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Apo-bovine lactoferrin nanoparticles supress hypoxia-angiogenesis pathway in macular degeneration model

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Aged macular degeneration (AMD) is the main cause of permanent vision loss in elderly1. The major challenge in the management of AMD is the development of the non-invasive therapy that able to cross the ocular barriers and reach to the posterior segment of the eye to exert the therapeutic efficacy. An iron-free form of bovine lactoferrin (Apo-bLf) has anti-angiogenic and anti-inflammatory activity. We assessed the protective role of Apo-bLf ultrasmall chitosan nanoparticles (Apo-bLf US-CHNP) as an anti-angiogenic agent and to suppress the hypoxia - angiogenesis pathway. Characterisation of the synthesized nanoparticles indicated that their size was around 14 nm, spheroid shape detected by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM). While Fourier Transforms Infrared Spectroscopy (FTIR) showed the presence of Apo-bLf protein and chitosan within prepared nanoparticles. Differential scanning calorimetry was used to determine the thermal stability and water holding capacity of the prepared nanoparticles. Encapsulation efficacy was more than 91%. Continuous protein release for Apo-bLf nanoparticles was displayed an initial release in PBS (pH 7.2 and 7.6) at 37 C during the first 24 h and then exhibited zero-order drug release kinetics up to 96h (detection time at the 2h interval). Apo-bLf US-CHNP downregulated effectively the common AMD biomarkers such as VEGF, HIF 1-α, Cas-3, Bax, MMP9 and augment α-Crystallin *in vitro* for RPE-19 when exposed to oxidative stress; moreover, they suppress angiogenesis (screening expression of 55 key angiogenic markers) within AMD models *in vitro* and ex vivo. Therefore Apo-bLf US-CHNP may hold the promise for the prolonged therapeutic management of retinal degenerative diseases since they possess anti-apoptosis, anti-oxidant and anti-inflammatory effects.

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