# MOLECULAR GENETIC STUDIES ON HEAT SHOCK RESPONSE IN *DROSOPHILA*

By

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

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# ABSTRACT

**Marwa Roushdy Sayed Mahmood. "Molecular Genetic Studies on Heat Shock Response in *Drosophila*". Unpublished M.Sc. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2017.**

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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| 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-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium 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 |