Drosophila melanogaster: A Model Organism to Understand Biological Activities of Nanoparticles

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Abstract

Nanoparticles exhibit remarkable physicochemical features not inevitably found in bulk arrangements; their size or coating modifications distinctly alter their physical, chemical, and biological attributes. Owing to these unique properties, there is a general inclination to explore nanoparticles in numerous fields, viz., in medicine and food industry. To that end, our environment and human health are also affected by its toxicity. To study the reaction of nanoparticles with human cells and complex system, a thorough understanding of the parameters is necessary for the nanoparticles to react within the cells. As human-based trials are difficult with ethical barriers, one extensively exploited laboratory model organism, viz., *Drosophila melanogaster*, is used as an in vivo model organism for the study of developmental biology, genetics, and recently host pathogenicity. *D. melanogaster* is very much genetically malleable organism with its short life-cycle, clear developmental stages and availability. *D. melanogaster*, possessing completely the experimental features like inclination of investigational manipulation proportional to vertebrate models, significant gene homology with developed and complex organisms, and simplicity of gaining mutant phenotypes, seems to be an ideal model organism for the study of nanoparticles activities in cells and nanotoxicological trials. The molecular trails with numerous developmental and behavioral factors can be assessed expending this model in different modes of throughput type tests.

Keywords

Nanoparticles · *D. melanogaster* · Host pathogenicity · Nanotoxicology · Gene homology

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10.1 Introduction

Nanoparticles exhibit remarkable physicochemical features which are not inevitably found in bulk arrangements; their size or coating modifications distinctly alter their physical, chemical, and biological attributes. Owing to these unique properties, there is a general inclination to explore nanoparticles in numerous fields, viz., in medicine and food industry. To that end, our environment and human health are also affected by its toxicity. To study the reaction of nanoparticles with human cells and complex system, a thorough understanding of the parameters is necessary for the nanoparticles to react within the cells. As human-based trials are difficult with ethical barriers, one extensively exploited laboratory model organism, viz., *Drosophila melanogaster*, is used as an in vivo model organism for the study of developmental biology, genetics, and recently host pathogenicity (Pandey and Nichols 2011). *D. melanogaster* is very much genetically malleable organism with its short life cycle, clear developmental stages, and availability (Greenspan 2004). *D. melanogaster*, possessing completely the experimental features like inclination of investigational manipulation proportional to vertebrate models, significant gene homology with developed and complex organisms, and simplicity of gaining mutant phenotypes, seems to be an ideal model organism for the study of nanoparticles activities in cells and nanotoxicological trials (Rand 2010). The molecular trails with numerous developmental and behavioral factors can be assessed expending this model in different modes of throughput type tests (Ahamed et al. 2010).

10.2 Insects and Nanoparticles

The progress of nanoparticles is now widely used in the various divisions of science to understand their toxic effects on expansion and anatomy of their organisms and environment (Ahamed et al. 2010). It has widespread applications in pest management of insects. As per research conducted, ferromagnetic substances have been recognized in the head, neck, thorax, and abdomen of *Solenopsis substituta* ant (Guan et al. 2008). The components of nanoparticles are found in the compound eyes of insects. The bright colors of wings of butterflies are nothing but the nanoparticles. In recent years, an innovative photodegradable insecticide involving nanoparticles have been developed (Garcia-Bellido et al. 1979; Guan et al. 2008).

10.3 Toxic Effects of Nanoparticles in Insects

Nanoparticles (NPs) invade by intracellular penetration through the exoskeleton and disrupt the organisms (Benelli 2016). Then, the nanoscale substances bind to sulfur or phosphorous from proteins or DNA, respectively, leading to the expeditious reconstruction of organelles and enzymes (Benelli 2016). The degradation of

Table 10.1 Toxic effects of nanoparticles on insect physiology

Insect name	Nanomaterial	Physiological effect	References
Aedes aegypti	Ag, Au, ZnO	Damages the epithelial in insects	Banumathi et al. (2017a, b, c)
		Damages the midgut, cortex, and gill	Kalimuthu et al. (2017)
		Reduces lateral hair	Sundararajan and Kumari (2017)
A. albopictus	Ag	Reduces the total number of protein in larval stage Reduces phosphatase enzyme, esterase, and acetylcholine	Ga'al et al. (2018)
Anopheles stephensi	Ag, Au, ZnO, Polystyrene, SiO_2	Damages the epithelial in insects	Banumathi et al. (2017a, b, c)
		Reduces lateral hair	Sundararajan and Kumari (2017)
		Damages the midgut and cortex	Kalimuthu et al. (2017)
		Reshapes the thorax	Abinaya et al. (2018)
		Abuses the structure of larval body	Sultana et al. (2018)
Culex pipiens	Ag	Reduces the total number of protein in larval stage Reduces acetylcholine, α and β carboxylase	Fouad et al. (2018)
Bombus terrestris	SiO_2	Midgut epithelial injury	Mommaerts et al. (2012)
Acheta domesticus	Graphene oxide	Activates catalase, glutathione, and peroxidase Activates heat shock protein (HSP70)	Dziewięcka et al. (2016)

membrane permeability and interruption in proton intent force cause reduction of the cellular function followed by cell death (Benelli 2018) (Table 10.1). A few experiments exhibit the toxic effects of nanoparticles on growth. A testing with 100 mg zinc oxide nanoparticles (ZnONPs) per litre resulted in 100% lethality in the *Aedes aegypti* mosquito production whereas, 1.57 mg/l of ZnONPs witnessed a low LC_{50} (Banumathi et al. 2017a, b, c). Several scientists exhibited that midgut epithelial injury occurred in intoxicated workers of *Bombus terrestris* due to silicon dioxide nanoparticles (SiO_2NPs) and in *A. aegypti* due to silver nanoparticles (AgNPs), respectively (Mommaerts et al. 2012; Kalimuthu et al. 2017) (Table 10.1). *A. aegypti* associated with gold nanoparticles (AuNPs), on a contaminated environment at the favorable concentration of high mortality results in harming the midgut, epithelial cells, and cortex (Sundararajan and Kumari 2017). Studies on *Acheta domesticus* revealed that the graphene oxide nanoparticles activate catalase, glutathione peroxidase, and heat-shock protein (HSP70) (Dziewięcka et al. 2016) (Table 10.1).

Table 10.2 Scientific classification of *Drosophila melanogaster*

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Diptera
Family	Drosophilidae
Genus	*Drosophila*
Subgenus	Sophophora
Species	Melanogaster

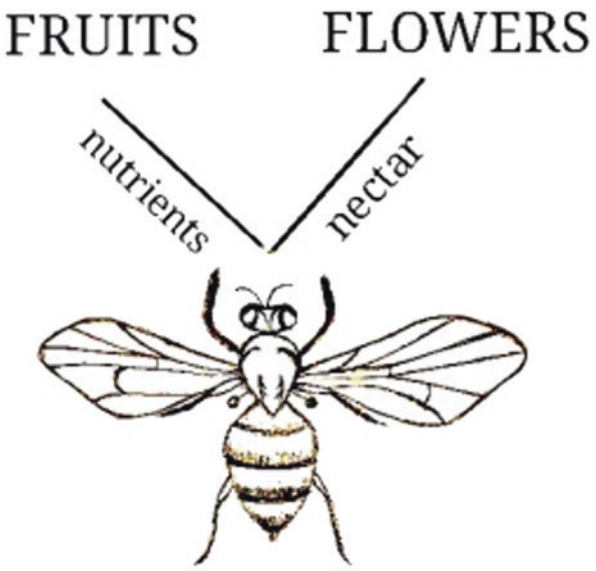

Fig. 10.1 The fruit fly, *Drosophila melanogaster*

10.4 *Drosophila* in Nanoparticle Study

Thomas Hunt Morgan first used *D. melanogaster* as a model organism in the time period of 1960–1990 (Morgan 1910). It was also used to study some human diseases and as a model organism used to study toxicology (Pandey and Nichols 2011). In the year of 2010, the term Drosophotoxicology was reported, as *Drosophila* has a short life span around 40–60 days (Rand 2010). The nanotoxicity can easily be studied for their genome stability, development, reproduction, and activity in several age periods of adult flies, utilizing any particular tissue or organ (Greenspan 2004). As an example, the *Drosophila* can be used in organogenesis to exhibit the toxins and developmental studies concerning cell determination and neurons in embryonic stage or the larval stage, during its applications in developmental and physical studies. In the stages between late larva and pupa, the toxin's mode of action to affect fictional discs is an advantage to study the unfavorable toxic effects

on end replication and physiological changes between the stages of larva to adult flies (Pandey and Nichols 2011; Stocker and Gallant 2008). Some organs of adult *Drosophila* such as the brain, heart, lungs, kidney, liver, gut, and reproductive tract are physically almost similar to humans. *Drosophila* fat body also has identical functions as the human liver (Pandey and Nichols 2011). The tracheal system of *Drosophila* (respiratory system) is nothing but a divided network of epithelial tubes, separated in every part of the body. It helps in transporting oxygen and other gases. As for these identical traits in organs between *Drosophila* and human, it can be the acceptable model organism of toxicology analysis, approved by the European Centre (Ahamed et al. 2010). Besides this, as a conclusion, we can say that the short life span, recognizable developmental stages in their life cycle, well-known genome sequence, accessible equipment, and reagents makes *D. melanogaster* an efficient in vivo model organism for toxicology. The scientific classification of *D. melanogaster* and the fly is shown in Table 10.2 and Fig. 10.1, respectively.

D. melanogaster belongs to kingdom Animalia, phylum Arthropoda, class Insecta, order Diptera, and family Drosophilidae. It is generally known as fruit fly. The first approach of using *D. melanogaster* as a model organism is registered by Charles W. Woodworth. It has a broad spectrum in genetics, physiology, and microbial pathogenesis researches. As per reports till 2017, eight Nobel Prizes are archived for researches regarding *D. melanogaster*. It has a worldwide geographic extent including islands. The flies belonging to the family Tephritidae are also known as "fruit fly," but these flies, *Ceratitis capitata*, are economic pests in Australia and South Africa.

10.5 Characteristics and Life Cycle

Female *Drosophila* is 2.5 cm in size, whereas the male *Drosophila* is shorter than them (Fig. 10.2). Male *Drosophila* appears with a darker back, black patch at the abdomen part, a row of dark hairs on the tarsus of first leg (sex-comb), and the various body colors which make a difference between male and female *Drosophila*.

Drosophila has a short life span around 50 days at the optimal room temperature of 25 °C (77 °F). But the developmental period depends on the temperature and the ectothermic species as well. The time period and the temperature increase proportionally due to heat stress. At the temperature of 28 °C (82 °F), *Drosophila* can develop from egg to adult stage just in 7 days (Ashburner and Thompson 1978; Ashburner et al. 2005); at 30 °C (86 °F), it takes 11 days; at 18 °C (64 °F), it takes 19 days; at 12 °C (54 °F), it takes over 50 days; and at 25 °C (77 °F), it takes 8.5 days, which is the ideal case. The developmental time period also depends on crowded environment. The time period gets increased (Chiang and Hodson 1950) such as female flies may lay 400 eggs at a time. They mainly lay eggs into the decaying material such as decaying fruits, mushrooms, and slap fluxes. Some specific eggs having 0.5 mm long in size hatch after 12–15 h at the ideal condition. As a result, the larvae complete their growth in 4 days. Within this period, after 24 and 48 h of hatching, molting occurs twice in the second instar larval stage and third

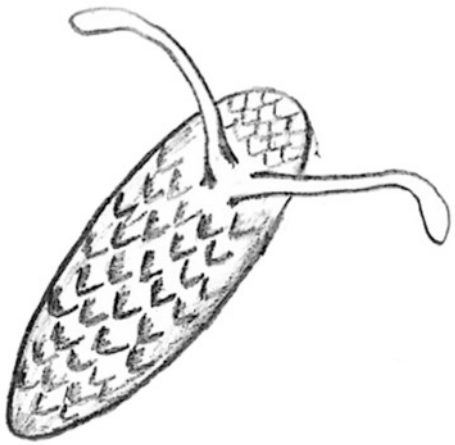

Fig. 10.2 Male *Drosophila melanogaster*

instar larval stage. They used to feed on the nutrients of decomposed materials, sugars from fruits, and nectar from flowers during their developmental period. The mother flies generally deposit their feces on the egg sacs with the intention to initiate an identical microbial configuration in the embryo's guts (Blum et al. 2013). Then the larvae get transformed into pupa, and subsequently after 4 days, the adult *Drosophila* emerges with some physiological changes (Blum et al. 2013).

10.6 *Drosophila melanogaster* Reproduction Mechanism

Male flies used to approach the female by five types of observable techniques. At first, they carol a courting song by vibrating and expanding their wings horizontally to orient them, and then the male fly places himself at the low back portion of the female fly's abdominal part with the motive to press and lick the female genitalia. And then they make their abdominal a coil-like structure to participate in mating, staying for around 15–20 min (Houot et al. 2010). Some specific neurons of abdominal nerve cord permit the female to hold their body during mating and control their mood to approach the male for mating. But the male can help them by their secret chemical substances, pheromone, to reactivate the nerves. They didn't mate in a poor environment (Dagaeff et al. 2016). Female prefer to mate with their brothers rather than other males (Loyau et al. 2012), and they used to follow the other females while choosing the male as a copulation partner. Both male and female flies can do multiple mating at the same time, to make sure about the fertilization known as polygamy (Haartman 1951). But conventionally with whom the initial mating is done, he is the 80% ancestor of the offspring. The priority of the last male can be registered over the both phenomenon dislocation and inability (Price et al. 1999). The advantages of mature males in mating are they can adopt a developed courtship dance while approaching recent females and as they are efficient in it they can complete their copulation quickly.

By this procedure, male flies can inject their 1.76 mm long sperms in the seminal fluid of the female flies (Gilbert 2006). The female flies get ready for the next mating after 8–12 h of development (Pitnick 1996). As they prefer a short-lived copulation than male, they can deny or refuse the male fly for copulation by quitting or kicking and ejecting their ovipositor (Connolly and Cook 1973). *Drosophila*'s reproduction mechanism doesn't follow the human's estrous cycle. Due to gonadotropic hormone, they used to follow a cyclic pathway which is correlated and produces stable and controlled offspring (Meiselman et al. 2017).

10.7 A Model Organism for Understanding the Biological Activities of Nanoparticles

Model organisms are a non-human species used to study easily biological phenomenon in the laboratory. *Drosophila* is one of the most efficient biological model organisms in genetics and developmental biology.

D. melanogaster as a model organism

- Adult flies are very small, 3 mm in length, so it can easily grow and be studied in the laboratory.
- It produces a large number of offspring by multiple mating (female fly lays around 400 eggs at a time).
- It has a short generation time period, around 14 days.
- Embryos grew up and get developed outside the mother's body, and the multicellular anatomy helps to study the developmental stages.
- The modified nervous system helps to study their characteristics.
- The genome sequence is well known, containing 13,600 genes (Adams et al. 2000).
- Some have physically identical organs, unlike humans.

The most acceptable technique to study the nanotoxicity is by analyzing their existence after the revelation of nanomaterials into the fly's body. The introduction of nanomaterials into the *Drosophila* can be done by the common way of ingestion. As an example, the effect of nanoparticles in *Drosophila* was studied after 6 h of fasting by influxing a specific concentration of 20 nm AgNPs through their food. After 10 days, the effects of before and after the introduction of nanoparticles into the flies were observed. As a result, it decreased the survival rate of flies. But in case of $AgNO_3$ with the same conditions, the results will be positive, concluding that specifically AgNPs (Tian et al. 2013) cause the toxic effects in *Drosophila*. It evades the capability of larva to develop in pupate. So the cycle of larva to pupation stage and then pupate to adult stage gets retarded.

Sometimes the toxicity of nanomaterials varies on their coat. Poly(maleic anhydride octadecene) and polyethylene glycol-coated nanomaterials are more venomous than the mercaptoundecanoic acid or poly(maleic anhydride octadecene)-coated nanomaterials (Galeone et al. 2012). CdSe-ZnS quantum dots exhibit toxic effects on *Drosophila* by decreasing their survival rate as CdSe-ZnS quantum dots are

coated with poly(maleic anhydride octadecene) and polyethylene glycol. By several experiments, it is considered that the integral quantities of nanoparticles are responsible for the toxicity, such as citrate-coated AuNPs, degrading the *Drosophila's* life span (Pompa et al. 2011). On the other hand, some nanoparticles play a positive role in *Drosophila*. Approximately around 20 nm silica nanoparticle did not show any toxic effects on flies, such as almost 80 nm of gallium phosphide (nanowires) (Adolfsson et al. 2013; Barandeh et al. 2012) and Gellan gum-PEI (nanocomposites) (Goyal et al. 2011) don't degrade their developments and survival rate, respectively. The insulin nanoparticles, having the function of transporting insulin, also didn't show any toxic effects to flies (Fangueiro et al. 2013). Dry nanoparticles, such as carbon black and unicellular nanotubes, can also be encountered to *Drosophila* by physiological contact. As *Drosophila* has similar traits like human, this process can be considered for the introduction of nanoparticles to the human skin. The introduction to the exterior part of the flies causes mortality to them within a couple of hours. If the nanoparticles specifically get exposure to the spherical open areas, then it causes respiratory retardation in flies, considering the main reason for death (Lehmann 2001; Liu et al. 2009).

Lastly nanoparticles can be inflamed by inhalation. Small nanoparticles can influx in *Drosophila* by a nebulizer-based technique through the spherical opened areas. These experiments conclude that some specific nanoparticles such as 24 nm-, 100 nm-, and 210 nm-sized FluoSpheres and 20 nm silver can transfer to the respiratory system of *Drosophila*, and it can be considered as the primary analysis of nanoparticle inhalation technique to the human also. As the embryo grows up outside the mother body, the various methods of introducing nanoparticles can be successfully studied in their different stages. It can therefore be concluded that *Drosophila* is an efficient model organism in nanomaterial studies (Fig. 10.3).

10.8 Gene Homology

Homology was first reported by Richard Owen. He registered that homology is nothing but the sharing of common organs or genes (Owen 1843) in various taxa, with the variety of structure and function (Gegenbaur 1878). The word "homology" is the joining of two meaningful words "homo" which means same and "logos"

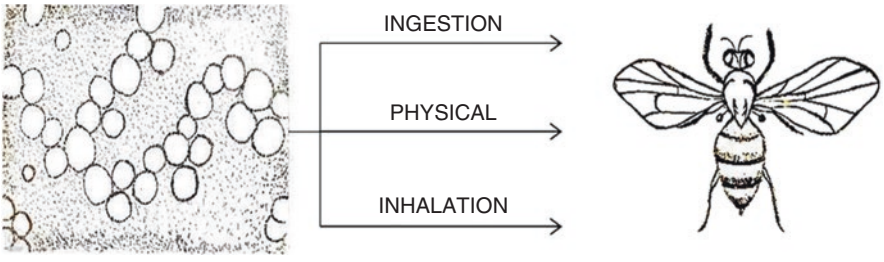

Fig. 10.3 *D. melanogaster* for nanoparticle study

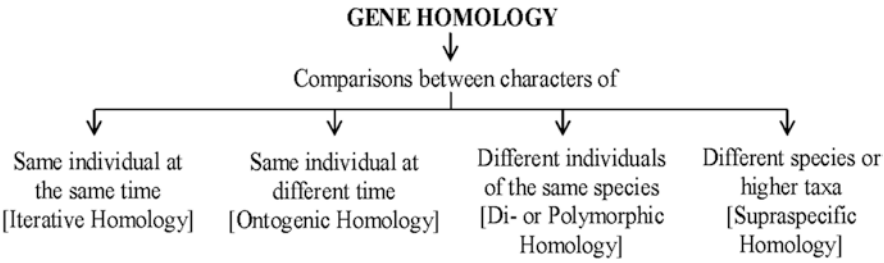

Fig. 10.4 Classification of gene homology

which means relation (Bower 1906; Williams and Forey 2004). As per developmental biology, the structure of homologs can be modified by the dynamic process of adaption to different motifs, resulting in enhanced descent from a common founder or ancestor. But later, the definition of homology restricts the homologous usage to supraspecific analogy (Ax 1989). Some examples of homologous structure are wings of bat, forelegs of dogs and horses, etc. A gene inherited in two species from a common founder is known as homologous gene. They may be having a same sequence, but it is not mandatory at all (Haszprunar 1992; Wagner and Laubichler 2000; Wagner et al. 2000). The traits of a homologous gene are known as homology. But the word "homology" applies for both homologous protein and gene in genetics. It forms the fundamental arrangements for provisional biology. Homologs vary due to mutations, as their identical founder. Homologous gene can derive their common founder. Homologous gene can derive though three specific circumstances as follows:

(a) Phylogenetic event: This helps to grow the orthologous gene.
(b) Genetic duplication event: This helps to grow paralogous gene.
(c) Horizontal gene transfer event: This helps to grow xenologous gene.

10.9 Classification of Gene Homology

The classification of gene homology is shown in Fig. 10.4.

10.9.1 Iterative Homology

Iterative homology is the specific type of homology which clarifies the typical and repetitious relationship into the fragment of the common organism (Webster 1913). Iterative homology is also known as serial homology. The occurrence of homoeotic mutations can encounter this type of homology. These individuals are as complicated as in numerous *Drosophila* mutants. But homoeotic mutation in *Drosophila* can be a matter of probabilities.

10.9.2 Ontogenic Homology

This is the type of homology in which there is specific arrangement in the common taxa, showing evolutionary similarities in growth. It can be recognized by pursuing the characteristic distinction and adaption, throughout the ontogeny (Haszprunar 1992). The phenomenon of naturally occurring neoteny as a mutation is occasional. But until now, it is considered in modifying phylogenies. It should be emphasized that this defines all characters of a definite ontogenic stage, ancestor homologue of the adult stage. As ontogeny is a part of the evolution, larval character gets reconstructed from an adult stage of a precursor in the cases of the biological and polyphasic life cycle, deduced for the pedigree of larval insect homolog along the ommatidia of the possible ancestor. But it doesn't count as a natural ancestor of the common entities. Similarly it can also be registered with protonephridia and metanephridia in larva and adults of coelomate metazoans, respectively (Ruppert and Smith 1988).

10.9.3 Di- or Polymorphic Homology

In this type of homology, species are arranged in the common taxa, which exhibit physiological similarities. This naturally occurs between subspecies, variants, and mutants, where the adaption of characters is not stable, whereas in dimorphism and polymorphism, the adaption of characters is stable. It also includes the examination of specific mutation or diagnostic experiments regarding sex or polymorphism. The instances of hypothetical polymorphism detection are generally possible in the adult bee. On the contrary, studies of polymorphism recognition in typical larvas show that they get triggered by food with the intention of becoming a queen. For example, studies of sex change along with all intermediary structures in gastropods, dog whelk (*Nucella lapillus*), and European sting winkle (*Ocenebra erinaceus*) (Gibbs et al. 1990).

10.9.4 Supraspecific Homology

This homology defines the contrast between the different species and higher taxonomy. As particularly, oncogenic and bi- or polymorphism homology is the type of phylogenetic homology, so this phase should evade for supraspecific homology. Nonetheless, it is a major part of phylogenetic reconstruction. During the meiosis cell division, the genetic conversions take place as the former varieties can obtain within the species. Although these occurrences are not within species, some exceptional examples are the procedure of transferring gene by vectors or conjunction (Stachel and Zambryski 1989).

10.10 Host Pathogenicity

10.10.1 Pathogenicity

Pathogenicity is interpreted as having the complete ability of infectious agent including bacteria, fungi, virus, protozoa, and helminth, responsible for disease in a host (Malcolm and Moore 2017). Pathogenicity means virulence, but in many cases, it is analyzed as a qualitative term. Pathogenicity is also being definite from the transmissibility of the virus, which measures the level of infection.

10.10.2 Pathogenic Treatment

Infectious diseases are inescapable disorders due to organisms (bacteria, virus, fungi, or parasites). Many of them also live in our bodies with different functions. They may be harmful or helpful for the host. There are some simple strategies, treatments, and global methods to prevent pathogenic diseases such as vaccines and medicines, antibiotics, and antivirals.

10.10.2.1 Vaccine

The vaccine is a biotic arrangement that provides an active and improved acquired immunity to a specific disease. Vaccines are basically made of by the exhausted

Table 10.3 Side-effects of vaccines

Vaccines	Side effects	Reference
1. DTaP (diphtheria, tetanus, acellular pertussis) vaccine	Redness, soreness, fever, coma, permanent brain damage, poor appetite	Regalado et al. (1990)
2. Hepatitis A vaccine	Low grade fever, headache, tiredness, soreness, redness, shoulder pain, allergic reactions	Tong et al. (1993)
3. Hepatitis B vaccine	Soreness, fever, shoulder pain, allergic reaction	Bircan and Rahmet (2017)
4. Influenza (inactivated) vaccine	Flu, soreness, redness, swelling, red or itchy eyes, fever, headache, fatigue, Guillain-Barre syndrome (GBS)	Hirve et al. (2016)
5. Influenza (live) vaccine	Runny nose, cough, fever, headache, wheezing, abdominal pain, vomiting, sore throat, chills, tiredness	Hirve et al. (2016)
6. Polio vaccine	Dizziness, ringing in the ear, shoulder pain soreness, allergic reaction	O'Reilly et al. (2012)
7. Rabies vaccine	Soreness, redness, swelling, nausea, abdominal pain, dizziness, muscle aches, pain in joints, nervous system disorder	Hsu et al. (2017)
8. Yellow fever vaccine	Soreness, redness, swelling, sever allergenic reactions and nervous system reaction	Amanna and Slifka (2016)
9. Anthrax vaccine	Tenderness, redness, itching, lump, bruise, headache, fatigue	Zai et al. (2016)

micros and their toxins and contain a factor that reorganizes the microorganisms, responsible for diseases. The factor helps the immune system to make it easier to recognize it, identify it as a foreign body, and eradicate the disease-causing microorganisms by provoking host's immune system. The vaccine itself is incapable of causing disease. But the host body reacts to vaccines as if it is a pathogen-containing agent. Many diseases are now cured by vaccine such as polio, measles, diphtheria, whooping cough, mumps, and tetanus (Table 10.3). The procedure is known as vaccination (Melief et al. 2015; Bol et al. 2016). Vaccinated host body constructs antibodies which counteract the disease-causing viruses and bacteria. As they are not much probable to being infected or transfer the infection to others, non-vaccinated people will also be saved by the immunity of the herd. The side effects of various vaccines are shown in Table 10.3.

10.10.2.2 Antibiotic

Antibiotic is an antimicrobial material containing medicine, powerful to fight against bacterial infection. It has the function to either kill or inhibit the bacterial reproduction in the host body. Antibiotics are universally used in the treatments. But it is necessary to use it properly to save lives. To prevent the development of bacterial resistance, it should be taken as directed and also after the symptoms disappear. Antibiotics are not effective for virus infections. Some antibiotics are penicillin,

Table 10.4 Side effects of antibiotics

Antibiotic	Side effects	Reference
Antibacterial		
(a) Penicillin G	Muscle spasm, nausea, vomiting, skin rash	Hitchings et al. (2015)
(b) Amoxicillin	Nausea, diarrhea, stomach pain, headache, rash	Gillies et al. (2015)
(c) Cephalexin	Dizziness, abdominal pain, joint pain, itching, diarrhea	Haberfeld (2009)
Anti-tumor		
(a) Doxorubicin	Darkening of skin and nails, puffy eyelid, eye redness, weakness, loss of appetite	Chaterjee et al. (2010)
(b) Bleomycin	Poor appetite and weight loss, phlebitis, pneumonitis, pulmonary fibrosis	Huls and Ten Bokkel Huinink (2012)
(c) Mitomycin	Pale skin, unusual bruising or bleeding, irritability, bloody diarrhea, rapid weight gain, no urinating	Charpentier et al. (2011)
Anti-fungal		
(a) Griseofulvin	Heart burn, numbness or tingling in hands or feet, stomach pain, rash	Harris (1976)
(b) Micafungin	Indigestion, constipation, trouble sleeping	Carver (2004)
(c) Nystatin	Mouth irritation, hives, skin irritation	Carver (2004)
Antiprotozoal		
Daunorubicin	Temporary hair loss, reddening within 1–2 days, mild itching, irregular heartbeat	Fornari et al. (1994)

cephalexin, etc. (Gould 2016; Foster and Raoult 1974). The side effects of various antibiotics are displayed in Table 10.4.

10.10.2.3 Antiviral Agents

As antibiotics are not effective for viral infections, antivirals are used in which it is a class of medicine, mainly used to prevent viral infections like flu, warts, cold, etc. It inhibits viral reproduction into the host and encourages body immune system to fight against viral contaminations. There are some classifications among the drugs of antivirals, specific to different types of infections such as abacavir used for HIV, amantadine used for influenza, oseltamivir, tamiflu, etc. (Rossignol 2014). The side effects of various antiviral medicines are displayed in Table 10.5.

10.11 Nanoparticles

Nanoparticles are a type of particle enclosed within the interfacial layer, ranging within 1–100 nm. Interfacial layer is a basic component of nanoscale, containing ions and organic and inorganic molecules. This inorganic nanoparticles when coated with organic fragments is known as stabilizer, surface ligand, etc. (Batista Carlos et al. 2015). The first basic researches started during the period of 1970–1980 in the USA (Granqvist et al. 1976), and in Japan, it was first studied during an ERATO project, known as ultrafine particles (UFP) (Hayashi et al. 1997).

10.11.1 Characterization

Characterization defines the physical and chemical components present in NPs. This characterization is done with the various motives like in nanotoxicology studies, exposure assessment, which shows their different nanomaterial's toxicity levels

Table 10.5 Side effects of antivirals

Virus	Anti-viral agents	Side effects	Reference
Herpes virus	Vidarabine	Burning, stinging, pain, irritation, itching, redness	Rossi (2013)
Herpes simplex	Acyclovir	Nausea, vomiting, diarrhea, headache, abdominal pain	Rossi (2013)
Retro virus (HIV)	Ritonavir	Diarrhea, stomach pain, dizziness, loss of appetite	Hayward (2017)
Influenza A	Amantadine	Dry mouth, insomnia, constipation	Singhal and Rahman (2002)
Influenza B	Relenza	Ear, nose, throat infection, nasal irritation, vomiting	Hayden (2001)
Hepatitis B and C	Interferon	Trouble sleeping, fever, nausea, weakness	Bhatti and Berenson (2007)
HCV, HSV	Ribavirin	Muscle pain, stomach pain, headache	Alvarez et al. (2006)

and constructing process control which is a fusion of control engineering and chemical engineering. These things can be estimated by the techniques of microscopy, spectroscopy, and particle counters (Hassellöv et al. 2008; Tiede et al. 2008). But many of the processes are unable to calculate the unfavorable effects on less concentrated nanomaterials. Electron microscopy and scanning probe microscopy are not that much efficient to examine nanomaterials because of their small size in visible light. Spectroscopy technique is used to calculate the concentration and morphological traits of nanoparticles by electromagnetic radiation, such as X-rays and UV rays. The chromatography, centrifugation, and filtration methods are used to separate the different sizes of nanoparticles for characterization. For some specific approaches, nanoparticles can be characterized in complicated matrices such as soil, water, food, polymers, blood, etc. (Linsinger et al. 2011).

10.11.2 Functionalization

Functionalization defines the polymers present over the nanoparticles. The stability, tragedy, physical, and chemical characterization depend on the coating over the nanoparticles, for example, coating of red blood cell can degrade the immune system. Nanoparticles coated with polymers are highly stable. If the coating will be polar, then the nanoparticles will be highly soluble in water. The coating that is highly activated produces non-specific binding. But the hydroxyl or methoxy end group that attaches to polyethylene glycol prevents non-specific binding (Prime and Whitesides 1991; Liu et al. 2010). Nanoparticles can attach to biotic components also, but it will only react on those specific organelles and the locomotion of some proteins and RNA for where it is actually tagged (Suzuki et al. 2007). Nanoparticles except monovalents contribute many selected groups. It can arrange the receptors in a gathered form, resulting in the signals that detect the cellular path getting charged and more attached. So the particular groups, monoclonal antibodies, aptamers, or peptides, must be attached sequentially and in a restricted number with the nanoparticles.

10.12 Nanotoxicology

Nanotoxicology, an important part of toxicology, defines the experiments and studies about the toxicity of respective nanoparticles (Buzea et al. 2007). But it is not mandatory to have toxic effects in all nanoparticles. Nanotoxicology experiments and analysis also explain the maximum levels of specific nanoparticles in which they will not exhibit any toxic effects or any kind of negative effects on environment and mankind (Mahmoudi et al. 2012).

10.12.1 Mechanisms of Toxicity

10.12.1.1 Oxidative Stress

Small-sized nanoparticles can have a large volume as well as a broad exterior part, helping them to actively participate in chemical and biological events. It causes the high production of reactive oxygen species (ROS) and free radicals (Jaeger et al. 2012; Ng et al. 2013). The rate of production varies on nanoparticles, such as carbon nanotubes, nanoparticle metal oxides, etc. The production of ROS and free radical can be considered as the preliminary method of nanoparticle toxicity. The greater yield of ROS causes intracellular effects on proteins, lipids, and DNA followed by cardiovascular diseases and neurological disorders (Turrens 2003). The overdose of nanoparticles can promote highly oxidative stress. But for ethical barriers, it is difficult to execute any experiment in in vivo mammalian model organisms. *Drosophila* can be the efficient model organism to study oxidative stress. After ingesting 5 nm-, 15 nm-, 40 nm-, and 80 nm-sized AuNPs, the intracellular ROS level can be measured from the 2,7-dichlorofluorescein diacetate (DCF-DA)-dyed fly's homogenate. AuNPs might be the cause of increasing production of ROS. But the various-sized AuNPs didn't affect the production of ROS. It concludes that the total exterior surface is not the major measuring factor in promoting oxidative stress (Vecchio et al. 2012). On the condition of introducing 10 and 100 mg/mL of indefinite silica nanoparticles to *D. melanogaster*, causing an increase in oxidative stress depends on time and concentration.

10.12.1.2 Cytotoxicity

Due to the negative effects of NPs, the sustainability of cells is driven by the state and naked surface of cell membrane. In case of metallic nanoparticles such as NPs, the cells in copper oxide provided 60% unfeasibility in them. An electrostatic interest attracted the positive charged metallic ions toward the cell membrane. It coats the membrane and inhibits the ability of producing basic needs such as fuels and wastes (Seabra and Durán 2015). The toxic effects on specific cells damage their mitochondria and promote oxidative stress resulting in cell death (apoptosis).

10.12.1.3 Genotoxicity

Metallic oxide nanoparticles such as copper oxide, uraninite, and cobalt oxide exhibit genetic effects. This effect on DNA causes genetic disorders that can be detected in future generations, e.g., cancer. Nowadays, it is an important study in scientific investigation. Since *Drosophila* is an excellent model organism in genetics, a well-known genome sequence, and has identical traits with human, it is used to study the interaction between nanoparticles and the genome of specific organism. The introduction of 15 nm sodium citrate-capped AuNPs to *Drosophila* affects the DNA fragments present in gastrointestinal tissue. Chronic genotoxicity can occur in future generations due to genetic disorders in germline cells by the AuNPs. As the

small particles can influx easily into the organisms, the larger particles, 40 and 80 nm of AuNPs, are less genotoxic than small particles having 5 and 15 nm size (Vecchio et al. 2012). AgNPs and CdSe-ZnS QDs are also responsible for genotoxicity. The genotoxicity can be estimated by SMART (somatic mutation and recombination test). The characterization of nanoparticles defines the reproducibility of toxicology and the mode of action of toxicity of nanoparticles (Powers et al. 2006). The properties of nanoparticles such as size distribution and agglomeration state vary on the components of toxicological studies. Among the common toxic researches in nanotoxicology, the probable toxin characteristic is challenging, but their biological methods are not yet well known. In microscopy methods, only the electron microscopy and atomic force microscopy allow to examine the nanoparticles. But, basically a specific characterization of physical (shape description, size, total quantity, vectors that are attached) and chemical components is needed to study the nanotoxicology. And this characterization should be examined on biological moist environment before its exposure to living organism (Powers et al. 2006).

10.12.2 Factors Affecting Toxicity

The physical and chemical factors affect toxicity. The size of the nanoparticles mainly defines the level of toxicity. But besides this, chemical composition, shape, surface structure, surface charge, aggregation, and solubility are also responsible for the toxic effect (Nel et al. 2006). The functional groups explaining the chemical reactions of molecules also affect toxicity. If the level of toxicity increases, then the exposure of this nanoparticle may be harmful for mankind.

10.12.2.1 Composition

Metal-Based NPs

Metal-based NPs means basically synthesized NPs, having the functions as semiconductors, thermoelectric materials, used in drug delivery mechanism. Various studies have been done on NPs, such as their small exterior region with comparison to their volume, concluding an adverse effect on biological environment (Schrand et al. 2010). But still, trials are in progress to find out such nanoparticles having toxic effects that causes genetic disorders, evade cell sustainability, necrosis, etc. after their exposure.

Carbon-Based NPs

In 2013, nanotoxicology studies were done with the carbon nanotubes, resulting in minor lung trouble. But it was observed that the introduction of nanoparticles is needed for a long time to conclude results according to pathology. But some experiments of fullerenes proved C60 as a non-toxic carbon-based nanoparticles.

Size

Size also defers toxicity. The large-sized nanoparticles can be less toxic of their small particles. The small particles can influx independently everywhere in an organism causing more toxicity.

Dispersion State

The nanoparticles can agglomerate or aggregate in environmental or biological fluid. Agglomeration and aggregation denote loosely and tightly bounded particles, respectively. Due to the excessive environmental ionic stability, the nanoparticles get agglomerated. It protects the counter-attractions, occurring due to nanoparticle alterations. But many nanoparticles may get agglomerated in the environment or on the host body, so it is necessary to study if agglomeration affects toxicity or not.

10.13 Conclusion

In *D. melanogaster*, while other nanoparticles display scanty effective reaction, AuNPs show an induced toxicity by decreasing fertility and life span. It was manifested by the presence of DNA fragmentation in the gastrointestinal tissue. AuNP on the basis of its size influences the surface chemistry. It also effects the localization, intracellular fate, and toxicological pathways in vivo. So, nano-safety is strongly required which is being increasingly exploited in commercialization and novel application. AgNPs are also being obtained from bio-reduction of silver nitrate. They are removed by using olive, fig, and mulberry leaf extracts. Silver nanoparticles have been characterized for using UV rays, FT-IR, and SEM analysis, which shows better stabilization (Armstrong et al. 2013). In *D. melanogaster*, the larvae, pupae, and adult mortality gets reduced by olive, mulberry, and fig AgNPs. A more detailed research in the need of the hour regarding studies on translocation and uptake at cellular and molecular level.

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