution Vd, ml	2173.90	47559.3	145.3	5763.53	305574	2108.7
Total clearance Clt ml/min	2.174	23.209	0.13	5.377	73.338	0.8
Area under "concentration-time" curve AUG	230001	468.243	7465.2	92982,2	6817.8	1113.2

As seen with the introduction of Biomyirin, the elimination half-life increases for all three isoniazid, ethambutol, and rifampicin preparations. The rate of elimination decreases. The period of semi-absorption increases, the rate of absorption decreases. For ethambutol, the equilibrium maximum concentration does not change, for isoniazid and its modified forms, the equilibrium maximum concentration decreases by 75%, and for rifampicin and its modified form, the equilibrium maximum concentration decreases by 92%.

The total clearance of Biomayrin for isoniazid is increased by 250% and for ethambutol it is increased by 320% and for rifampicin by 615%. This difference in clearance is associated with greater bioavailability of drugs in the composition of Biomayrin.

CONCLUSION

Thus, the results of the study showed that Biomayrin, being less toxic than low-molecular analogs, has a pronounced anti-tuberculosis effect. With a two-fold lower dose in the calculation of the active substance in

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comparison with Mayrin, it exhibits almost identical activity against the strain MBT Humanis.

The results of pharmacokinetic studies have shown that with the introduction of Biomayrin, the therapeutic concentration of active substances (isoniazid, ethambutol and rifampicin) in the blood lasts longer than that of their low-molecular counterparts. It should also be noted that the introduction of isoniazid into the polysaccharide matrix slows down its metabolism to the therapeutically inactive acetylisoniazid. The results obtained reliably confirmed the prolonged action of Biomayrin.

REFERENCES

1. Khomenko A.G. Modern chemotherapy of tuberculosis // Clinical pharmacology and therapy. 1998. № 4. P. 16-20.
2. Strachunsky L.S., Kozlov S.N. Modern antimicrobial chemotherapy. A guide for doctors. - M.: Borges, 2002. – P. 432.
3. Application for patent IAP 20160219 dated 07/06/2016 Shomurotov Sh.A., Akhmedov O.R., Turaev A.S. "Combined long-acting anti-TB drug."
4. Recommendation for laboratory diagnosis of tuberculosis. Omsk, 1988. – P.64.
5. Yaschenko T.N. Mecheva I.S. Manual of laboratory tests for tuberculosis. – M.: Medicine, 1973. – P. 53-77.
6. Methodical recommendations for preclinical study of the general toxic effect of drugs. / Guidelines for conducting preclinical studies of drugs. Part one. P.13-23 // ed. Mironova A.N.- M.-2012. - p.944
7. "Encyclopedia of medicines" 2017. Register of medicines of Russia. 25th edition.
8. Slivkin A.I., Selemenev V.F., Sukhoverkhova Ye.A. (1999) Physico-chemical and biological methods for assessing the quality of medicines. Voronezh: VSU,. 215-218.
9. Laboratory of Applied Pharmacokinetics (1995) USC * Pack P.C. Collection Clinical Research Programs. Los Angeles. University of Southern California. School of Medicine. Version 10.7.