Evaluation of Nanovenom complexes formed by Flower-like Silica Nanoparticles and *Crotalus durissus terrificus* venom for antivenom production

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In Argentina, ophidism is a major public health problem with *Crotalus durissus terrificus* (*C.d.t.*) being one of the species of greatest medical importance due to the high lethality of its venom (V). Currently, the generation of antivenoms (AVs) is a costly and difficult task, and the potential use of Silica Nanoparticles (SiNPs) as carriers for V toxins (complex called nanovenom -NV-) appears to be a new tool to produce them. Therefore, in this work we study the adsorption capacity, cytotoxic and indirect hemolytic activity of NVs formed by Flower-like Silica Nanoparticles (Fw NPs) and *C.d.t.* V.

The physicochemical properties of the Fw NPs were previously analyzed, showing a hydrodynamic diameter of 300.4 ± 2.1 nm (measured by DLS), and a zeta potential of -33.4 ± 1.6 mV. TEM and SEM micrographs were also obtained, as well as FT-IR spectra (showing silica absorption peaks at 793, 950, 1047, 1630 and 3375 cm^{-1})[1]. With this nanomaterial, we performed adsorption isotherms, incubating different concentrations of V (0.25 - 2.5 mg/ml) with 10 mg of Fw NPs, applying the Langmuir and Freundlich models. From the data obtained, it was observed that the NVs fit both models well (R^2=0.71, Qmax=0.26 mg V/mg Fw NPs, KL=3.45 for Langmuir and R^2=0.73, K=0.26, n=0.59 for Freundlich). Moreover, more than 70% of the V, in each concentration analyzed, was adsorbed to the surface of the Fw NPs. Regarding indirect hemolytic activity, NVs exhibited high activity (89%), with the V serving as the positive control, while Fw NPs showed no activity whatsoever. The cytotoxic activity was analyzed by MTT assay in the RAW 264.7 murine cell line, revealing high cytotoxicity from Fw NPs, with this effect being reduced in NVs. These results prove that this type of NPs can load a large amount of V due to its large surface area; furthermore, the indirect hemolytic activity showed that the V remains effective after being adsorbed onto the Fw NPs. In addition, NVs were found to be less cytotoxic than the Fw NPs.

These properties could be ideal for the generation of AVs in horses, since the high adsorption capacity of Fw NPs could lead to better antigen presentation and, therefore, improved production of neutralizing antibodies. Furthermore, it is necessary to verify whether the observed cytotoxicity correlates with an increased release of pro-inflammatory cytokines that enhance the animal's immune system, improving the response and, consequently, yielding more effective antivenoms.

Referencias

1. Ostapchuk, G. O. et al. *Environmental toxicology and pharmacology* 120 (2025) 104857