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IMMUNOLOGICAL CONTROL OF HISTAMINE SECRETION IN TB/HIV INFECTED HUMAN PATIENTS BY METAL NANOPARTICLES-A CASE STUDY

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ABSTRACT

Silver nanoparticles (Ag NPs) with particle size 42-44nm function as a fast and sensitive marker for detection of histamine resulting from biological samples of TB and HIV patients. Characterization of AgNPs is carried out by using different analytical techniques such as UV–vis, FTIR, and TEM. The possibility of AgNPs, a nanomaterial pervasively used in nanotechnology and pharmaceutical industries, to directly induce histamine secretion without prior allergen sensitization has remained uncertain. Our investigation suggests that systemic circulation of Ag NPs may prompt histamine release at different locales causing abnormal changes in the secretion of histamine, which regulates immunity in TB and HIV infected patents. The *Mycobacterium tuberculosis* (Mtb) has developed methods to evade the immune system and survive. With the discovery of nanoparticle (NP)-based drugs, it is necessary to research their antimycobacterial properties and bactericidal mechanisms. In this study, we synthesized mixed AgNPs and tested their ability to inhibit *Mtb* growth into macrophages and investigated the cytotoxic effects of NPs in THP-1 cells. The *in vitro* experiment, involving 20 strains of the tuberculosis mycobacteria, exhibited a potentiating effect of silver nanoparticles on known antituberculous preparations in respect of overcoming drug-resistance of the causative agent.

Keywords: **Histamine, TB, HIV, Nanoparticles**

INTRODUCTION

Patients co-infected with HIV-1 and tuberculosis (TB) are at risk of developing TB-associated immune reconstitution inflammatory syndrome (TB-IRIS) following commencement of antiretroviral therapy (ART). TB-IRIS is characterized by transient but severe localized or systemic inflammatory reactions against Mycobacterium tuberculosis antigens. The role of innate immunity in TB-IRIS pathogenesis has attracted increased attention in recent years [1]. The immune system can defend against foreign antigens, which has been divided into two general types of immunity: innate immunity and adaptive immunity. Innate immunity is the nonspecific and first line of the body's defense system, which relies on pattern recognition receptors (PRPs) to recognize broad and conserved molecular patterns found on pathogens (pathogen-associated molecular patterns, PAMPs) [2].Therefore, the innate immune system plays an essential role in the early recognition and subsequent proinflammatory response. The adaptive immune system is antigen specific and reacts only with the organism that induced the response. Innate and adaptive immunity can be thought of as two equally important aspects of the immune system. Innate immune systemconsists of different cells andproteins that arenonspecific and first line of defense system. The main components of the innate immune system are including physical epithelial barriers, phagocytic cells (monocyte/macrophages, dendritic cells, and polymorphonuclear leukocytes), phagocytic leukocytes, basophils, mast cells, eosinophils, natural killer (NK) cell, and circulating plasma proteins [3]. The main function of the immune system is to protect hosts from infectious pathogens by recognizing self from non-self [4]. Many environmental substances, such as metals, organic pesticides, and gaseous pollutants, disrupt the immune response and increase susceptibility to infectious disease [5]. The immune system is comprised of various cells, cell products, tissues and organs, which together form an efficient, interactive and intricate network that protects the host from infectious agents. Before encountering the immune systems, the pathogen encounters different barriers, comprising of external (skin etc.) and chemical barriers (stomach acids etc.) [6]. The immune system consists of two types of immunity, namely; the innate immunity, that is present from birth and acquired immunity that only develops after birth and upon contact with a specific pathogen [7]. The investigation into the effects of silver (Ag) and silver nanoparticles (AgNPs) have increased considerably as there have been dramatic increases in commercial products

which contain AgNPs. In 2014 alone 420 tons of AgNPs were produced and this amount is expected to rise in the future. Products containing AgNPs range from health and fitness, cleaning products, and food [8]. However, relatively little is known about the potential adverse effects of AgNPs. In recent decades, many studies have rapid progress in toll-like receptor of innate system, which induce expression genes of involved inflammation. Moreover, toll-like receptors activate both innate and adaptive immune system and play an important role in antiviral and anti-immunity [9]. Mast cells (MC) are inflammatory cells typically found in relatively large numbers in the mucosa of the respiratory, gastrointestinal, and urinary tracts and near blood or lymphatic vessels. Since these sites are also common portals of infection, MC are likely to be one of the first inflammatory cells to make contact with invading pathogens and, following activation, release a myriad of proinflammatory cytokines, proteases, and inflammatory mediators. These MC mediators are crucial for mobilizing and recruiting various other inflammatory cells to the site of infection [10]. The vital role of MC in triggering the innate immune response has been demonstrated following infections with *Escherichia coli* and *Klebsiella pneumoniae* [11]. In vitro studies have also suggested that MC may be involved in host defense against the highly infectious respiratory pathogen Bordetella pertussis [12]. The aim of this project is to utilize mammalian cell cultures to monitor the effects of the heavy metals silver on the immune system, utilizing in vitro techniques. Once the in vitro cell cultures have been optimized by using the heavy metals, the effects of various nanoparticles (AgNPs) on the immune system will be evaluated.

Materials and Method

Human Samples (Study group): The samples for this study comprised normal human volunteers (Control) (n=12) and TB patients (n=20) – including untreated (TB1= 4), under treatment (TB2, n=16) with anti-tuberculosis therapy (ATT). 8 HIV infected Patients (3Female/3 Male) were also included in this study were from Govt. Chest and TB Hospital, Mangalagiri, and Govt. General Hospital, Guntur, India. Blood samples were collected from all subjects and following parameters were estimated with prior written permission from Patients and respective authorities in the period of December 2017 to June 2018, characteristics of study group given in table1.

Characterization of Silver Nanoparticles: Chemical bonding and functional group in Ag Nps analyzed using Fourier transform infrared spectrophotometer (FT-IR) (Perkin Elmer,

Waltham, MA, USA). The FT-IR spectrum was recorded in the range of 400–4000 cm−1 with 4 cm−1 spectral resolution. The UV-Visible absorption spectrum of AgNPs has been recorded by using Elico made double beam spectro photometer and surface morphology ny transmission electron microscopy (TEM, Philips CM200).

Chemicals: Synthetic Silver Nitrate (100ml) nanoprticle solution purchased from Sigma-Aldrich (USA) with an average partile size 45nm, and other chemicals used in this study are AR grade purchased from Merck co Ltd, India. The Veratox® kit ELISA, dedicated to histamine quantification, was purchased from Neogen® Corporation. All aqueous solutions required in the electrochemical studies were freshly prepared from the solid and pure compounds with double distilled water obtained in the lab with professional equipment.

HIV Detection and Estimation of Histamine Levels in TB/HIV infected Patients: 8 HIV patients were divided into 2 groups depending on the level of CD-4 lymphocytes. The first group included 4 patients with CD-4 cell count more than 500 cell/mm3, second group consisted of 2 patients with CD-4 cell count less than 500 cell/mm^3. Histamine analysis was done by the method proposed by Meshyarikova S.A [13]. According to this technique, histamine was determined by the DeNovix QFX Fluorometer, India. The method is based on the formation of fluorescence condensation products with *o*-phthalic aldehyde. Selected HIV infected patients were registered, the diagnosis of all patients was confirmed clinically and by the laboratory methods of ELISA and Western blot method. Histamine levels were measured in TB patients cell pellets and supernatants using also a fluorometric assay. Histamine release was expressed as percentage of the total cellular histamine content and calculated by the formula:

$$\text{\% histamine release} = \frac{\text{histamine in supernatant}}{\text{histamine in supernatant and pellet}} \times 100$$

In all experiments, total histamine content was calculated, and the spontaneous release was subtracted from stimulated cell release

Silver nanoparticles against Mycobacterium tuberculosis

Drug preparation: Twenty clinical isolates of Mycobacterium tuberculosis obtained from Govt TB Hospital, Managalagiri and were sub cultured on Middlebrook 7H11 agar. Suspensions were prepared in 0.02% (v/v) Tween 60-0.4% bovine serum albumin, so that their turbidities matched that of a McFarland no. 1 turbidity standard. Suspensions were further diluted 1:25 in 7H9GC broth, 25 ml of 10% (vol/vol) glycerol, 1 g of Bacto Casitone (DIFCO), 880 ml of distilled

water, and 100 ml of oleic acid, albumin, dextrose, and catalase. General antibiotics used in TB treatment, Isoniazid (INH), rifampin (RMP), streptomycin (SM), and ethambutol (EMB) were obtained from Merck. Stock solutions of INH, SM, and EMB were prepared in double distilled water, and RMP was prepared in DMSO. Stock solutions were diluted in 7H9GC broth to two times the maximum desired final testing concentrations prior to their addition to microplates finally; Minimum Bactericidal Concentration (MBC) results were given in Table 4.

Anti-TB activity using Alamar Blue Method: The antimycobacterial activity of antibiotics and nanosilver were assessed against *M. tuberculosis* using Microplate Alamar Blue Assay (MABA) [14]. This methodology is non-toxic, uses a thermally stable reagent. Briefly, 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The drug concentrations ranges between 8 and 64 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. The strains which is having IC90 values of >16 µg/ml, were considered as resistance strains, these resistance strains were tested against nanosilver particles by MABA method as mentioned above, the concentration of nanosilver was taken as 1.5 µg/ml.

RESULTS AND DISCUSSION

1. UV–vis Spectroscopy analysis: A preliminary analysis provided a quick and easy screening of the synthesis of the AgNPs by identifying the localized surface plasmon resonance peak typical of nano-Ag. The Ag colloid was characterized by strong absorption in the visible region at 439nm. The position of the maximum absorption and width of the absorption band provided information about the form, average size, and size distribution of the NPs. The average size of AgNPs was 43 ± 2 nm.

2. DLS and TEM analysis: DLS has proven to be a suitable and simple technique for the characterization of multi-modal AgNPs suspensions and is one of the most frequently used methods to obtain an average diameter of NPs dispersed in liquids. DLS is a quick, simple, and nondestructive method that can

simultaneously probe many particles [15]. The DLS size distribution of myricetin-mediated synthesized AgNPs ranged from 35 to 60 nm (Figure 1B). The calculated average particle size distribution of the AgNPs was 44 nm. The broad spectrum of the DLS pattern confirmed the comparability of the particle size with the sharp SPR peak (439 nm) obtained in the UV-VIS spectra. TEM micrograph of AgNps (Figure 1a) show the particle aggregation for NPs despite the fact that nanoparticles still spherical. The precise reasons for the aggregation have not been established; however, they likely involve hydrophobic interactions. Dynamic light scattering (DLS) analysis was performed to determine the size distribution of the AgNPs synthesized using myricetin. To corroborate the evidence gained from the DLS size distribution, transmission electron microscopy (TEM) was done to reveal the size and morphology of the AgNPs purchased from Sigma Aldrich (Figure 1a). The prepared particles were nearly spherical in shape and uniform in size.

3. FT IR Analysis: Fig 3 shows the FT IR spectra of silver nanoparticles in the range of 4000-400 cm^{-1}. The FTIR spectrum shows absorption bands at wave number (cm-1) 2991, 1610, 1466, 1358, 1092, 1010 and 850-750cm^{-1}(broad) for chemically prepared AgNPs, which refers to amine or amide N-H stretching of hydrazine hydrate, N-N stretching, NH_2 deformation and nitrate stretching [16]. The presence of C–H stretching of aromatic compound was also ascertained by the absorption peak at 2991 cm^{-1} [17]. Additionally, the peaks at 1610 cm^{-1} and 1466 cm^{-1} are ascribed to –C=C– and =NH stretching of amide bonds, respectively [18]. This indicated that the amino groups are involved in the encapsulation and stabilization of AgNPs.

Immunological studies of Silver Nano particles

Some researchers have investigated the efficacy of silver nanoparticles as anti-leishmanial agents. Ameneh et al., demonstrated that combination of UV light with silver nanoparticles resulted in inhibition of the proliferation and metabolic activity of promastigotes by 2- to 6.5-fold [19]. Some viral infections can be cleared from the body via the immune system. However, some are persistent and the infections can last for years such as herpes, hepatitis, HIV, etc. Presently, very few drugs can be used to hinder the spread of viral invaders, indicating that there is a pressing need to develop new drug systems which can be effective for the treatment of viral infections. The combination also inhibited the survival of amastigotes in host cells significantly.

Quantitative determination of Histamine levels

The four groups of subjects were studied between 2017 and 2018, and their demographic characteristics are displayed in Table I. All subjects, excluding the TB patients, Control, and HIF infected patients with without/with treatment, had a normal physical examination and a normal laboratory workup, including complete blood count. Control group (n=12) 4 female, 8 male with an average age group 32.5 years, TB1 (n=4) 1 female, 3 male with age group 27 to 38 (mean33.7), TBs group (n=12) 6 female, 10 female with age group 26-41 years (mean =34.2) and HIV infected group (n=8) 3 female and male each with age group 22-40yrs (mean 37.4yrs) considered for the current investigation.

HIV infected Patients: Histamine levels estimated in the samples collected from HIV infected samples before after treatment with AgNPs- Zidovudine (AZT) complex given in table 2.

In group of patients with CD-4 cells count less than 500 cells/mm^3 histamine level was increased and average level was 1.41± 0.02 □mol/L ($p<0.001$). Furthermore, level of histamine was increased in all patients of this group irrespective to their clinical conditions. The level of histamine in this group was between 0.62-1.38 □mol/L. In group of patients with CD-4 cells count more than 500 cells/mm^3 histamine level was reliably increased and average level was 1.22±0.03 μmol/L ($p<0.001$). In this group of patients histamine level was increased in all of them regardless to their clinical conditions and varied from 0.6 9 to 2.01 μmol/L. In Table 2, the comparison of histamine level in different groups of HIV infected patients has shown the greatest increase of its level in the group of patients with CD-4 cells count more than 500 cells/mm^3. The level of histamine was decreasing as CD-4 cells count was reducing. This data indicates the active role of T-helpers in immediate hypersensitivity reaction at the presence of HIV infection. It is interesting to notice that most of the HIV infected patient (n=1) treated with AgNPs-Zidovudine complex the highest histamine level had a viral load of minimum on ARV therapy. Similar results also observed in the study of Akhmedjanova Z.I., et al (2012). It is known that histamine acts through H2 receptors of lymphocytes, activates adenylate cyclase, increases the intracellular level of cAMP. Through this system histamine acts as an immune-modulator. In particular, experiences in vitro have shown that increased level of intra cellular cAMP caused by the histamine, blocks spontaneous rosettes formation. Basing on this, we can assume that histamine participates in suppression of receptors activity of immune competent cells. A healthy immune response is a key to good health, but ongoing immune

activation and inflammation due to a persistent threat such as chronic HIV infection can lead to many different problems throughout the body [20]. Early responders release additional cytokines, including interferon-gamma that promotes longer-term immune activation mediated by the lymphocytes: T-cells, B-cells, and natural killer cells. Antigen-presenting cells such as macrophages capture pathogens and display pieces of them (antigens) on their surface. Lymphocytes interact with these cells and learn to recognize and directly target those particular pathogens. This adaptive branch of the immune system responds to specific threats. And just as some chemical messengers promote immune activation, opposing signals act to dampen responses. Anti-inflammatory cytokines include IL-4, IL-10, and transforming growth factor-beta (TGF-beta) [21]. Discovered increased level of histamine in all HIV-infected patients suggests impaired tolerance to histamine which leads to various physiological reactions in the form of dilatation of blood vessels and disturbance of microcirculation.

Histamine assay in TB Patients: The levels of histamine in the skin-chamber fluids were assessed by a radioenzymatic assay, as previously reported by Bedard PM, et al (1986) [22]. This assay reliably detects histamine concentrations as low as 100 pg/ml in all biological fluids and the dosage is not affected by the presence of compound 48/80 in the specimen. All samples of chamber fluids, obtained were assayed on a single occasion at the end of study. Histamine is a potent mediator released from mast cells and basophils under numerous conditions. Histamine is best known for its role in immediate hypersensitivity reactions because it is a potent smooth muscle spasmogen and mucus secretagogue and rapidly increases vascular endothelial permeability. Details of Histamine concentration in lavage and serum analyses on all 20 subjects (TB1 & TB2) and 12 controls are presented in Table 3.

From the Table 3, it was identified that, control blood samples showing the normal histamine sample range (25-65ng/ml), but in TB1 patients histamine values are very low indicates the low immune power, when exposed to AgNPs all TB1 & TB2 patients showing the higher histamine concentrations nearly normal range, indicating that mast cells will generate histamine secretion when exposed to AgNPs. Hence AgNPs are considered as alternative medicine to increase the immune levels in TB patients.

Histamine release from Mast Cells after Treatment with AgNPs

One milliliter aliquots of washed fraction blood cells in Tris-buffered saline were prepared, and 10-fold dilutions of AgNPs

from 10 to 0.01 mg/ml were added. These cells and controls without the AgNPs were incubated for 40 min at 37°C. Histamine in the control supematant and cells was determined fluorometrically [23]. Since AgNPs interferes with the fluorometric procedure, the test cells were washed twice and extracted in 0.4M HClO4, and residual histamine was determined as procedure describved by Patterson et al.(1981) [24]. The released histamine was calculated by the difference of test cell histamine from the total histamine in the control aliquot. Control experiments showed that an aliquot of test cells that had been incubated with 1 mg/ml AgNPs and washed contained insufficient NPs levels to interfere with subsequent assay of the histamine in an aliquot of untreated cells when both were mixed prior to determination of the cellular histamine.

Anti Tuberculosis effect of AgNPs: MBC test results for all of the clinical isolates of M. tuberculosis were available by the 7th day of incubation. After 4 days of incubation, the Alamar Blue reagent was added to the control wells. Following incubation Blue reagent was added to the control wells. Following incubation at 37°C for 24 h, most control wells became pink. For those that remained blue, Alamar Blue was added to the nextcontrol well and the plates were re-incubated for another 24 until all control wells were pink (indicating sufficient growth to determine drug susceptibility). Alamar Blue was then added to all remaining wells, and the results were determined on the following day (day 6 or 7). 20 strains were susceptible to INH, SM, EM and RMP 20 Isolates were resistant. RMP+AgNPs gives more than 90% activity against M. tubersulosis. From Table 4, it was identified that Silver nanoparticles along with antibiotic shows low MBC values indicates the AgNPs acts more aganst M. tuberculosis when compared with without NPs.

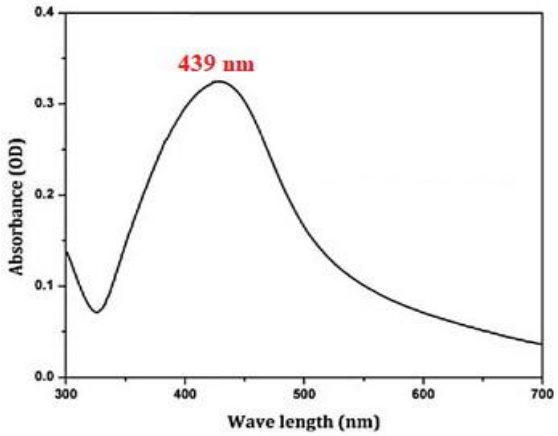

Figure 1: Ultraviolet-visible spectra of AgNPs

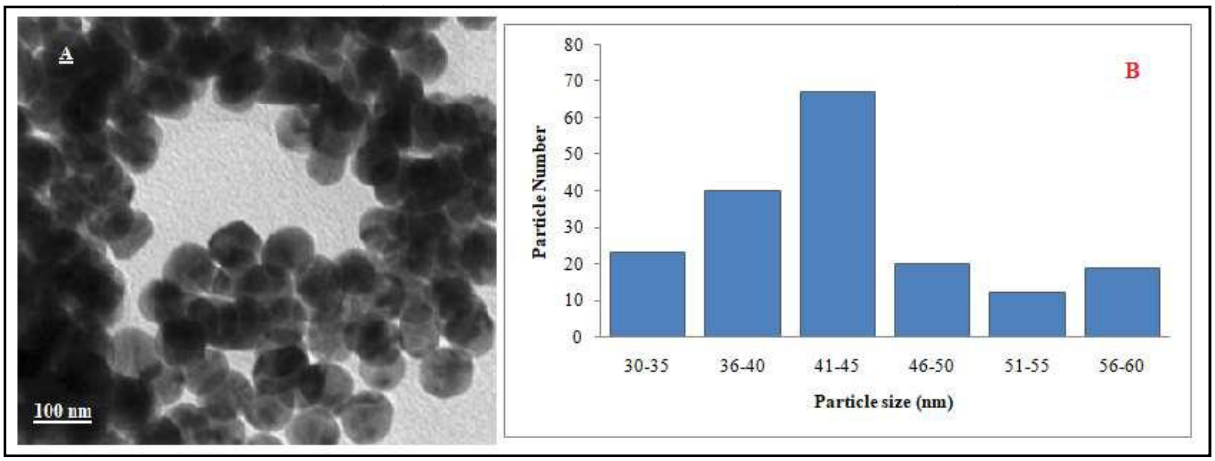

Figure 2: (A) Ag NPs characterization using TEM. A representative TEM image of the Ag NPs with an average diameter of 43± 2nm (n = 67). (B) A bar graph (DLS pattern) showing the size distribution of Ag NPs based on TEM images with average particle size 44 nm (n = 64).

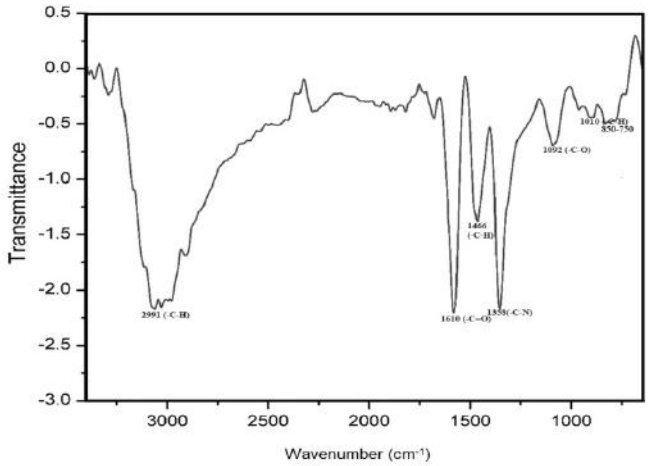

Figure 3: FT-IR spectrum of silver nanoparticles

Table 1: Characteristics of the study groups

Patient Group	n	Age (years)		Sex F/M
		Mean	Range	
Control	12	32.5	29-42	4/8
TB1	4	33.7	27-38	1/3
TB2	16	34.2	26-41	6/10
HIV	8	37.4	22-40	3/3

Table 2: Histamine concentration in HIV infected patients before and after treatment

	Histamine Concentration in HIV Infected patients µmol/L		Control µmol/L
	CD-4 cell count < 500 cell/mm^3	CD-4 cell count > 500 cell/mm^3	
Before treatment	1.41± 0.02 (p<0.001)	1.22±0.03 p<0.001	0.38±0.001
After Treatment with AgNPs-Zidovudine complex	1.46± 0.03 (p<0.001) (n=1)	1.27± 0.03 (p<0.001) (n=1)	-

Table 3: Histamine concentration (%) in 20 TB isolates and 12 controls

Sample Name	Blood Histamine (%)	Control ng/ml	After Reaction with Ag NPs	p value
TB1-I2	13.98	29.84	27.13	<0.001
TB1-I3	16.61	35.44	32.21	<0.001
TB1-I4	17.04	36.37	33.07	<0.001
TB2-I1	14.91	31.81	28.92	<0.001
TB2-I2	15.65	33.40	30.36	<0.001
TB2-I3	17.89	38.18	34.71	<0.005

TB2-I4	**14.91**	**31.81**	**28.92**	**<0.001**
TB2-I5	**15.81**	**33.74**	**30.67**	**<0.001**
TB2-I6	**23.52**	**50.19**	**45.63**	**<0.001**
TB2-I7	**21.98**	**46.90**	**42.63**	**<0.001**
TB2-I8	**19.28**	**41.14**	**37.40**	**<0.001**
TB2-I9	**21.59**	**46.08**	**41.89**	**<0.005**
TB2-I10	**20.82**	**44.43**	**40.39**	**<0.001**
TB2-I11	**17.34**	**37.00**	**33.64**	**<0.001**
TB2-I12	**13.77**	**33.39**	**26.71**	**<0.001**
TB2-I13	**41.31**	**12.95**	**23.13**	**<0.001**
TB2-I14	**12.24**	**30.87**	**23.75**	**<0.001**
TB2-I15	**11.22**	**23.94**	**21.77**	**<0.001**
TB2-I16	**18.87**	**40.27**	**36.61**	**<0.001**

Table 4: MBC values in different TB isolated treated with AgNPs+ INH/SM/EM/RMP and individual antibiotics

Sample Name*	**MBC values in %**							
	INH	**INH+AgNP**	**SM**	**SM+AgNP**	**EM**	**EM+AgNP**	**RMP**	**RMP+AgNP**
TB1-I1	**31.70**	**5.71**	**37.74**	**6.79**	**32.02**	**3.52**	**3.59**	**0.36**
TB1-I2	**27.42**	**4.94**	**32.64**	**5.88**	**27.69**	**3.05**	**3.11**	**0.37**
TB1-I3	**32.56**	**5.86**	**38.76**	**6.98**	**32.88**	**3.62**	**3.69**	**0.37**
TB1-I4	**33.42**	**6.01**	**39.78**	**7.16**	**33.75**	**3.71**	**3.79**	**0.30**
TB2-I1	**29.23**	**5.26**	**34.80**	**6.26**	**29.52**	**3.25**	**3.31**	**0.33**
TB2-I2	**30.69**	**5.52**	**36.54**	**6.58**	**31.00**	**3.41**	**3.48**	**0.35**
TB2-I3	**35.08**	**6.31**	**41.76**	**7.52**	**35.43**	**3.90**	**3.98**	**0.48**
TB2-I4	**29.23**	**5.26**	**34.80**	**6.26**	**29.52**	**3.25**	**3.31**	**0.33**
TB2-I5	**31.00**	**5.58**	**36.90**	**6.64**	**31.31**	**3.44**	**3.51**	**0.28**
TB2-I6	**46.12**	**8.30**	**54.90**	**9.88**	**46.58**	**5.12**	**5.23**	**0.52**
TB2-I7	**43.09**	**7.76**	**51.30**	**9.23**	**37.49**	**4.12**	**4.21**	**0.42**
TB2-I8	**37.80**	**6.80**	**45.00**	**8.10**	**32.89**	**3.62**	**3.69**	**0.44**
TB2-I9	**42.34**	**7.62**	**50.40**	**9.07**	**36.83**	**4.05**	**4.13**	**0.41**
TB2-I10	**40.82**	**7.35**	**48.60**	**8.75**	**35.52**	**3.91**	**3.99**	**0.32**
TB2-I11	**34.00**	**6.12**	**30.60**	**5.51**	**29.58**	**3.25**	**3.32**	**0.33**
TB2-I12	**27.00**	**4.86**	**24.30**	**4.37**	**23.49**	**2.58**	**2.64**	**0.26**
TB2-I13	**81.00**	**14.58**	**72.90**	**13.12**	**70.47**	**7.75**	**7.91**	**0.95**
TB2-I14	**24.00**	**4.32**	**21.60**	**3.89**	**20.88**	**2.30**	**2.34**	**0.23**
TB2-I15	**22.00**	**3.96**	**19.80**	**3.56**	**19.14**	**2.11**	**2.15**	**0.17**
TB2-I16	**37.00**	**6.66**	**37.74**	**6.79**	**32.19**	**3.54**	**3.61**	**0.36**

*** TB1/TB2: without treatment/with treatment; I1-16: Isolate 1-16; MBC: Minimum bacterial count**

CONCLUSION

The increasing use of AgNPs in biomedical products has prompted global concern regarding their toxicity and their impact to biological systems. All the above make it possible to assume that histamine takes a part in the regulation of immunologic reactivity, in the development of adaptive and compensatory reactions of immune system and in conservation of reserved abilities of the body. Rising concentration of histamine can indicate the specific adaptive reactions caused by adjusting to a series of factors, and a strain

of various systems of an organism. The role of histamine in this case is dilation of capillaries and augmentation of their permeability to way out various metabolites from an organism. The increase in histamine level occurs due to the changes in permeability of ionic channels of the membranes of histamine contained cells as a response to intrusion of the virus of immunodeficiency. The performed research scientifically establishes efficacy and safety of the nanocomposite application in combination therapy of patients suffering from drug-resistant tuberculosis. The data, obtained in the course of the *in vivo* experiment made it possible to conclude with statistical validity that the antituberculous activity of the silver nanoparticles in isolated variant as well as used jointly with RMP possess a dose-dependent character.

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