



# ***Approaches toward Enhancement of Avian Influenza H5N1 Vaccine Formulation***

*Thesis*

Submitted for the degree of M.Sc. in Science  
As a partial fulfillment for requirements of the Master of Science  
(Biochemistry)

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**2013**

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**Date of examination:**     /     /2013

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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

صدق الله العظيم

سورة البقرة آية 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 (HPAI) 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<sup>+</sup> or CD8<sup>+</sup> 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<sup>+</sup>/CD8<sup>+</sup> 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.

## ACKNOWLEDGEMENT

I offer my thanks always to **Allah**, for his great care and guidance in every step of my life and for giving me the ability to complete this work.

I wish to express my sincere thanks, deepest gratitude and appreciation to **Prof. Dr. Ahmed Osman Mostafa**, Professor of Biochemistry, Faculty of Science, Ain shams University, for his generous supervision, great support, helpful advice and patience to produce this work.

I am greatly indebted and grateful to **Prof. Dr. Amany Sayed Maghraby**, Professor of Immunology and Parasitology, Therapeutical Chemistry Department, National Research Center, for her sincere guidance, valuable discussion, great support, abounding patience, effort and time she spent supporting every step of this work.

My appreciation is expressed to **Prof. Dr. Mohamed Ahmed Ali**, Professor of Virology, Water Pollution Department, National Research Center, for his great support, kind help, strong encouragement and effort reviewing this thesis.

Grateful appreciation is also extended to **Prof. Dr. Mahmoud Mohamed Bahgat**, Professor of Biochemistry, Therapeutical Chemistry Department, National Research Center, for continuous encouragement, kind help and effort reviewing this thesis.

Special deep appreciation is given to my colleagues in the NRC for their kind help and valuable contribution in this work.

Finally, unlimited cardiac thanks to my beloved family for the great help and encouragement.



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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 <sub>2</sub> O	Double distilled water
DIVA	differentiation of infected from vaccinated animals
<i>E. angustifolia</i>	<i>Echinacea angustifolia</i>
<i>E. pallida</i>	<i>Echinacea pallida</i>
<i>E. purpurea</i>	<i>Echinacea purpurea</i>
ELISA	Enzyme linked immunosorbent assay
FAO	Food and Agriculture Organization
FCS	Fetal calf serum
FITC	Fluorescence 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