

# The interaction of silver-enriched clinoptilolite with mammalian immunocompetent cells



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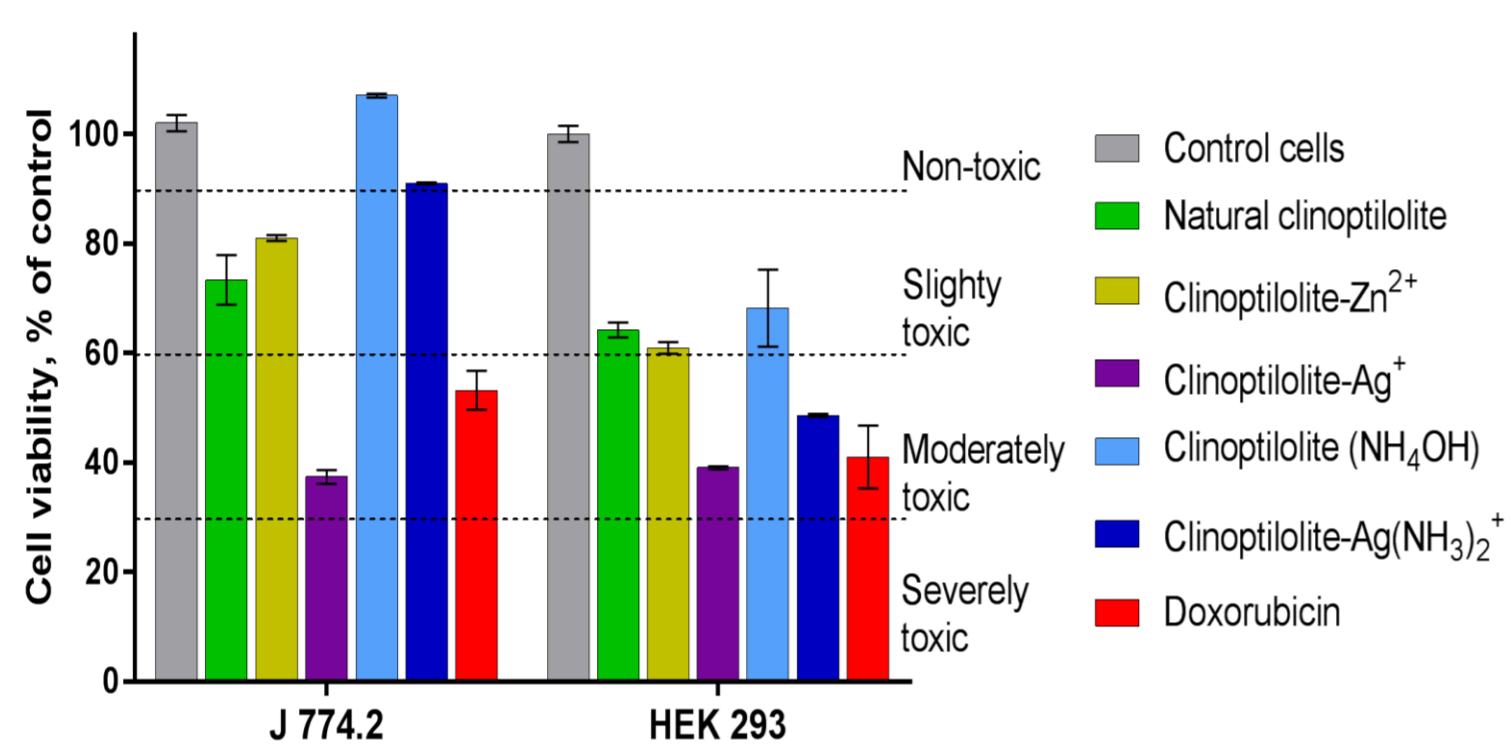
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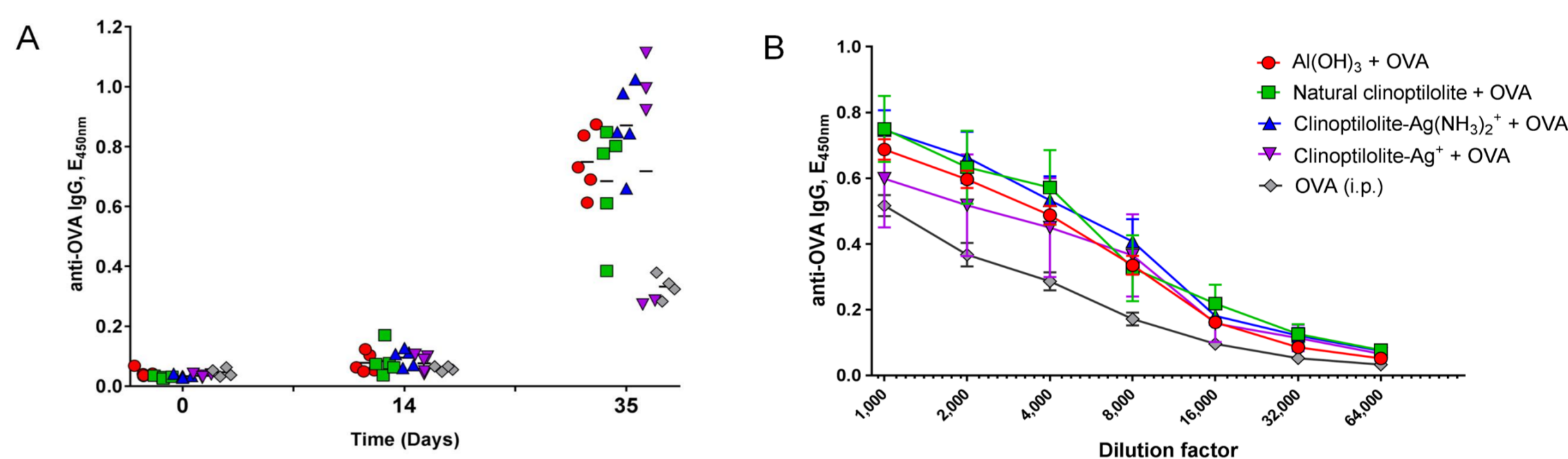
**Introduction:** Owing to their unique chemical and physical properties, zeolites are widely used in agriculture, industry, medicine, cosmetology and other fields. Natural zeolite – clinoptilolite, is permitted to be utilized for medical and veterinary applications [1]. In spite of the fact that Ag-doped clinoptilolite possesses toxic impact on tumors and pseudo-normal mammalian cells *in vitro* [2], its influence on immune cells, as well as on the development of immune response has still to be thoroughly studied.

Our research **was aimed** at interaction between silver-enriched natural clinoptilolite (from the deposit near the Sokirnytsia village in the Transcarpathian region of Ukraine) and mammalian immune cells.

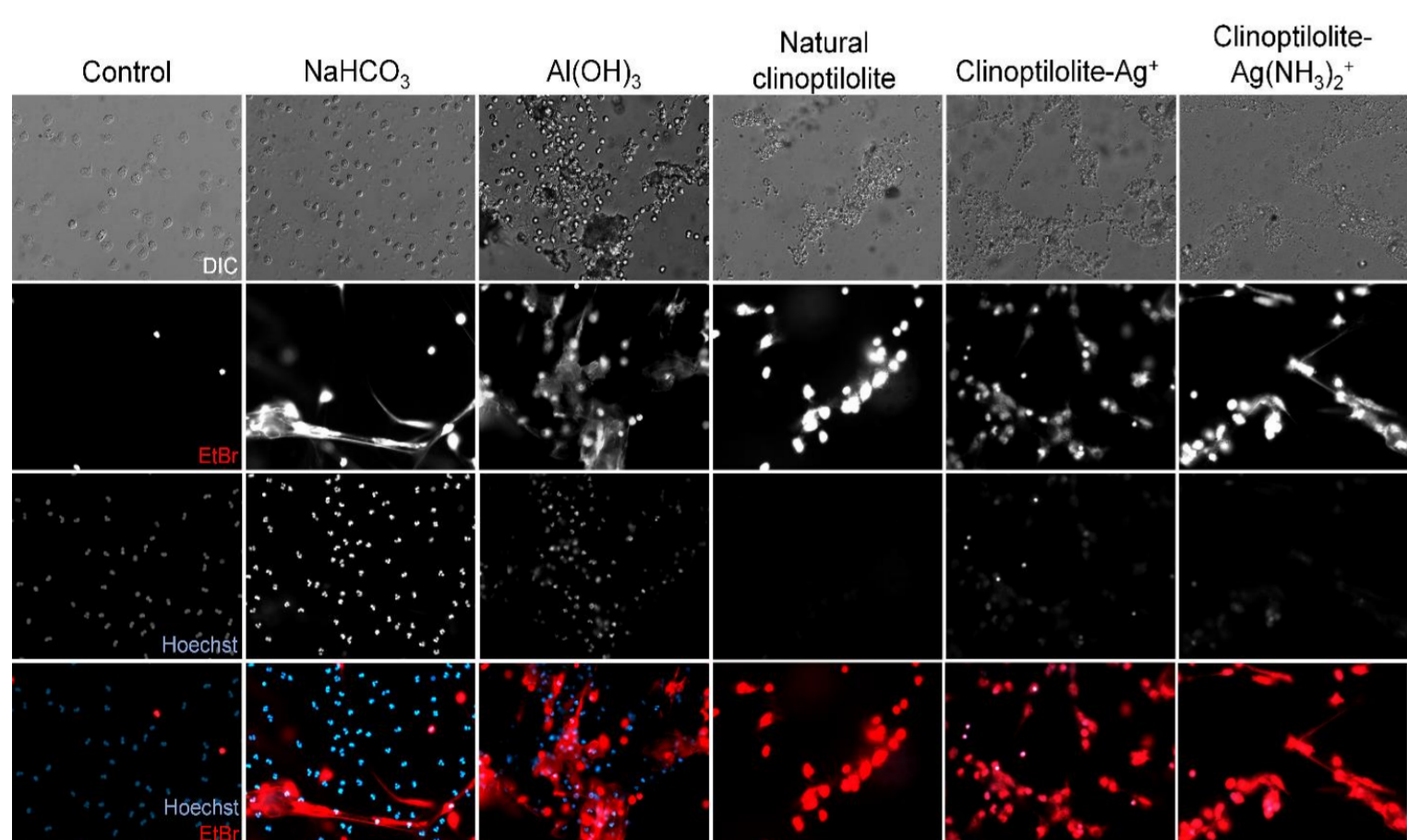
**Methods:** Cytotoxic effect of the clinoptilolite samples was quantitatively estimated by means of MTT assay. Human granulocytes were isolated from the freshly obtained heparinized venous blood of normal healthy donors (approved by the Bio-Ethics committee at the Institute of Cell Biology, NAS of Ukraine (protocol No 2/07102020) using ficoll-triombast medium with gradient density  $\rho = 1.08 \text{ g/cm}^3$ . The immunogenic effect of clinoptilolite samples was evaluated by mice immunization and the air-pouch model. Immune responses were investigated by ELISA. All the works including housing and care, method of euthanasia were conducted in accordance with the established experimental protocols and requirements of Ethics committee of Institute of Cell Biology NAS of Ukraine, protocol № 3/07102020.



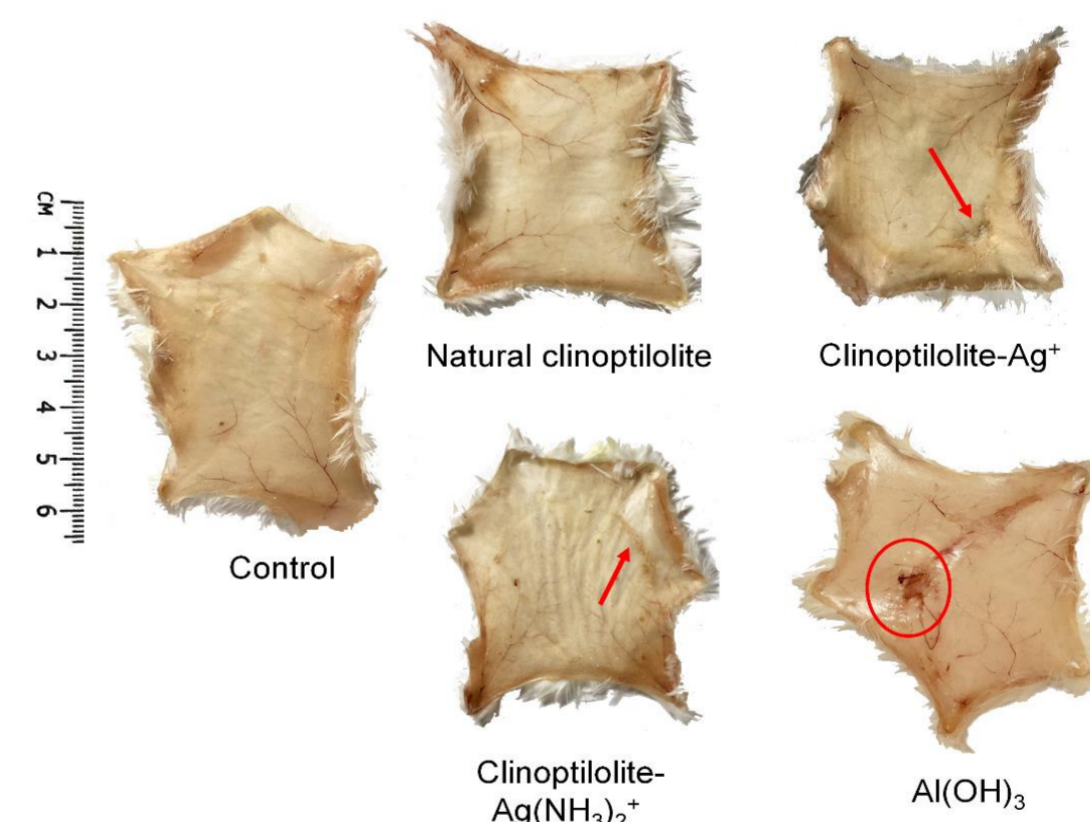
Viability of human embryonic kidney cells of HEK 293 line and murine monocytes/macrophages of J774.2 line treated with indicated clinoptilolite samples (5 mg mL<sup>-1</sup>).



Humoral immune responses in Balb/c mice provoked by OVA using the Alum, natural clinoptilolite, clinoptilolite-Ag (NH<sub>3</sub>)<sub>2</sub><sup>+</sup>, clinoptilolite-Ag<sup>+</sup> or OVA alone acting as adjuvants. (A) Anti-OVA IgG levels in serum (humoral immune response); (sera dilution 1:2,000). (B) Titers of anti-OVA IgG antibodies after immunization with indicated compounds.



Natural and Ag-enriched clinoptilolite (2.5 mg mL<sup>-1</sup>) induce activation of the neutrophils with further formation of NETs. Extracellular DNA was stained with the Ethidium bromide (red).



Demonstration of the interaction between the clinoptilolite samples and immune cells in the air-pouch model. The arrows mark particle aggregates, while the damaged vessels and edema are encircled.

DIC imaging is shown in the upper row and fluorescence microscopy with Ethidium bromide, EtBr (red) and Hoechst (blue) - in the lower rows.

The investigated samples of the clinoptilolite and aluminium hydroxide activated neutrophil granulocytes obtained from the organisms, but no inflammation was observed in the air-pouches.

**Conclusion:** In this study, the immunostimulatory effects of natural zeolite, the clinoptilolite, were studied. It was shown that the clinoptilolite and its silver-doped composites provoked an increased humoral immune response acting *in vivo* like an adjuvant and leading to stimulation of antibody producing plasma cells. We found that the clinoptilolite acts *in vitro* on the neutrophil granulocytes with their further activation. The immunostimulatory effects of natural clinoptilolite and its Ag-doped composites correlated with the antifungal action against *Candida albicans*. Taken together with relatively low cytotoxicity of these agents, the obtained results suggest a possibility for their use in medicine as modulators of the immune response and antifungal remedies.

- References:**
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  - Vasylechko V., Klyuchivska O., Manko N., Gryshchouk G., Kalychak Ya., Zhmurko I., Stoika R. Novel nanocomposite materials of silver-exchanged clinoptilolite with pre-concentration of Ag (NH<sub>3</sub>)<sub>2</sub><sup>+</sup> in water possess enhanced anticancer action // Appl Nanosci.-2020.-10, N 12.-P. 4869-4878.

**Acknowledgements:** This work was partially funded by the Ministry of Education and Sciences of Ukraine (Grant KhA-87F, 2019-2021).