DROSOPHILA MELANOGASTER AS A MODEL ORGANISM FOR NANOPARTICLE TOXICITY RESEARCH

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Abstract

Nanotechnology is a rapidly developing interdisciplinary field that combines branches of science such as engineering, physics, chemistry, biology, computer science, biotechnology, medicine, and pharmacy. Due to the rapid growth of interest in nanotechnology, new methods are needed to study the effects of nanoparticles on living organisms. In combination with in vitro and in vivo studies on vertebrate animals, valuable research data can be obtained through in vivo studies on invertebrates. Drosophila melanogaster (D. melanogaster), widely known as the fruit fly, has long been a cornerstone of genetic and developmental biology research. Its popularity owes to the short life cycle and approximately 13,600 genes, many of which are homologous to human genes. In recent years, the use of D. melanogaster has also been extended to the rapidly growing scientific field of nanotechnology. As a model organism, D. melanogaster offers a unique combination of genetic tractability and conservativeness of biological pathways, making it an ideal candidate for studying the biological impacts of nanoparticles. This article discusses the types of nanoparticles as a drug delivery system, one of their classifications, and use in pharmacy. It also reviews the growing role of D. melanogaster in nanoparticle research, highlighting its potential to provide insights mainly into nanoparticle toxicity, biodistribution, and therapeutic applications.

Keywords: *nanotechnology, Drosophila melanogaster, nanoparticles, toxicity, model organism*

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Nanotechnology and Nanoparticles

Nanotechnology is a rapidly developing interdisciplinary field that combines branches of science such as engineering, physics, chemistry, biology, computer science, biotechnology, medicine, and pharmacy. Although significant progress in the development of nanotechnology has occurred over the past decade, its beginnings are attributed to a lecture given in 1959 by Professor Richard Feynman entitled “There is a plenty of room at the bottom”. The term “nanotechnology” was introduced by Professor Norio Taniguchi in 1974 [1-3].

Nanotechnology refers to the manipulation, design, and application of materials and devices on an atomic scale. Nanoparticles (NPs) produced this way range in size from 1 to 100 nanometers (a nanometer is one-billionth of a meter, 1 nm = 10^{-9} m) [1,3,4]. In the literature, the term “nanoparticles” is sometimes applied to structures whose dimensions exceed 10^{-9} m. Therefore, there is also a classification of nanoparticles based on the differences in the number of dimensions, whose size is expressed in nanometers [5]. The classification of NPs is presented in TABLE 1.

TABLE 1. Classification of nanoparticles based on differences in the number of dimensions [5].

Dimension	Definition	Example
0D	3 directions (x, y, z) are at the nanometer scale	Quantum dots
1D	The 2 perpendicular directions (x, y) are both represented at the nanometer scale	Nanorods
2D	Only one size direction (x) is expressed at the nanometer scale	Nanodisks
3D	None of the dimensions are on the nanometer scale	Polycrystals

In the context of biology, NPs can be designed with specific properties to interact with biological systems in a highly controlled manner. These particles can be made from various materials, including metals, lipids, and polymers, and can be tailored to specific shapes, sizes, and surface properties [1,5,6].

Inorganic nanoparticles

NPs are classified based on the materials from which they are made. One of the main materials used for synthesizing inorganic NPs is various types of metal alloys. Metal-based nanoparticles, such as nanorods, exhibit a wide range of structures, sizes, and geometric variants. They are distinguished by unique optical, electrical, and magnetic properties, primarily determined by the type of metal used in their preparation. Commonly utilized compounds include metals, metalloids, and their oxides, such as gold, iron, and silicon oxide (silica). These NPs exhibit greater stability compared to those synthesized from organic polymers and phospholipids; however, their application is limited due to poor solubility and potential toxicity [7].

Silver NPs (Ag NPs) are used to create coatings for dressings and as biosensors for pathogens, glucose, enzymes, specific tumour markers, and as adjuvants in vaccines. However, their use is limited due to the cytotoxicity of silver toward skin, liver, and respiratory cells, as well as its ability to cross the blood-brain barrier and damage neurons in the central nervous system. Their widespread application is driven by their antibacterial and antifungal properties [8].

Zinc oxide NPs (ZnO NPs) are commonly used in consumer products such as sunscreens and cosmetics due to their UV-absorbing capabilities. They exhibit strong antimicrobial and photocatalytic properties. However, concerns about their toxicity arise, particularly regarding the effects on the respiratory and immune systems after exposure, which are influenced by particle size, ion release, and the generation of reactive oxygen species (ROS) [9,10].

Titanium dioxide NPs (TiO_2 NPs) are used in commercial products such as sunscreens, cosmetics, and food due to their high stability, UV-blocking ability, and overall good biocompatibility. They are particularly effective in biomedical applications, such as drug delivery systems, because of their surface modification potential to enhance adhesion and cell proliferation. Although low concentrations generally do not show toxicity, higher doses (e.g., 150-200 μg/mL) can induce apoptosis, particularly in human alveolar cells. Studies on various cell lines indicate that cytotoxic effects depend on factors such as particle size, aggregation state, and the specific type of exposed cell [9].

Gold NPs (Au NPs) are applied in biological and medical fields due to their unique optical properties and biocompatibility. However, their toxicity remains a topic of debate and depends on several factors, including particle size, concentration, aggregation state, and interactions with cell membranes. At lower concentrations, Au NPs typically exhibit limited cytotoxicity, but higher concentrations are associated with oxidative stress and DNA damage in lung fibroblasts. Surface charge and electrostatic interactions with cell membranes significantly influence their biological effects [9].

Quantum dots (QDs) are inorganic colloidal particles ranging in size from 1 to 12 nm, typically composed of materials with semiconductor and metal properties. They can absorb infrared radiation and emit light when excited by a light beam of an appropriate wavelength. As the size of QDs increases, the energy gap between the conduction band and the valence band also increases. Consequently, QDs larger than 10 nm emit shorter wavelengths (blue) of the electromagnetic spectrum compared to smaller QDs (2-10 nm). These larger particles (100 nm) are used as drug delivery systems because they are not suitable for bioimaging due to low quantum efficiency [11,12].

Their drawbacks include non-selectivity during transport to diseased sites, leading to accumulation in healthy tissues, which necessitates the development of functionalization strategies (where specific molecules or antibodies are attached to the surface of NPs, enabling them to detect specific cells or antigens). Moreover, the inherent presence of heavy metals in their structure limits their application in medicine due to potential toxicity [12].

Organic nanoparticles

Organic NPs are produced from organic substances, most commonly polymers or lipids. When NPs are used as drug carriers, biodegradable synthetic polymers are primarily employed, e.g. poly(lactic acid), poly(lactic-co-glycolic acid), polycaprolactone, poly(glycolic acid), as well as natural polymers e.g. albumin, fibroin, chitosan, alginate [13,14].

Among organic NPs, carbon-based structures such as fullerenes, nanotubes, and graphene can also be distinguished [15]. Two main classes of carbon NPs are characterized by fullerenes and carbon nanotubes (CNTs) [16]. Fullerenes are an allotropic form of carbon. They form a closed, symmetrical, and hollow shape such as a sphere, ellipsoid, or tube. The most commonly used fullerene consists of 60 carbon atoms [2,3]. They are characterized by their ability to conduct electricity and their high tensile strength [16].

CNTs are cylindrical structures derived from fullerenes with diameters ranging from one to several nanometers and lengths reaching up to several centimeters. CNTs exhibit exceptional electrical properties, are highly resistant to tensile forces, and are excellent conductors of heat. Due to their hollow structure, they are used as carriers for therapeutic substances [2-4]. CNTs can undergo chemical functionalization by introducing specific functional groups either at the ends or on the surface of the CNTs [16,17]. However, functionalization methods may alter the physicochemical properties of CNTs, such as their electrical conductivity. In medicine, they are used as drug carriers to treat disorders of the central nervous system. Studies by Gonzalez-Carter et al. [18] have shown that multi-walled carbon nanotubes with a negative charge have the greatest ability to cross the blood-brain barrier compared to positively charged or electrically neutral nanotubes [18].

Graphene is a flat structure composed of carbon atoms arranged in hexagons. With a thickness of just one atom, it is approximately a two-dimensional structure. Layers of graphene stacked one on top of another form the structure of graphite, another allotropic form of carbon [4].

Drug delivery system

NPs can be designed to deliver drugs directly to specific cells or tissues in the body, reducing side effects and interactions with surrounding tissues, and improving the effectiveness of treatment. For example, in cancer treatment, NPs can be used to deliver anticancer drugs directly to cancer cells, sparing healthy cells and minimizing the toxic effects of chemotherapy [1,2]. Furthermore, certain NPs can generate heat when exposed to specific types of light or magnetic field, which can be used to destroy cancer cells [2].

Depending on the application, nanocarriers should have specific carrier sizes, physical properties, surface characteristics, biodegradability, and the presence of suitable functional groups [19].

Polymeric NPs have found extensive use in drug delivery. It has been demonstrated that they are stable in the bloodstream, do not activate the immune system or inflammatory processes, and are not degraded by the reticuloendothelial system. Biodegradable polymers are most widely used to reduce the occurrence of side effects. To reduce opsonization of the carrier, polymers are coated with a layer of non-ionic surfactants. The pharmacokinetics of the carrier is significantly influenced by its shape. One type of polymer is dendrimers, which contain many functional groups that facilitate drug immobilization, making dendrimers fascinating as carriers [20,21].

Due to the possibility of chemical functionalization, the most commonly used carbon nanomaterials are carbon nanotubes. Additionally, they are characterized by high mechanical strength, good conductive properties, and a large specific surface area. To render carbon nanotubes water-soluble and non-immunogenic, their surface can be modified by PEGylation, attachment of amphiphilic copolymers, immobilization of poly(amidoamine) (PAMAM) dendrimers, and functionalization with hydroxyapatite [19,23].

Among magnetic NPs, only iron NPs (mainly its oxides – magnetite, maghemite) exhibit biocompatibility. These can act as simple carriers or as a core component in core-shell structures. The coating on the magnetic core improves the chemical and physical properties of the carrier. The multifunctional character of the particle, given by the presence of specific ligands, enables targeted therapy applications. A particle acting as both a biosensor and a carrier allows for diagnosis and therapy, significantly accelerating the progress of anticancer therapies [19].

Use in the imaging studies as potential contrast agents

NPs can be used as contrast agents in medical imaging techniques such as magnetic resonance imaging (MRI) or computed tomography (CT). They can also be designed to detect specific molecules or cells, making them useful in diagnostics [1,4].

Naturally occurring NPs such as viruses, lipoproteins, and ferritin can be used as carriers of materials generating contrast as an alternative to synthetic nanoparticles. These have precisely defined sizes, are biocompatible and biodegradable, and do not stimulate an immune response [23].

Because of their unique electronic and optical properties, gold NPs can be used to enhance contrast in imaging or to detect disease biomarkers at the organ, tissue, and single-cell levels using optical or electron microscopy. This is achieved through a functionalization process [4].

Thanks to their magnetic properties, iron NPs can be utilized as contrast agents in diagnostic techniques, including those for cancer. In the method of magnetic resonance imaging, iron oxide NPs combined with antibodies that specifically bind to c-erB2 tyrosine kinase receptors, characteristic of breast cancer cells, were used. Biotinylated targeting molecules bound to the extracellular domains of cancer cell receptors. The contrast observed in the image was directly proportional to the expression of the specific receptor on the surface of breast cancer cells. This allows observation of cancer cells while avoiding the penetration of the contrast agent into their interior [24,25].

In vitro studies of nanoparticles

In vitro studies of NPs are a key factor in the process of evaluating their safety, efficacy, and interactions with cells. The purpose of such studies is to understand how NPs interact with cells and tissues, as well as their potential effects on living organisms in the context of biomedical applications such as drug delivery or cancer therapy [26].

In a study conducted by Coccini et al. [27], mesenchymal stem cells derived from the umbilical cord lining membrane (CL-MSC) were used to assess *in vitro* toxicity of magnetite. Cytotoxicity, internalization, differentiation, and proliferative capacity were evaluated following exposure to various concentrations of magnetite nanoparticles. The data were compared with those obtained from studies on bone marrow-derived stem cells (BM-MSC). Early passages showed that cytotoxicity occurred at 10 µg/mL in CL-MSC and 100 µg/ml in BM-MSC (no differences in toxicity were observed between CL- and BM-MSC at higher doses, 100-300 µg/mL), reduced cell density and loss of monolayer characteristics were affected at concentrations ≥50 µg/mL only in CL-MSC; and NPs uptake was concentration-dependent in both MSC types. Upon exposure to 100 µg/mL of magnetite NPs, proliferative capacity was reduced in CL-MSC without morphological changes. Additionally, a progressive decrease in intracellular magnetite NPs was observed over the course of cultivation. Surface antigen expression and multilineage differentiation remained unaffected. These findings suggest that CL-MSC can serve as a reliable cellular model for assessing magnetite NPs toxicity and support the validity of this approach to enhance confidence in the safety of nanoparticles when predicting health effects [27].

The cytotoxic effects and antibacterial efficacy against *Staphylococcus epidermidis* of silver NPs, microparticles (MPs) and Ag^+ ions were investigated on osteoblasts (OB) and osteoclasts (OC). Silver NPs showed strong cytotoxic effects on OB and OC, whereas weak cytotoxic effects were observed for silver MPs. Cytotoxicity was primarily mediated by size-dependent Ag^+ release. Antibacterial effects occurred at Ag^+ concentrations that were 2-4 times higher than those inducing cytotoxic effects. Such adverse effects on OB and OC viability may negatively impact the biocompatibility of orthopaedic implants [28].

In an *in vitro* study conducted by Kumaran et al. [29], genes associated with ROS, glutathione S-transferase (GST), and catalase were quantified using real-time polymerase chain reaction (real-time PCR) and molecular beacon technology. Cultures of human liver carcinoma epithelial (HepG2) cells were treated with monodisperse MgO NPs (at concentrations 25, 75, and 150 µg/mL, and incubation times 24, 48, and 72 hours) as potential therapeutic agents. Both the genetic and cellular cytotoxicity screening methods provided that the expression of GST and ROS catalase genes was maximized when treated with NPs at the highest used concentration with 48-hour incubation. However, the genotoxic effects of MgO NPs were not significant compared to control experiments, indicating their substantial potential applications in nanomedicine as both diagnostic and therapeutic tools [29].

The human breast cancer cell line (MCF-7) was used as a model to study the effects of metallic NPs, silver NPs (Ag NPs), and gold NPs (Au NPs) as potential anticancer agents were investigated. The treatment of cells with IC_{50} concentrations of Au NPs generated progressive accumulation of cells in sub-G1 and S phase, and inhibited the entrance of cells into the M phase of the cell cycle. Engineered Ag NPs effectively inhibit the proliferation of MCF-7 *in vitro* at high concentrations (1000 µM) through apoptotic mechanisms [30].

Drosophila melanogaster

D. melanogaster, commonly known as the fruit fly, has been used as a model organism in biological research since the early 20th century. It has played a significant role in many scientific discoveries, particularly in genetics, developmental biology, immunology, neuroscience, and evolutionary biology [31,32].

One of the key reasons why *D. melanogaster* is such a popular model organism is its size and short life cycle of approx. 10 days, with the fly hatching from an egg and maturing at a standard temperature of 25°C (FIG. 1). The fruit fly is only about 2-3 mm long, making it easy to handle and cultivate in laboratory conditions. This simple life cycle allows scientists to study multiple generations in a short period, an important characteristic of model organisms [31,33].

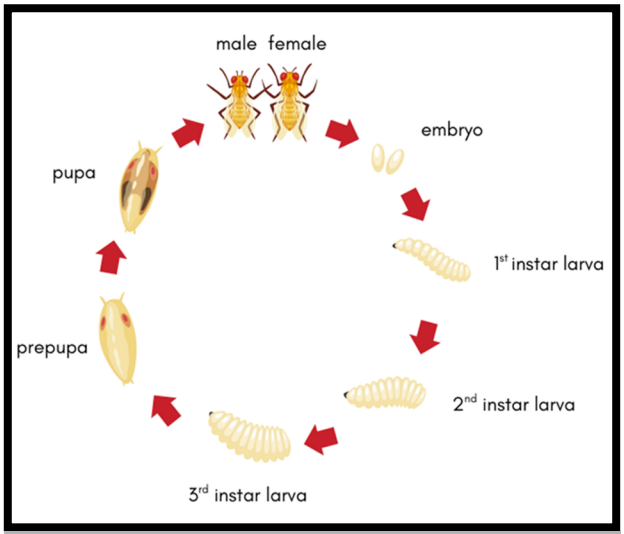

FIG. 1. Life cycle Drosophila melanogaster [34]. Made with the Canva.com platform.

D. melanogaster also demonstrates a remarkable capacity for rapid reproduction. After fertilization, females can lay hundreds of eggs, providing large sample sizes for experiments. The high reproductive rate enables the study of inheritance patterns across multiple generations in a relatively short time frame [31,33].

From a genetic perspective, *D. melanogaster* is particularly advantageous. It has four pairs of chromosomes, including three pairs of autosomes and a pair of sex chromosomes, and its entire genome has been sequenced. The fruit fly genome consists of approximately 13,600 genes, many of which are homologous to human genes. This genetic similarity means that research on *Drosophila* can provide insight into human biology [31,34]. Another significant advantage of *D. melanogaster* is the conservation of many biological pathways and processes between fruit flies and higher organisms, including humans. These conserved pathways include signal transduction, developmental processes, and fundamental cellular mechanisms such as apoptosis and cell division. Thus, studies on *D. melanogaster* have contributed to understanding various human diseases, such as neurodegenerative disorders and congenital abnormalities [31-33].

Despite its simplicity, *D. melanogaster* exhibits complex behaviours, making it a valuable model for studying the genetic and neuronal bases of behaviour. Research on fruit flies has provided insights into learning and memory, circadian rhythms, and sensory processing. Moreover, *D. melanogaster* is cost-effective to maintain and requires minimal space, allowing for rapid and efficient large-scale genetic studies. Transparent embryos of *D. melanogaster* also allow easy observation of developmental processes in real time [31-33,34-36].

Overview of *in vivo* studies using a model organism

The potential harmful effects of using nanomaterials pose a challenge to the development of nanotechnology. The number of *in vivo* studies on nanomaterials remains limited due to high costs and ethical considerations. A robust and economical model for *in vivo* nanomaterial studies is *D. melanogaster*. The use of this organism is ethically less controversial than the use of higher-model organisms. A significant advantage is its genetic similarity to humans [37].

In a study conducted by M. Alaraby et al. [38], *D. melanogaster* was used to assess the potential harmful effects of zinc oxide NPs. Toxicity, genotoxicity, internalization, oxidative stress induction, and deregulation of gene expression were evaluated following exposure of *D. melanogaster* to zinc oxide NPs. Three different *D. melanogaster* strains were employed: the wild-type Canton-S strain, the multiple-wing-hairs strain, and the flare-3 strain. The latter two strains were used in the wing spot assay and carried phenotypic wing markers. The effects of zinc oxide NPs were compared with those caused by $ZnCl_2$ to determine the potential role of released ions [38].

No significant toxicity associated with the presence of zinc ions in either form was observed. However, zinc oxide NPs were shown to be internalized in the midgut of *D. melanogaster* larvae. The uptake did not stop at the intestinal barrier but continued into the hemolymph, underscoring the potential of this organism as a model system. Neither ionic nor NPs forms of zinc induced reactive oxygen species production. Furthermore, no genotoxic effects of zinc oxide NPs were observed, as evidenced by the comet assay for primary DNA damage and the wing spot test for permanent genetic damage. Changes in the expression levels of the Hsp70 and p53 genes were observed, indicating a stress response to zinc oxide NPs exposure [38].

Silver NPs (Ag NPs) are among the most extensively studied due to their complex antimicrobial properties. A study conducted by A. Panacek et al. [39] focuses on the *in vivo* evaluation of the acute and chronic toxic effects and toxicity thresholds of Ag NPs on the eukaryotic organism *D. melanogaster* [39]. Ag NPs were prepared as a solid dispersion using the microencapsulation method, in which mannitol was used as the encapsulating agent. The solid dispersion with a high concentration of Ag NPs was used to prepare a standard D. *melanogaster* culture medium with silver concentrations ranging from 10 mg/L to 100 mg/L Ag. It has been used for acute toxicity testing and at a concentration of 5 mg/L Ag for chronic toxicity evaluation [39]. The acute toxic effect of silver NPs on *D. melanogaster* was observed at a silver concentration of 20 mg/L. At this concentration, 50% of the tested fly population was unable to emerge from the pupal stage, thus failing to complete their developmental cycle. Chronic toxicity of Ag NPs was evaluated based on prolonged exposure of eight successive generations of *D. melanogaster* to silver NPs. Long-term exposure to Ag NPs affected the fertility of *Drosophila* in the first three generations of offspring; however, the fertility of flies in subsequent generations increased to the level observed in the control group, demonstrating the flies' ability to adapt to exposure to silver NPs [39].

Ag NPs are synthesized using various methods, sometimes involving chemical substances (chemical methods) and biological units (biological methods). Synthesis methods are being improved to reduce toxicity and costs and to increase nanoparticle stability. In a study conducted by Tyagi et al. [40], biological and chemical synthesis methods were compared. Additionally, the antimicrobial potential of Ag NPs against three pathogenic fungi, *Alternaria solani*, *Fusarium spp.*, and *Carsespora cassicola*, was evaluated. The toxicity of Ag NPs was tested on the model organism *D. melanogaster* [40]. It was observed that Ag NPs synthesized using the biological method were more stable, had a uniform shape, and were smaller compared to those synthesized using the chemical method. Both types of NPs exhibited antifungal activity, with the inhibitory effect being dose-dependent, and dependent on the growth medium and fungal species. Furthermore, it was found that Ag NPs synthesized using the biological method were non-toxic to *D. melanogaster*, proving that they are safer to use compared to those synthesized using the chemical method [40].

To mitigate the toxic effects of Ag NPs on the organism, a study was conducted using *D. melanogaster* as a model organism. It was done to determine whether the addition of curcumin, a biologically active polyphenolic compound present in turmeric with various therapeutic properties, could alleviate the toxic effects caused by silver NPs consumption. It was found that the simultaneous administration of Ag NPs with curcumin in the food of *D. melanogaster* could mitigate the harmful effects caused by the consumption of Ag NPs. The addition of curcumin counteracted the reduction in feeding, pupation, metamorphosis, pigmentation, and fertility caused by Ag NPs consumption. Ovarian development impairment observed in flies fed a diet enriched with Ag NPs was also partially restored by the simultaneous administration of curcumin. Furthermore, a significant reduction in reactive oxygen species levels was observed in the larvae tissues after supplementing the food with curcumin. It was concluded that curcumin, when administered with Ag NPs, may counteract the harmful symptoms of consuming these nanoparticles [41].

Both Ag NPs and titanium dioxide nanoparticles (TiO_2 NPs) induce oxidative stress *in vivo* and *in vitro*. A study conducted by Posgai et al. [42] evaluated whether these nanoparticles affect the development, reproduction, and survival of the model organism *D. melanogaster*. It was also investigated whether the adverse effects of their use are reversible after the application of antioxidants [42]. Consumption of TiO_2 NPs during the larval stage of the life cycle did not alter development or survival up to a dose of 200 µg/mL. On the other hand, consumption of Ag NPs had a dose-dependent, size-dependent, and coating-dependent effect on each of these life aspects (uncoated particles were more toxic than coated ones, and smaller particles were more toxic than larger ones). The adverse effect was partially or completely reversible with the use of vitamin C. Larvae growing on Ag NPs supplemented with vitamin C showed more than a twofold increase in survival and a threefold increase in reproductive success compared to flies raised solely on Ag NPs. The use of vitamin C also eliminated cuticle and pigmentation defects in flies fed with Ag NPs. Biochemical assays for superoxide dismutase and glutathione showed that these markers respond to oxidative stress induced by TiO_2 NPs and Ag NPs, and this response is reduced by vitamin C. It was also shown that antioxidants can reduce the toxic effects of Ag NPs use. Another important conclusion drawn from the lack of toxicity of TiO_2 NPs is the fact that oxidative stress does not necessarily cause whole-organism effects, strengthening the idea that NPs toxicity must be studied at different levels of biological organization [42].

In studies conducted by Pompa et al. [43], the effect of citrate-coated gold NPs after consumption by the model organism *D. melanogaster* was evaluated. The NPs solution was administered to the experimental group along with food, while the control group received food without the nanoparticle mixture. *In vivo* studies showed a clear toxic effect of Au NPs. They caused a reduction in lifespan and a drastic weakening of fertility, which was not related to sex. Furthermore, the presence of DNA fragmentation and excessive expression of stress proteins, including the highly conserved HSP 70, was proven. The presence of Au NPs in the tissues of *D. melanogaster* (digestive tract tissue, ovaries, testes) was observed using transmission electron microscopy (TEM). A significant amount of NPs was localized in endosomes near the lamellar structures of the rough endoplasmic reticulum (RER). Evidence of the high toxicity of NPs in *in vivo* studies raises important questions in fields such as nanomedicine and materials science [43].

K. Sood et al. [44] conducted a study on the toxicity of graphene oxide (GO) and zinc oxide (ZnO) NPs in relation to the model organism *D. melanogaster*. The study compared the toxicity of chemically and biologically synthesized ZnO and GO nanoparticles. The synthesized particles were characterized using techniques such as scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HR-TEM), Fourier-transform infrared spectroscopy (FT-IR), UV-VIS spectroscopy, energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), and dynamic light scattering (DLS). These techniques allowed for the analysis of physicochemical properties and the investigation of their potential cytotoxic and neurotoxic effects [44]. Toxicity studies were conducted using various tests, including MTT, mortality analysis, larval crawling test, climbing test, and protein content analysis. The results indicated that chemically synthesized ZnO exhibited the highest level of cellular toxicity, while biologically synthesized ZnO showed lower cytotoxicity. GO, on the other hand, exhibited intermediate toxicity. However, in neurotoxicity tests, biologically synthesized ZnO had the greatest impact on neuromuscular coordination, while GO proved to be the least harmful [44]. The study highlights the various toxic effects resulting from the presence of ZnO and GO NPs in the environment, particularly in the context of their potential applications and the risk of uncontrolled release. The authors suggest that differences in toxicity may arise from the size, shape, and specific surface properties of the particles, as well as the method of synthesis used, which could be crucial for future research on the safety of NPs in industry and environmental protection [44]. In addition, the study outlines the diverse toxic effects resulting from the presence of ZnO and GO NPs in the environment, particularly in the context of their potential applications and the risk of their uncontrolled release. The authors propose that differences in toxicity may arise from the size, shape, and specific surface properties of the particles, as well as the method of synthesis used, which could be crucial for future research on the safety of NPs in industry and environmental protection [44].

In the study by Singh et al. [45], the biosynthesis of green silver NPs (Ag NP) using the extract of *Urtica dioica* (UD) and their antibacterial and antioxidant properties as well as their effect on *D. melanogaster,* were discussed. The aim of the study was to develop a more sustainable method of NPs synthesis, avoiding the use of harmful chemicals such as ethylene glycol and sodium borohydride. The UD extract served as a reducing agent for Ag+ ions from silver nitrate, and the produced nanoparticles were analyzed using UV-Vis spectroscopy, FTIR, and SEM techniques [45]. The analysis revealed that the NPs have a spherical or oval structure, with a size range from 29 to 70 nm, and their chemical composition was confirmed by EDS spectroscopy (79% silver with trace amounts of oxygen, chlorine, and nitrogen). In biological studies, UD-Ag NP demonstrated significant antioxidant properties and inhibited the growth of *Escherichia coli* and *Pseudomonas putida* bacteria. Furthermore, the UD NPs, compared to $AgNO_3$, reduced cytotoxicity in *Drosophila* tissues, assessed by trypan blue staining, and enhanced biochemical activity (e.g., acetylcholinesterase) and improved motor behaviours in climbing and jumping tests [45]. The study indicates the potential applications of green Ag NPs as natural antioxidants and antibacterial agents, which could be useful in medicine and the production of environmentally friendly nanomaterials [45].

G. Vecchio et al. [46] studied the mutagenic effects of Au NPs on *D. melanogaster*, particularly in terms of genetic toxicity and the impact on the phenotype of subsequent generations. The experiment used stable nanoparticles with a diameter of 15 nm coated with citrate, which were administered through the diet of the flies. The results showed a significant decrease in fertility and survival, as well as phenotypic deformities, such as wing, eye, and thorax malformations in subsequent generations. Selected DNA analyses revealed genomic damage and apoptosis of hemocytes, suggesting an increase in ROS levels as a potential mechanism of toxicity [46]. Phenotypic observations showed that some mutations were inherited in the next generation, confirming the mutagenic effects of Au NPs on the germline. These results highlight the need for a detailed safety assessment of nanomaterials before they are introduced for wider use, especially in medicine [46].

Araj et al. [47] conducted research on the toxicity of Ag NPs and sulfur nanoparticles (S NPs) to various developmental stages of *D. melanogaster*. The NPs were obtained through green synthesis using plant leaf extracts such as olive, fig, medlar, pistachio, mulberry, and citrus. The study analyzed the effects of different concentrations of NPs (10, 50, 100, 200 ppm) on larvae, pupae, and adult flies. The results demonstrated the high toxicity of Ag NPs, especially from olive and mulberry leaves, in causing larval and pupal mortality and reducing egg-laying rates [47]. The research showed that Ag NPs were more effective than S NPs in terms of toxicity to insects, particularly at higher concentrations. Ag NPs caused high mortality among larvae and pupae and significantly reduced the number of eggs laid by females. Additionally, it was found that green synthesis of NPs is more environmentally friendly and beneficial, especially from an agricultural perspective, as an alternative to conventional pesticides [47].

The study conducted by D. Sabat et al. [48] focuses on the toxic effects of titanium dioxide NPs (TiO_2 NPs) on the behaviour and development of *D. melanogaster*. Various concentrations of TiO_2 NPs (50, 100, 200, 250 mg/L) were administered through the diet to analyze their impact on the survival, life cycle, and mechanosensory behaviour of the flies. It was observed that at higher concentrations (above 200 mg/L), the TiO_2 NPs delayed larval development and caused a decrease in the number of pupae. Changes in behaviour were also noted, particularly in the crawling activity of larvae and climbing behaviour of adult flies, indicating an effect of TiO_2 on the nervous system and mechanosensory organs. Additionally, higher concentrations of TiO_2 NPs led to DNA damage and increased oxidative stress, suggesting a toxicity mechanism related to the generation of ROS [48]. Microscopic observations revealed abnormalities in the wing and bristle phenotypes, which could result from disturbances in the signaling pathways involved in the development of these structures. These results highlight the potential hazard that TiO_2 NPs may pose to living organisms, suggesting the need for further research on their toxicity and a cautious approach to their use in food and cosmetic products [48].

Conclusions

Nanotechnology is a rapidly developing field of science focused on the design and application of materials at the nanometer scale. Since its origins in the 1950s, it has significantly expanded possibilities in medicine, electronics, and engineering, enabling the creation of precise tools for diagnosis, therapy, and innovative materials with unique properties [49]. This article discusses the use of the fruit fly (*Drosophila melanogaster*) as a model organism in nanotechnology research. Due to its biological and genetic characteristics, the fruit fly allows for the analysis of nanoparticle toxicity and their impact on living organisms. The importance of these studies for the development of nanomedicine, including cancer therapies and diagnostics, is emphasized, while also highlighting the need to assess toxicity risks and search for ways to minimize them.

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