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Development of *Drosophila Melanogaster* for Assessing Metal Nanoparticles Interaction

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Abstract—*Now a day, the rapid development of nanotechnology allowed the fabrication of a wide range of different nanomaterials, raising many questions about their safety and potential risks for the human health and environment. In current nanotoxicology research mostly based on in vitro analysis is not standardized, hampering any comparison or reproducibility of the obtained results. Toxicity research has focused on acute exposures both in vitro and in vivo. Few in vivo studies on chronic lifetime effects of NP exposure are available. Drosophila melanogaster provides a powerful model for investigating human health and nanotoxicity of nanoparticles. D. melanogaster model offers several important advantages, such as a relatively simple genome structure, short lifespan, low maintenance cost, readiness of experimental manipulation comparative to vertebrate models from both ethical and technical points of view, relevant gene homology with higher organisms, and ease of obtaining mutant phenotypes. We have developed an in vivo chronic nanotoxicity model using Drosophila melanogaster. The effects of silver nanoparticles exposure of Drosophila adults and larval stages on reproduction, development, and survivorship, were assessed based on different concentrations of silver nanoparticles. We've found that chronic exposure to silver NPs via ingestion has toxic effects on fly viability, DOD and reproductive effort.*

Keywords*: Drosophila melanogaster; nanotoxicity; silver nanoparticles; reproducibility*

1. INTRODUCTION

The fast emergence of nanotechnology allowed the obtaining of a wide range of different nanoparticles (NPs) for specific applications in the pharmaceutical, cosmetic, and other biomedical product industries, as wells as for developing imaging diagnosis techniques and photothermal therapy. However, many questions arise regarding safety and latent risks for human health and environment [1,2]. Taking into account that the huge potential of NPs for different applications is the result of the fact that they are more reactive than conventional-sized particles, it is also possible that they may also exhibit a higher cytotoxicity. The current nanotoxicology research uses in vitro models that do not offer information about the fate of NPs in the host organisms (biodistribution, accumulation, metabolism, persistence, elimination etc.) [3]. The in vivo studies are using mostly aquatic organisms, such as rainbow trout (Oncorhynchus mykiss), zebra fish (Danio rerio), nematode (Caenorhabditis elegans), algae, and daphnids [4–7]. Additionally, the protocols used in different studies for assessing the nanotoxicity are not standardized regarding the many variables occurring in this field of research (variations in size, fabrication procedures, aggregation, solubility, intracellular uptake, and cellular and animal models). These aspects impede on any comparison or reproducibility of the obtained results, raising the necessity of standardization and of setting up in vitro/in vivo experimental models for the characterization of NPs cytotoxicity and biocompatibility. Establishing of various standard pharmacological parameters, such as dosage, administration route, metabolism, etc. [8–10] is also required.

2. WHY DROSOPHILA *MELANOGASTER?*

D. *melanogaster is* certainly the most famous non mammalian model organism used in the field of biomedical research. The fruit fly owes its fame to T.H. Morgan, who chose it as a model for his studies on the genetic inheritance in the early 1900s. Since then, the reputation of Drosophila grew rapidly, making this insect the model organism preferred by geneticists from around the world. In the following years, the innovation and development of tools for gene discovery and genetic analysis in Drosophila has permitted a deep knowledge of the relationships between the causes and effects of gene mutations at biomolecular level [11]. Today, Drosophila is considered one of the most effective tools for analyzing the function of human disease genes. It should be, in fact, mentioned that about the 75% of human disease-related genes are believed to have a functional homolog in the fly [12], including those

responsible for developmental and neurological disorders, cancer, cardiovascular disease, metabolic and storage diseases, as well as genes required for the function of the visual, auditory and immune systems [13]. The great similarities between human and flies were recognized and appreciated by scientists working in biology and medicine, making Drosophila the non-mammal model organism par excellence, even in those disciplines where mammal model organisms are considered irreplaceable (i.e. pharmacology [14] and genotoxicology [15]. A large part of the success of this model organism is due to the advantages that Drosophila offers with respect to the vertebrate animal models. As an example, the care and culture of this fruit fly is simple, inexpensive, it has a short generation time (about 10 days at 25^{o}C), large no of mutations, less no of chromosomes, high fecundity, and the offspring become sexually mature within one week. All these characteristics enable to study several generations in few weeks.

3. INVESTIGATION OF NPS TOXICITY BY USING D. *MELANOGASTER*

In this scenario, the Drosophila has been chosen as a model organism to study the toxic effects of nanoparticles. Today's the Drosophila has quickly attracted the attention of many research groups, establishing as the standard model organisms in the field of nanotoxicology research. Quickly, the toxic effects of some nanoparticles were tested using fruit flies and, turning the tide of some pre conceptions, results have confirmed the predictions about the risk to human health and the environment due to the indiscriminate use of nanoparticles/nanomaterials. In this context, a particular example is represented by gold nanoparticles (AuNPs), which displayed significant toxicity, despite the well-known biocompatibility of gold in bulk form [16]. In fact, it has been observed that AuNPs administered by ingestion to Drosophila were equally distributed along various organs and tissues, causing a strong reduction in lifespan and fertility of flies [17], and disorder in gene expression [18] and metabolism [19]. However, the most striking result obtained during the analysis of the effects induced by AuNPs in Drosophila was the discovery of aberrant phenotypes in the untreated progeny derived from flies fed with nanoparticles [20]. Among the first NPs tested for their cytotoxicity on D. *melanogaster* model were carbon nanotubes [21, 22]. Dietary uptake of fullerene C60, carbon black, or single-walled or multi-walled nanotubes, fed to the larval stage, had no deleterious effect on egg-to-adult survivorship, although these nanomaterials are incorporated in tissues. When administered to adults, carbon black or single-walled nanotubes proved an intensive capacity of adherence to fly body surface, impairing the grooming behavior and locomotion and inducing increased mortality. These results show that nanomaterials superstructure and aggregation state influence its toxicity, and the adhesion of NPs to the fly body surface activates the grooming behavior leading to the nanoparticle transport inside the body [23]. Gallium phosphide (GaP) nanowires ingested by D. *melanogaster* larvae and/or adults did not (i) accumulate in the fly tissues; (ii) stimulated the immune response; (iii) modify the gene expression; or (iv) affect the life span or the somatic mutation rate [24].The effects of magnetite or iron oxide (Fe_3O_4) NPs capped/modified/coated with pristine citricacid and 3-aminopropyltriethoxylsilane in concentrations of 300–600g/g have been investigated using the D. *melanogaster* model. The uptake of Fe_3O_4 NPs caused a significant decrease in the female fecundity, and a developmental delay at the egg-pupae and pupae-adult transitions. Additionally, adult uptake of Fe_3O_4 NPs disturbed the oogenesis period, induced ovarian defects, delays in egg chamber development, reduced the eggs length and of the nurse cells. Furthermore, Fe, Ca, and Cu trace element imbalances, along the anterior-posterior axis of the fertilized eggs were found [25]. The titanium dioxide (TiO_2) and silver (Ag) NPs have been shown to induce a decrease of survival rate and fecundity, delays in development and the occurrence of distinct phenotypes [26–30].

4. *IN VIVO* STUDIES OF SILVER NANOPARTICLE

4.1 Silver nanoparticles ingestion effects on Drosophila survivorship and developmental time: A study of silver nanoparticles ingestion were completed and found the major, concentration-dependent (20%, 50%, 70% and 100%) effects on survivorship egg to adult stages of D. *melanogaster*. Higher concentrations of NPs (100%) were more toxic only 50% hatchability were recorded (7 larvae emerges out of 10) and 20% viability (2 flies emerges out of 10) flies. In concentration of 70% and 50% results has no significant differences were observed in both conc. the 75% hatchability and 60% viability were recorded. While the 20% concentration was showing less toxic for the development of Drosophila (hatchability and viability) only 10% toxic effects was observed. On the other hand, the 90% hatchability and 80% viability were recorded at 20% conc. of AgNPs. Times to pupation were slowed by nano-silver ingestion in an increasing concentration of NPs.

4.2 Silver nanoparticles ingestion effects on Drosophila adult cuticle development and melanization time: Silver nanoparticles ingestion during the larval stage resulted in cuticular and melanization defects in adults (Fig. . 1). Flies that survived higher concentration of AgNPs ingestion had a soft, non-pigmented cuticle. No such effect was observed in normal-fed flies as control. On the other hands, the control flies has higher pigmented in all abdominal segments as compare to AgNPs feds flies in both sexes. Un-pigmented flies was observed in 100% AgNPs treated feds, light pigmented flies was observed in 70% AgNPs treated feds no significant differences observed in 70 and 50% AgNPs treated feds, less pigmented flies was observed in 20% AgNPs treated, its pigmentation score is near to control flies. As epidermal pigments are secreted by the cuticle, the cuticle defect is likely the root cause of these phenotypes.

4.3 Mating success is reduced by nano-silver ingestion: Silver nanoparticles ingestion during the larval stage reduced mating success in adults. Lower concentration of NPs were slightly disrupt to mating propensity and mating speed. On the other hands progenies reproduce less as compared to control flies. Increase the pattern to produced lesser progenies in correspondence to increase AgNPs concentration. Results of this research indicate the 100% AgNPs feds fly did not fit for produce progenies in large numbers.

Fig. 1: Silver nanoparticles ingestion shows concentration-dependent effects on adult melanization and cuticular development.

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