

**Cytotoxicity and immunological responses following oral vaccination of nanoencapsulated avian influenza virus H5 DNA vaccine with green synthesis silver nanoparticles.**

ABSTRACT

DNA formulations provide the basis for safe and cost effective vaccine. Low efficiency is often observed in the delivery of DNA vaccines. In order to assess a new strategy for oral DNA vaccine formulation and delivery, plasmid encoding hemagglutinin (HA) gene of avian influenza virus, A/Ck/Malaysia/5858/04 (H5N1) (pcDNA3.1/H5) was formulated using green synthesis of silver nanoparticles (AgNP) with polyethylene glycol (PEG). AgNP were successfully synthesized uniformly dispersed with size in the range of 4 to 18 nm with an average size of 11 nm. Cytotoxicity of the prepared AgNP was investigated in vitro and in vivo using MCF-7 cells and cytokine expression, respectively. At the concentration of  $10^{-5}$  log<sub>10</sub>AgNP, no cytotoxic effects were detected in MCF-7 cells with 9.5% cell death compared to the control. One-day-old specific pathogen-free (SPF) chicks immunized once by oral gavage with 10  $\mu$ l of pcDNA3.1/H5 (200 ng/ml) nanoencapsulated with 40  $\mu$ l AgNP ( $3.7 \times 10^{-2}$   $\mu$ g of Ag) showed no clinical manifestations. PCR successfully detect the AgNP/H5 plasmid from the duodenum of the inoculated chicken as early as 1 h post-immunization. Immunization of chickens with AgNP/H5 enhanced both pro inflammatory and Th1-like expressions, although no significant differences were recorded in the chickens inoculated with AgNP, AgNP/pcDNA3.1 and the control. In addition, serum samples collected from immunized chickens with AgNP/H5 showed rapidly increasing antibody against H5 on day 14 after immunization. The highest average antibody titres were detected on day 35 post-immunization at  $51.2 \pm 7.5$ . AgNP/H5 also elicited both CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the immunized chickens as early as day 14 after immunization, at  $7.5 \pm 2.0$  and  $20 \pm 1.9$  percentage, respectively. Hence, single oral administrations of AgNP/H5 led to induce both the antibody and cell-mediated immune responses as well as enhanced cytokine production.

**Keyword:** Avian influenza virus; H5 DNA vaccine; HI antibody; T cell; Cytokine; Green synthesis AgNP