MOLECULAR GENETIC STUDIES ON HEAT SHOCK RESPONSE IN DROSOPHILA

By

MARWA ROUSHDY SAYED MAHMOOD

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MARWA ROUSHDY SAYED MAHMOOD

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This thesis for M. Sc. degree has been approved by:

Dr.	Ayman Ali Diab Prof. of Genetics, Faculty of Biotechnology, October University for Modern Sciences and Arts (MSA)
Dr.	Abdel-Fatah Abdel-Kader Mohamed Awad
Dr.	Nermin Mahmoud Abdel-Gawad Prof. of Genetics, Faculty of Agriculture, Ain Shams University
Dr.	Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams University

Date of Examination: 24/1/2017

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MARWA ROUSHDY SAYED MAHMOOD

B.Sc. Agric. Sc. (Biotechnology), Ain Shams University, 2010

Under the supervision of:

Dr. Fatthy MohamedAbdel-Tawab

Professor Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University (Principal Supervisor)

Dr. Nermin Mahmoud Abdel-Gawad

Professor of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University

Dr. Naglaa Mohammed Ebeed

Associate Professor of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University

ABSTRACT

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The heat shock proteins (HSPs), abundantly expressed in insects, are important modulators of insect survival and are used as sensitive biomarkers for xenobiotics. Increased nanomaterial productions, including silver nanoparticles (AgNPs) and their wide range of applications imply a higher risk of human and environmental exposure.

The aims of the present study were to: 1. Investigate the impact of AgNPs exposure in *D. melanogaster* with regard to changes in the expression of heat shock (*hsp*) genes (*hsp23*, *hsp26*, *hsp27*, and *hsp60*).

2. Assess the correlations between *hsps* genes, antioxidant systems and oxidative stress as reflected by changes in the expression of antioxidant enzymes (SOD, CAT and GSH).

3. Assess the ability to tumors development by monitoring the changes in the expression of tumor suppressor gene (p53).

4. Detect some genetic biomarkers associated with biological stress in fruit flies.

Characterization of AgNPs by using transmission electron microscopy (TEM) and dynamic light scattering (DLS) analysis revealed agglomeration of silver particles in water, spherical shape, and uniform size with an average diameter of between 15-70 nm and the hydrodynamic diameters were 74.35 nm.

Larvae exposure to different concentrations of AgNPs resulted in significant changes in body color and some toxic effects such as melanization, necrosis and malformations and the larvae failed to pupate at higher concentrations. The acute toxic effect of AgNPs on *D.melanogaster* was observed at the AgNPs concentration of 1600 µg/ml. Silver concentration, resulted in 50% of the tested flies unable to leave the pupae, and they failed to complete their developmental cycle.

Antioxidant activity was tested by DPPH scavenging assay after larvae consumption of AgNPs resulted in significant decrease in the activity of antioxidants. Meanwhile, AgNPs promoted the generation of reactive oxygen species (ROS).

Quantitative real-time PCR (qRT-PCR) analysis of *hsp* genes, antioxidant genes and tumor-suppressor p53 gene expression revealed that the analyzed markers responded significantly at 1600 µg/ml of AgNPs. AgNPs appeared to up-regulate the expression of *hsp26*, p53 and GSH genes, whereas the expression of *hsp23*, *hsp27*, *hsp60*, CAT and SOD genes appeared to be down-regulated. Finally, as *Drosophila*, is considered an established genetic model system, it is recommended that it could be utilized for further understanding of the biological effects of nanoparticles.

Keywords: Heat shock proteins (Hsps); Nanotoxicity, Drosophila, gene expression; Antioxidant system; Reactive oxygen species (ROS).

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LIST OF ABBREVIATIONS

ABBREVIATION

DEFINITION

AgNPs	Silver nanoparticles
TEM	Transmission electron microscopy
NM	Nano material
DLS	Dynamic light scattering
DPPH	2, 2-diphenyl-1-picrylhydrazyl
IC50	Half maximal inhibitory concentration
LC50	Half maximal lethal concentration
MTT	$3\hbox{-}(4,5\hbox{-}dimethyl thiazol\hbox{-}2-yl)\hbox{-}2,5\hbox{-}diphenyl tetrazolium bromide}$
ROS	Reactive oxygen species
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
gDNA	Genomic deoxyribonucleic acid
TBE	Tris/Borate/EDTA
cDNA	Complementary DNA
HSPs	Heat shock proteins
PCR	Polymerase chain reaction
RT-PCR	Reverse transcriptase polymerase chain reaction
Ct	Cycle threshold