Approaches toward Enhancement of Avian Influenza H5N1 Vaccine Formulation

Thesis

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BY

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

" قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا
إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْحَكِيمُ"

صدق الله العظيم

سورة البقرة اية 32

Dedication

To

My Family

My great father and my Kind mother

Thank you for supporting me with kindness

patience and love

Abstract

Objective: The puzzling inefficiency of the available anti-highly pathogenic avian influenza (HPIA) vaccines to protect Egyptian poultry against seasonal waves of viral infections was reported. Therefore the ultimate goal of the present work was to try to improve the immune efficacies of two anti-HPAI vaccine preparations by including extracts from two plants from the Egyptian *Flora* namely *Echinacea purpurea* or *Nigella sativa* in their formulations and monitor the effect on mice immune responses. **Material and method**: two formulations of oil emulsion inactivated vaccines: V1 (mineral oil emulsion inactivated rg (A/chicken/Egypt/Q1995D/2010 (H5N1)) vaccine) and V2 (rg (A/chicken/Egypt/Q1995D/2010 (H5N1)) with Montanide ISA 70 VG) were prepared in combination with either *E. purpurea* or *N. sativa* and used to immunize female Swiss albino mice and booster immunization was administered two weeks later. Control groups included mice that received immunization with the unformulated vaccines without the plant extracts and normal group which non immunized with vaccine or plant extract. Sera were collected from various mice groups and studied by enzyme linked immunosorbent assay for IgG and IgM Levels. Moreover, immunophenotyping for $CD4^+$ or $CD8^+$ T- lymphocytes from mesenteric lymph nodes (MLN) and thymus (T) from vaccinated and control animals was carried out. **Results:** Two weeks post booster immunization level of IgM (0.54±0.06; fold=6.75) in sera from mice intramuscular immunized with V2 was significantly higher ($P \leq 0.001$) than in other mice groups, while, one week post booster immunization, level of IgG (0.77±0.02; fold=10.43) in sera from mice that received formulated V1 with *N. sativa* was significantly higher ($P \leq 0.001$) than in other animals. The ratio of MLN- or T-$CD4^+/CD8^+$ cells was >1 in all mice. **Conclusion**: Introducing *N. sativa* to the V1 formulation at concentration (10%)

stimulate mouse immune responses compared to plant extract free vaccine.

Key words: *Highly pathogenic avian influenza, Echinacea purpurea*; *Nigella sativa*; ELISA; lymphocytes.

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List of abbreviations

AGP	Agar Gel Precipitation
AI	Avian influenza
AIVs	Avian influenza viruses
BALT	bronchus-associated lymphoid tissue
CELO	Chicken Embryo Lethal Orphan
CMI	Cell Mediated Immunity
CPE	Cytopathic effect
CTL	Cytotoxic T Lymphocytes
ddH_2O	Double distilled water
DIVA	differentiation of infected from vaccinated animals
E. angustifolia	*Echinacea angustifolia*
E. pallida	*Echinacea pallida*
E. purpurea	*Echinacea purpurea*
ELISA	Enzyme linked immunosorbent assay
FAO	Food and Agriculture Organization
FCS	Fetal calf serum
FITC	Fluorescene isothiocyanate
FPV	Fowlpox Virus
GALT	gut-associated lymphoid tissue
HA	Haemagglutinin
hAd5	human Adenovirus 5
HALT	head-associated lymphoid tissue
HPAI	Highly pathogenic avian influenza
IFN	Interferon
Igs	immunoglobulins
ILTV	laryngotracheitis herpes virus
IM	Intra-muscular