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ИЗВЕСТИЯ

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Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабарлары. Химия және технология сериясы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Webof Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабарлары. Химия және технология сериясы Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді химиялық ғылымдар бойынша контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Известия НАН РК. Серия химии и технологий» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Известия НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному контенту по химическим наукам для нашего сообщества.

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STUDY ON THE EFFECT OF THE DRUG FS-1 ON ACTIVITY OF BACTERIAL ANTIOXIDANT SYSTEM

Abstract. The effect of iodine-containing complex FS-1 on the antioxidant system in *M. smegmatis* is described. It is shown that FS-1 under *in vitro* experimental conditions at doses of 2 μg/ml and 4 μg/ml inhibits the functional activity of bacterial superoxide dismutase enzyme on the example of atypical mycobacteria *M. smegmatis*, i.e. resistance to oxidative stress.

Keywords: Iodine-containing complex FS-1, antioxidant system, oxidative stress.

Introduction. The widespread introduction of anti-infectious drugs in medical and veterinary practice has resulted in the emergence of various resistance mechanisms in microorganisms that neutralize or reduce their effect [1-3]. Currently, this problem has acquired a global scale and led to a decrease in the drug treatment effectiveness or its complete loss [4-6].

The reason for the spread of infection is not only the resistance of the causative agent to the drugs used, but also the acquisition of resistance and adaptation to the effects of the defense mechanisms of the immune system in the macroorganism [7-9]. It has been established that the course of the disease and nature of the treatment for a number of infectious diseases are influenced by free radical oxidation processes [10, 13]. The protective role of the immune system in the macroorganism and, first of all, phagocytosis with an oxygen-dependent mechanism of action on bacteria is known [11]. In this case, phagocytes kill the absorbed microbes by various superoxide radicals through the action of the antioxidant enzyme system in microorganisms. The antioxidant enzyme system in microorganisms is composed of catalase, peroxidase, antioxidant superoxide dismutase (SOD), etc., involved in the neutralization of free radicals. Catalase and SOD protect microorganisms from exogenous and endogenous oxidative stresses by neutralizing superoxide radicals. The superoxide toxic radical formed in the cells as a result of metabolic processes by means of the SOD and catalase enzymes is converted into hydrogen peroxide, followed by its decomposition into harmless molecular oxygen and water. Microorganisms, in turn, acquire resistance and adaptation to oxidative stress, as a result of which they survive in the nidus of inflammation, frequently inside phagocytes [12-15].

The cells of microorganisms in their structure and functions are a full-fledged unicellular organism responsible for physiological regulation and self-reproduction [16]. The mechanism of action of modern chemotherapeutic drugs on the microbial cell is complex and multiform. It is associated with their effects on the microbial enzyme system, respiratory rate, metabolism, on the processes of reproduction and vital activity of bacteria. Therefore, the need to study the effect of chemotherapeutic drugs on the microbial cells, their morphological features, emerging endogenous and exogenous oxidative stresses during both metabolic and infectious processes, seems relevant.

This paper presents the results of a study on the effect of the new iodine-containing drug FS-1 (Patent of the Republic of Kazakhstan No. 28746, 2014) developed at JSC Scientific Center for Anti-Infectious Drugs on the activity of the bacterial antioxidant system in mycobacteria.

Materials and Methods. As a model microorganism, a fast-growing atypical culture *Mycobacterium smegmatis* ATCC 607, obtained from the American Type Culture Collection, was used. The examined concentrations of the drug FS-1 were chosen based on MBC against this test culture, and amounted to 2 and 4 μg/ml. 0.1% solution of adrenaline hydrochloride (FSUE Moscow Endocrine Plant, Russia), 0.2 M bicarbonate buffer (pH 10.65) were used in this study.

The effect of the substance under study on the antioxidant system in bacteria was determined by *in vitro* autooxidation of adrenaline [17]. During the autooxidation of adrenaline in alkaline medium at room temperature, an adrenaline oxidation product was formed, which absorbed at a wavelength of 347 nm. The formation of this product was inhibited by bacterial superoxide dismutase. It was found that the emergence of the adrenaline oxidation product significantly outpaced the formation of adrenochrome (480 nm). It was therefore proposed to use the determination of this substance to measure the antioxidant activity of various compounds.

To this end, 2 ml of bicarbonate buffer (pH 10.65) were poured into test tubes, 2 ml of a suspension of *M. smegmatis* ATCC 607 at a concentration of $1.5x10^8$ CFU/ml prepared in physiological saline (pH 7.2) were added, followed by the introduction of the examined concentrations of FS-1 and 0.1 % solution of adrenaline hydrochloride. The experimental tubes were incubated for 15 min at room temperature and further centrifuged at 5,000 rpm for 5 min. A sample without adding the substance under study, i.e. containing 2 ml of bicarbonate buffer, 2 ml of physiological saline, and 0.2 ml of 0.1% adrenaline hydrochloride solution, served as a control.

The optical density of the supernatant was measured every 30 seconds for 10 min (20 cycles) in the spectral range from 200 to 500 nm using the Perkin – Elmer Lambda 35 double-beam spectrophotometer (USA). The operating principle for this device is based on measuring the ratio of two light fluxes that passed through the comparison channel (blank - 2 ml of bicarbonate buffer, 2 ml of saline solution) and the sample channel in the cuvette compartment, which allows the background values to be cut off.

The degree of impact of the substance under study on the antioxidant system in bacteria was calculated according to the following formula [17]:

$$\text{Percentage of inhibition (activity units} = [1 - (OD_{control} / OD_{exp})] \times 100\ \% \quad (1),$$

where $OD_{control}$ is the average value (n=20) of the optical density of the control sample ; OD_{exp} is the average value (n=20) of the optical density of the experimental sample.

The values above 30% were considered as a significant suppression of the activity of the bacterial antioxidant system under the effect of the substance under study.

The results of measuring the kinetics of the process for autooxidation of adrenaline in alkaline medium and in the presence of the examined concentrations of the drug FS-1 were presented as mean values from 2 independent experiments. In a statistical analysis of the control and experimental samples with GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA), the obtained data were checked for normal distribution using the Shapiro-Wilk test. When confirming the null hypothesis, the further data processing was carried out by the parametric method (One-way ANOVA). The results were considered significant at $p \leq 0.05$.

Results and Discussion

The effect of the drug FS-1 on the antioxidant system in *M. smegmatis* by *in vitro* autooxidation of adrenaline was studied. Spectral studies of the control sample in the range 200 to 500 nm revealed 3 absorption maxima at 242 nm, 292 nm, and 347 nm (Fig. 1). An aqueous solution of adrenaline gives a maximum absorption at 279 nm, while in alkaline medium (bicarbonate buffer, pH 10.65) there is a slight shift in the absorption maximum in the UV region to 292 nm [17]. As shown in Figure 1, by measuring the entire spectrum for 10 min, it was possible to detect the dynamics of spectral changes at 347 nm, which increased in direct proportion to the measurement time. An increment in the optical density of accumulation of the primary adrenaline oxidation product was 0.01-0.02 OU (optical density)/min; when measuring immediately, the optical density at 347 nm was equal to 0.18 A, after 10 minutes it was 0.35 A. Moreover, the optical density in the spectrum characteristic of adrenaline in alkaline medium (292 nm) decreased only by 0.01 A (from 1.41 A to 1.40 A) for 10 min.

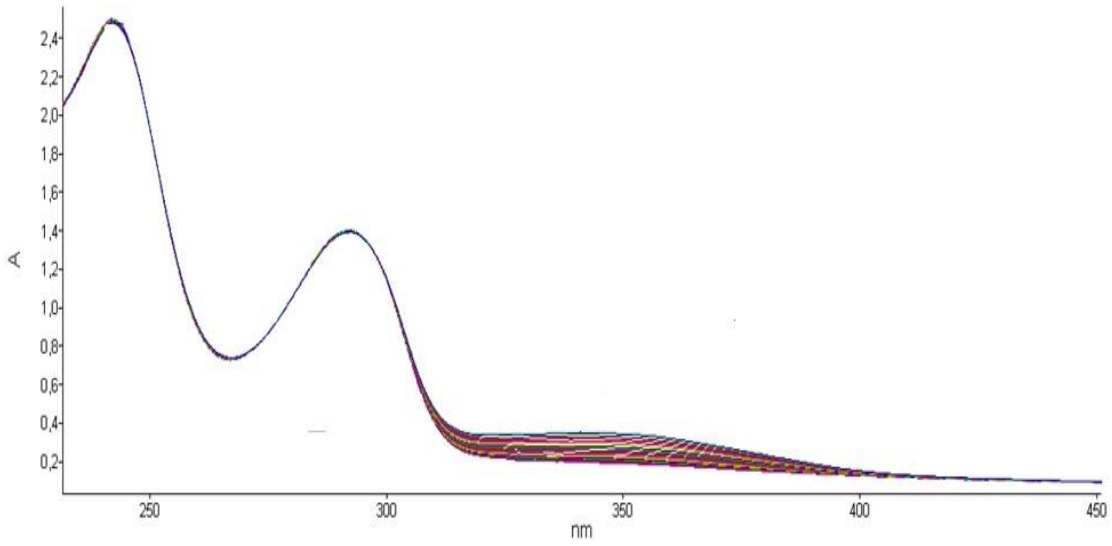

Figure 1 - UV

Spectral studies of the experimental samples in the range of 200 to 500 nm revealed that the introduction of the drug FS-1 in the examined concentrations resulted in a slight shift in the wavelength from 242 nm to 240 nm, 292 nm to 295 nm, and 347 nm to 333 nm (Fig. 2, 3).

Figure 2 shows the results of UV spectroscopy of the experimental sample containing 2 μg/ml of FS-1 where a time-dependent dynamics of spectral changes at 347 nm is clearly visible. An increment in the optical density of accumulation of the primary product of adrenaline autooxidation under the effect of the drug FS-1 at a concentration of 2 μg/ml was on average 0.05 OU/min. The optical density of the experimental sample containing 2 μg/ml of FS-1 with immediate measurement at 347 nm was equal to 0.54 A, after 10 minutes it was 1.05 A.

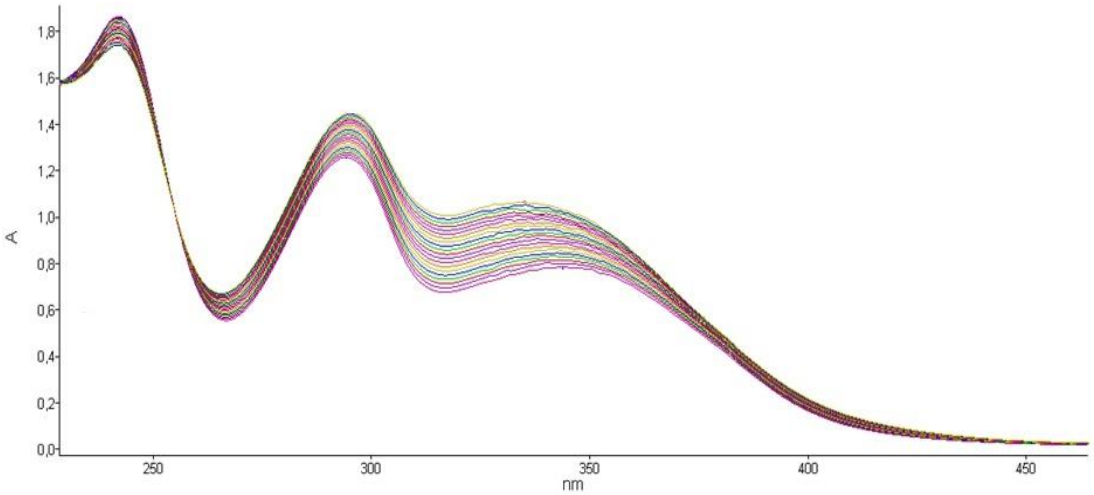

Figure 2 - UV absorption spectrum of the experimental sample containing 2 μg/ml of FS-1

Spectral measurements of the experimental sample containing 4 μg/ml of FS-1 at 347 nm for 10 minutes showed a time-dependent increasing dynamics of spectral changes (Fig. 3). An increment in the optical density of accumulation of the primary adrenaline oxidation product under the effect of the drug FS-1 at a concentration of 4 μg/ml was on average 0.035 OU/min; when measuring immediately, the optical density at 347 nm was equal to 0.83 A, after 10 minutes it was 1.18 A.

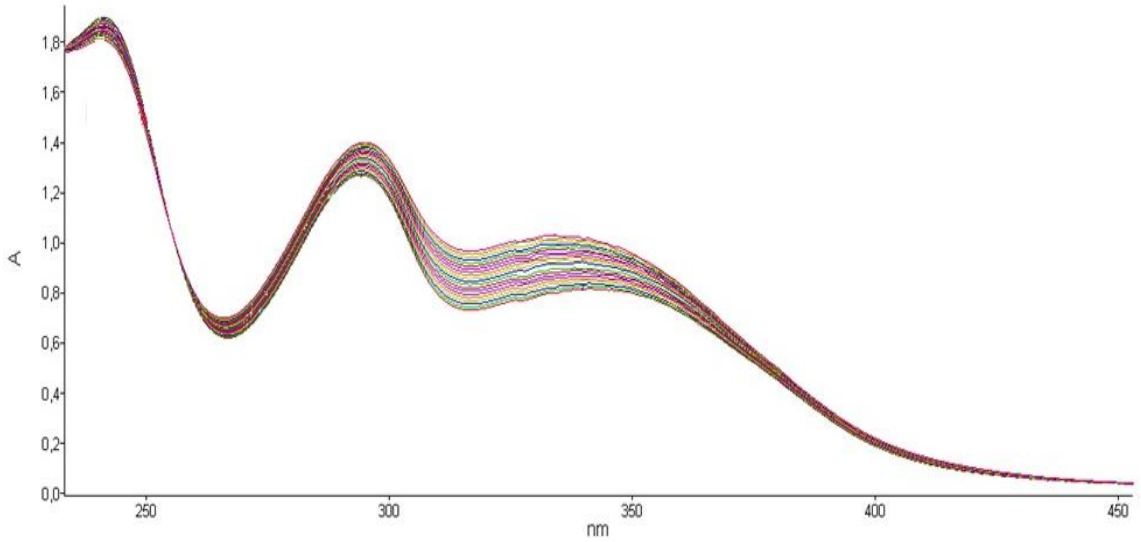

Figure 3 - UV absorption spectrum of the experimental sample containing 4 μg/ml of FS-1

Figure 4 presents summary data showing the dynamics of changes in the optical density of the control and experimental samples at a wavelength of 347 nm for 10 minutes.

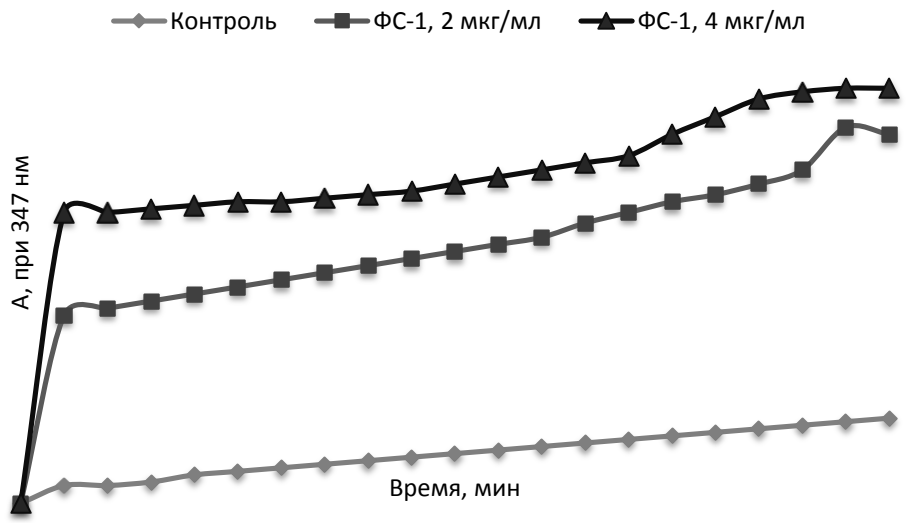

Figure 4 -Dynamics of changes in optical density of the control and experimental samples at a wavelength of 347 nm

As can be seen in Figure 4, the introduction of the drug FS-1 into the experimental samples at concentrations of 2 and 4 μg/ml compared with the control sample enhanced 2.5 ($p < 0.0001$) and 1.8 ($p < 0.0001$) times, respectively, the accumulation of the adrenaline autooxidation product in the supernatant, which is toxic to *M. smegmatis*.

The degree of impact of the drug FS-1 at a concentration of 2 μg/ml on the antioxidant system in bacteria calculated by the formula (1) was 80%, which indicated a significant inhibition of the functional activity of the bacterial superoxide dismutase enzyme under the effect of the examined substance.

The concentration of the drug FS-1 of 4 μg/ml also inhibited the functional activity of superoxide dismutase in *M. smegmatis* ATCC 607; the degree of impact of the examined substance on the antioxidant system in the test culture was 85%.

Bacteriological control of the supernatant after exposure to FS-1 at doses of 2 and 4 μg/ml on the bacterial antioxidant system showed a lack of growth on the Levenshtein-Jensen egg-based culture medium.

The studies thereby found that the drug FS-1 under *in vitro* experimental conditions at doses of 2 and 4 μg/ml exhibited a pronounced inhibitory activity on the antioxidant enzyme system in atypical mycobacteria *M. smegmatis*. This led to a loss of resistance to oxidative stress in bacterial cells and their death.

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БАКТЕРИАЛДЫ АНТИОКСИДАНТТЫ ЖҮЙЕСІНІҢ БЕЛСЕНДІЛІГІНЕ ФС-1 ДӘРІЛІК ЗАТЫНЫҢ ӘСЕР ЕТУІН ЗЕРТТЕУ

Аннотация. Бұл жұмыста *M.smegmatis* бактериясының антиоксидантты жүйесіне құрамында иод бар ФС-1 кешенінің әсер ету нәтижелері ұсынылды. Эксперименттік *in vitro* жағдайда ФС-1 дәрілік заты 2 және 4 мкг/мл дозасында әсер еткен кезде *M.smegmatis* – атипті микобактериялар мысалында супероксиддисмутаза бактериялық ферментінің функционалдық белсенділігін, яғни тотығу күйзелісіне тұрақтылығын төмендететіні көрсетілді.

Түйін сөз: құрамында иод бар ФС-1 кешені, антиоксидантты жүйесі, тотығу күйзелісі.

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