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Mixed metal oxide nanoparticles inhibit growth of *Mycobacterium tuberculosis* into THP-1 cells

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ABSTRACT

Objective/background: Humans have been in a constant battle with tuberculosis (TB). Currently, overuse of antibiotics has resulted in the spread of multidrug-resistant *Mycobacterium tuberculosis* (MDR), leading to antibiotic ineffectiveness at controlling the spread of TB infection in host cells and especially macrophages. Additionally, 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 metal oxide NPs and tested their ability to inhibit Mtb growth into macrophages and investigated the cytotoxic effects of NPs in THP-1 cells.

Methods: Silver (Ag) NPs and zinc oxide (ZnO) NPs were synthesized by chemical reduction and chemical deposition in aqueous solution, and the diffraction light scattering, scanning electron microscopy, transmission electron microscopy, and ultraviolet–visible light-absorption spectra were used to identify NP properties. Ag and ZnO NPs were mixed together at a ratio of $8_{ZnO}/2_{Ag}$ and diluted into Löwenstein–Jensen medium followed by the addition of bacteria and incubation for 28 days at 37 °C. The toxicity of NPs to THP-1 cells was assessed by MTT test, and macrophages were infected with Mtb for 4 h at 37 °C under 5% CO_2.

Results: Nano-sized particles were estimated at ~30–80 nm, and the initial concentration of Ag NPs and ZnO NPs were estimated at ~20 ppm and ~60 ppm. The minimal inhibitory concentration ratio of $8_{ZnO}/2_{Ag}$ NPs against Mtb was detected at ~1/32 of the initial concentration. Ag NPs in the range of concentrations exhibited no anti-Mtb effects, whereas ZnO NPs showed potent antibacterial activity at ~1/128 of the initial concentration. ZnO NPs at all concentrations showed cytotoxic activity, whereas 100% of THP-1 cells remained viable in the presence of Ag NPs at ~1/32 and ~1/64 of the initial concentrations. However, at ratios of $8_{ZnO}/2_{Ag}$, ~39.94% of the cells at ~1/16 of the initial concentration remained viable, with 100% of THP-1 cells at ~1/32 of the initial concentration remaining viable.

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Conclusion: Although Ag NPs exhibited low cytotoxicity, they were unable to inhibit Mtb growth *in vitro*. ZnO NPs exhibited strong anti-Mtb activity and inhibited bacterial growth, but exhibited high cytotoxicity to human macrophage cells. By mixing Ag and ZnO NPs at a ratio of $8_{ZnO}/2_{Ag}$, we acquired a mixture that exhibited potent antibacterial activity against Mtb and no cytotoxic effects on THP-1 cells, resulting in inhibition of both in vitro and ex vivo Mtb growth Figs. 1–3, Tables 1–3.

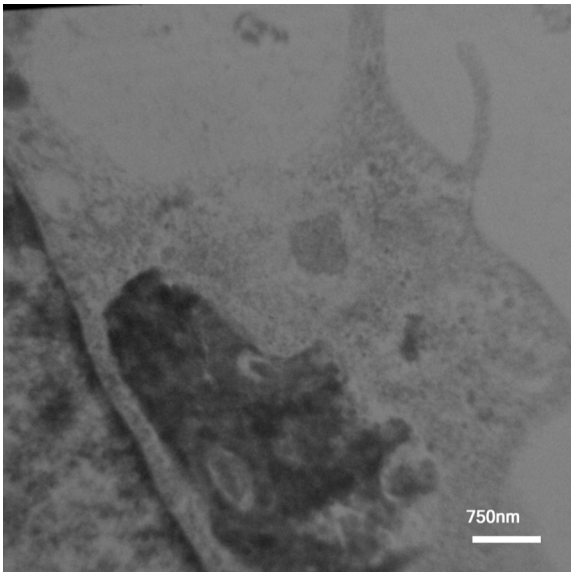

Fig. 1 – The TEM microscopic image of Ag & ZnO NPs into the human macrophage (THP-1) cell, infected with *Mycobacterium tuberculosis* on a scale of 750 nm.

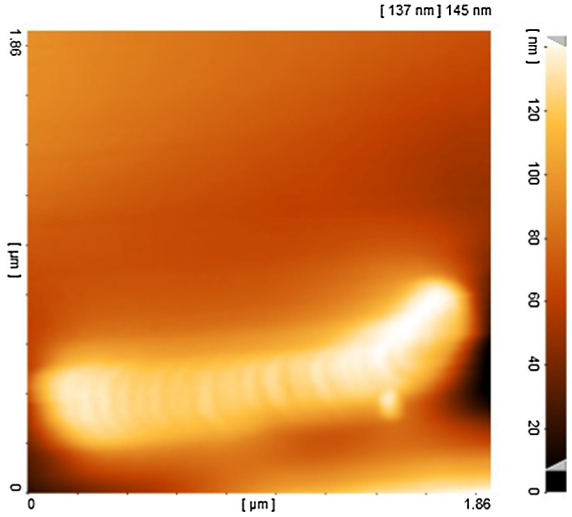

Fig. 2 – The AFM microscopic image of *Mycobacterium tuberculosis*, treated with Ag:ZnO NPs.

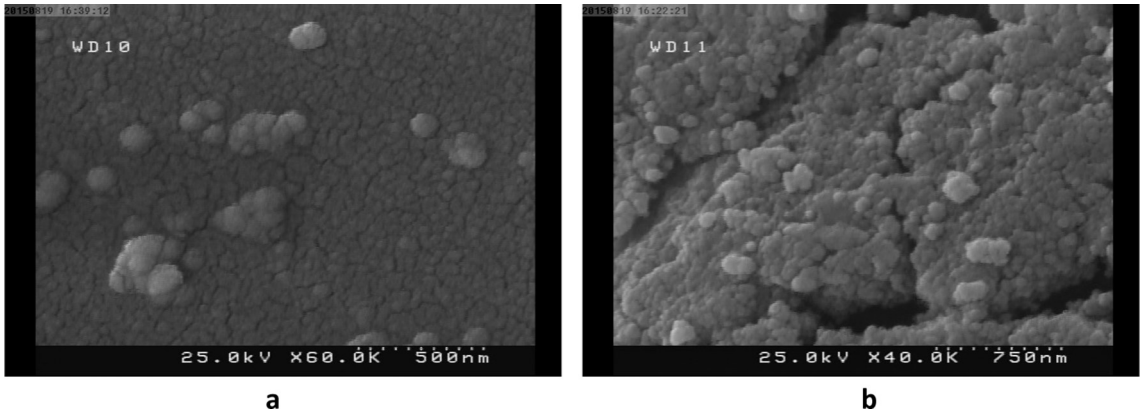

Fig. 3 – The SEM images of Ag (a) and ZnO (b)NPs, distribution of particle size was done with a 60 K magnification on a scale of 500 nm (a) and 750 nm (b).

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Table 1 – The results of anti-mycobacterial tests (MIC) with different dilution of Ag, ZnO and mixed Ag:ZnO NPs ($2_{Ag}/8_{ZnO}$) in contraction with *Mycobacterium tuberculosis* (*H37Rv Mtb*).

Dilution	Ag:ZnO									
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	Control
$2_{Ag}/8_{ZnO}$	No growth	No growth	No growth	No growth	MIC	150 ⩽ (CFU)	150 ⩽ (CFU)	150–300 (CFU)	150–300 (CFU)	500 ⩾ (CFU)
Ag	150 ⩽ (CFU)	150 ⩽ (CFU)	150 ⩽ (CFU)	150 ⩽ (CFU)	150–300 (CFU)	150–300 (CFU)	150–300 (CFU)	150–300 (CFU)	150–300 (CFU)	500 ⩾ (CFU)
ZnO	No growth	No growth	No growth	No growth	No growth	No growth	MIC	150 ⩽ (CFU)	150–300 (CFU)	500 ⩾ (CFU)

Table 2 – The mean and SD of different ratios/dilution of NPs light absorbance in contraction with the THP-1 cells in 24 h follow-up time.

Dilution	NPs						
	1:2	1:4	1:8	1:16	1:32	1:64	Control
Ag	0.242 ± 0.006	0.356 ± 0.011	0.419 ± 0.027	0.610 ± 0.010	0.715 ± 0.013	0.779 ± 0.009	0.782 ± 0.006
ZnO	0.103 ± 0.006	0.150 ± 0.009	0.203 ± 0.015	0.246 ± 0.011	0.302 ± 0.007	0.345 ± 0.005	0.552 ± 0.010
$2_{Ag}:8_{ZnO}$	0.226 ± 0.025	0.236 ± 0.032	0.303 ± 0.251	0.360 ± 0.055	0.786 ± 0.040	0.830 ± 0.052	0.785 ± 0.213

Table 3 – The IC_{50} and SD of different ratios/dilution of NPs in contraction with the THP-1 cells in 24 h follow-up time.

NPs	Minimum ± SD	Maximum ± SD	IC50 ± SD	Hill coeff. ± SD
Ag	0.258 ± 0.037	0.846 ± 0.072	3.644 ± 0.328	3.704 ± 1.193
ZnO	0.127 ± 0.048	35.954 ± 5331	42.630 ± 266	2.488 ± 2.947
$2_{Ag}:8_{ZnO}$	0.254 ± 0.022	0.807 ± 0.02805	4.287 ± 0.168	20.792 ± 10.32

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