Nanoparticle mediated ROS as the key contributor for the toxicity observed in *Drosophila melanogaster*

Monalisa Mishra*

Neural Developmental Biology Lab, Department of Life Science, National Institute of Technology, Rourkela, Odisha, India,769008

*Corresponding author- mishramo@nitrkl.ac.in,

Abstract

Nanoparticles (NPs) have revolutionized the society being a part of numerous products of day to day uses. However, its potential to act as an adverse entity was greatly ignored. Recently, the toxic effect of nanoparticles on biological system is checked using various model organisms. Among various models fruit fly *Drosophila melanogaster* has turned up as a promising model since numerous diseased genes and signalling pathways are similar to that of a human being. Effects of many NPs were tested using *Drosophila* and results suggest the deleterious effect of NPs on the various physiological system. NPs cause defects in genetic, molecular, phenotypic, developmental as well as behavioral level in *Drosophila*. Any chemical compounds that can cause abnormalities in offspring when ingested in prenatal stages are known as teratogens. In this review we have summerised the toxic effect generated by NPs tested using *Drosophila* model and propose NPs as a teratogen.

Keywords: Nanoparticles; Teratogens; Toxicity; Behavioral teratogenesis; *Drosophila*

Introduction

Nanoparticles (NPs) are engineered materials having one or multiple components and its size varies from 1-100 nanometer (nm)(Savolainen *et al.*, 2010). NPs include various carbon compounds, nanocrystals, metal oxide, quantum dots, silica and transition metals (Murray *et al.*, 2000, Dreher, 2004). The large surface area and high reactivity offer a characteristic physical, chemical and biological properties (Dobrovolskaia and McNeil, 2007, Hirano, 2009) to the NPs used in various technological, medicinal and industrial applications.

In the meantime, due to its physicochemical properties (surface chemistry, size, charge, shape, dispersion status etc.) various reports suggests that NPs may interact with living organisms including human beings (Singh *et al.*, 2009b, Panacek *et al.*, 2011). A study from various laboratory suggest the cytotoxic and genotoxic effects of NPs (Stone and Donaldson, 2006, AshaRani *et al.*, 2008, Mortensen *et al.*, 2008, Poland *et al.*, 2008, Napierska *et al.*, 2009, Singh *et al.*, 2009b). Thus the toxic effect of NPs on biological system (Aillon *et al.*, 2009, Zhao and Castranova, 2011, Chatterjee *et al.*, 2014b, Hawkins *et al.*, 2015, Kermanizadeh *et al.*, 2016) has introduced "nanotoxicology" a new field to decipher the mode of action of NPs (Liu *et al.*, 2009a). A range of *in vitro,* cell culture study and many model organisms are used to understand the toxicity of NPs.

Various model organisms to decipher the mode of nanotoxicity

Various studies used cell cultures to understand the underlying mechanisms of nanotoxicity (Lin *et al.*, 2006, Long *et al.*, 2006, Ahamed *et al.*, 2008, AshaRani *et al.*, 2008, Napierska *et al.*, 2009, Asakura *et al.*, 2010, Hackenberg *et al.*, 2011, Bondarenko *et al.*, 2013, de Melo Reis *et al.*, 2015). However, a cell culture model cannot mimic the effect of NPs on an organism with respect to bio distribution, accumulation, metabolism, persistence and its elimination. Thus the accuracy of effect in the living organism is compromised (Hu *et*

al., 2009). This limitation is overcome by various *in vivo* models which allow us to know the internalization, cellular uptake and tissue distribution of nanoparticle in the living being (Yadav *et al.*, 2010, Yadav *et al.*, 2011). Among various models mice, round worm (*Caenorhabditis elegans*), zebra fish (*Danio rerio*), *Daphnia magna, Rhinella arenarum, Xenopus laevis, Hydra and Drosophila melanogaster* are widely used to study the toxicity of NPs (Wang *et al.*, 2008, Contreras *et al.*, 2012, Song *et al.*, 2012, Hunt *et al.*, 2013, Völker *et al.*, 2013, Chatterjee *et al.*, 2014a, Chatterjee *et al.*, 2014b, He *et al.*, 2014, Meyer and Williams, 2014, Santo *et al.*, 2014, Dedeh *et al.*, 2015, Ibarra *et al.*, 2016, Murugadas *et al.*, 2016, Webster *et al.*, 2016, Barbero and Yslas, 2017, Colombo *et al.*, 2017, Coll *et al.*, 2018, Lajmanovich *et al.*, 2018). Toxicity study from various models proposes NP can alter the developmental and behavioural process in the offspring thus acts as a teratogen. All these models are used for teratogenic testing predominantly (Coyle *et al.*, 1976, Bailey *et al.*, 2013, Almeida *et al.*, 2016). Primitive animal like *Dictyostelium discoideum* is also used for tertogenicity testing (Maeda, 1970).

Among all the model organisms *Drosophila melanogaster*, is proved as a robust model to study the toxicity of various NPs (Leeuw *et al.*, 2007, Liu *et al.*, 2009a, Posgai *et al.*, 2009, Ahamed *et al.*, 2010a). Its fast reproduction, short life cycle, fully sequenced genome along with genetic and immunological similarities with vertebrates (Medzhitov *et al.*, 1997, Affleck and Walker, 2008) has established it as a model in the field of nanotoxicology. Furthermore, many fundamental biological mechanisms, molecular pathways, genes and transcription factors essential for development are conserved between *Drosophila* and mammals (Pandey and Nichols, 2011a, Wang *et al.*, 2012, Campos-Ortega and Hartenstein, 2013). More importantly, there exists 75% homology with disease related genes of humans. This includes similarity with more than 700 different diseases at the genetic level (Reiter *et al.*, 2001, Khurana *et al.*, 2006, Koh *et al.*, 2006, Wolf *et al.*, 2006).

Various studies of NPs using *Drosophila* suggest that NP causes detrimental effect on development at cellular, molecular, genetic and behavioural level. Exposure of NP at embryonic level alters the developmental process and brings out an alteration in adult for a successive generation without causing any lethality (Posgai *et al.*, 2011, Vecchio *et al.*, 2012, Anand *et al.*, 2017). Can we include NP as a teratogen? The result of NPs from various studies suggests that NPs act as a teratogen. If we look at the explanation of teratogens then these are any external factors (chemical, physical), which hamper the developmental process if ingested during the prenatal development period and the effects are sustained throughout the life of the organism (Coyle *et al.*, 1976). Anomalies caused by teratogens can be anatomical, morphological, physiological or behavioural (Coyle *et al.*, 1976) which are combined effect of molecular, cellular and biochemical alterations (Wilson, 1968). The whole process of alteration of the developmental process and bringing about modifications is known as teratogenesis. In this review we are summarizing all the studies which use *D. melanogaster* as a model organism where NP effect acts as a potential teratogen.

Mode of administration of nanoparticles in *Drosophila*

Route of administration is an important factor to determine any kind of toxicity (Williams *et al.*, 1982, Grassian *et al.*, 2007). Three major modes of exposure of NPs is used to expose *Drosophila* towards NPs. Various routes include (1) inhalation through respiratory tract, (2) epidermal absorption and (3) ingestion via food material (Key *et al.*, 2011).

Only handfuls of literature are available which exposes NP through the respiratory route. NPs present in the environment may enter into the human body via the respiratory tract. *Drosophila* respiratory organ, the trachea is formed by fusion of 10,000 tubules which are interconnected with each other (Gervais *et al.*, 2012). The genes involved in the trachea development share similarity with vertebrates (Horowitz and Simons, 2008). Furthermore, unlike mammalian trachea *Drosophila* trachea is formed of only single type of epithelial cells

and thus offer the system acts like a cell culture system within *Drosophila* (Horowitz and Simons, 2008). Further the structure of the trachea allows it to be used for various respiratory related issues of vertebrates (Pandey and Nichols, 2011b). To mimic the effect of NPs internalized via respiratory system, Posgai et al., developed a method to study the effect of NPs via respiratory tract of *Drosophila* (Posgai *et al.*, 2009). They exposed fluospheres, red fluorescent CdSe/ZnS nanoparticles and silver nanoparticles into tracheal-specific (*breathless*) GFP reporter strain of *Drosophila*. This method is known as a nebulizer nanoparticle delivery system which later changed to direct microtransfer method. (Vega-Alvarez *et al.*, 2014). This method ensures the uniform release of NP to the specific target without any tissue damage. This further allows determining the minimum toxic dose in a large number of replicates with less risk of false positive results.

However, among all the methods oral administration of NPs is extensively used to determine nanoparticle toxicity. There are several reasons for choosing the oral route for NP intake. (1) Various food and medicine contains NPs thus there is a high chance that NPs can enter into the body via oral route. (2) *Drosophila* gastrointestinal tract shares a homology with humans in many respects. Like vertebrate *Drosophila* digestive tract has various pH in different parts of the digestive system. It also possesses peritrophic membrane (PM), an analogous structure of mammalian digestive tract which helps to protect the midgut epithelium from foreign particles and microbes (Tellam, 1996, Hayakawa *et al.*, 2004, Hegedus *et al.*, 2009) (Fig.1B). The charge of the NP has a role in determining the toxicity of NP since the NP's charge may change the pH of the gut and thus its functionality (Sabat *et al.*, 2016). Positively charged NPs uptake at posterior midgut of *Drosophila* causes a lumen-negative transepithelial potential of 35-45mV (Shanbhag and Tripathi, 2009, Chen *et al.*, 2015). Various modifications of NPs allow their intake in an easier way in gut which is now days being used for effective drug delivery purposes. Jiang *et al*. demonstrated how surface

coating of Poly D,L-lactic-co-glycolic acid (PLGA) can enhance cellular uptake of NPs by increasing the retention period of NPs in posterior midgut of *Drosophila* larva (Jiang *et al.*, 2015). As chitosan is positively charged, it allowed electrostatic interaction with the alkaline posterior part of midgut and subsequently uptake of those nanoparticles occurred. This shows a relation between various modifications of NPs and their toxicity in *Drosophila* model. However, the paper is silent about the teratogenic effect of the concerned NP. Copper oxide nanoparticles (CuO NPs) when taken orally downregulate genes essential for proper functioning of intestinal barrier(Alaraby *et al.*, 2016). CuO NPs accumulate within the gut cells and downregulate genes like *Dual oxidase (Duox), Hemolectin (Hml), Prophenoloxidase 2 (PPO2)* and *Unpaired 3 (Upd3).* Alongwith that various stress genes *Hsp70, Sod2, Cat, p53* (Alaraby *et al.*, 2016) also found to be downregulated. Accumulation of CuO NPs affect translocation through intestinal barrier to haemolymph in *Drosophila* larvae. The mechanism of toxicity as well as teratogenicity of NPs taken via oral route is associated with the mode of absorption. In case of vertebrates, three different pathways are reported for the absorption of various sized NPs in gut (Fig.1A). They include (1) paracellular (2) transenterocytic and (3) M-cell mediated pathway (Yu *et al.*, 2016). Paracellular pathway can merely contribute to NP transport as the intercellular space is very less and restricted by tight junctions. Transenterocytic pathway has been found to be most common pathway of NPs due to the involvement of enterocytes which are abundant in gut lining. These cells are generally responsible for transport of small sized NPs (50-100 nm) compared to large ones (500 nm, 1 μm, 10 μm)(Desai *et al.*, 1996). M cell mediated pathway is responsible for transport of large sized NPs (Desai *et al.*, 1996). Various polymeric NPs having polystyrene, found to be transported via M cells (Eldridge *et al.*, 1990). On the contrary, in spite of large numbers similarity with vertebrates gut, *Drosophila* gut has some structural differences (Fig.1B). M cell is found to be absent in *Drosophila* gut. In the absence of M cells how large

sized NPs are being absorbed in gut opens a new challenge (Lucchetta and Ohlstein, 2012, Buchon *et al.*, 2014). Large sized NPs are being transported through enterocytes to haemolymph, homologue of human blood (Ahamed *et al.*, 2010a, Alaraby *et al.*, 2015a). Endocytosis and direct penetration through enterocytes is found to be one of the mechanism for NP transport in *Drosophila* (Pandey *et al.*, 2013). However, the mechanism of NP transport needs further investigation to have the complete picture.

Drosophila developmental process includes egg, larvae, pupae and adult stage.The egg stage lasts for one day. Next it hatched to 1^{st} inster larvae. The 1st inster larvae stay at that stage for two days and then changed to 2^{nd} inster larvae. The inster larvae after two days transformed to 3^{rd} inster larvae. The 3^{rd} inster larvae after two days changed to pupae stage. The pupae stage last for 3 days and after pupation the adult fly hatched. The is the normal life cycle of the *Drosophila* at 25^{O}C(Fig.2). However, various environmental and physiological factor factors can alter the life cycle of *Drosophila*. The effect of any factor can be assessed in all the four developmental stages (Hsu and Schulz, 2000). Whichever defect in any of the stage may lead to an abnormal adult. The larvae are a voracious feeder which consumes the maximum amount of food in their feeding phase for energy requirement during pupation. During this period their mouth opening ranges from ~50 to~200 μm (Liu *et al.*, 2009a) which is large enough to uptake nano sized materials. Thus the oral intake of NP on physiology and development can be studied at the larval stage. Since larvae are transparent the fate of nanoparticles can be studied by feeding the larvae with fluorescent tagged NPs. The larval walking behaviour acts as a ruler to determine the toxicity assessment of NPs (Sabat *et al.*, 2016, Mishra *et al.*, 2017, Pappus *et al.*, 2017). A defective larvae crawling indicates the defect in mechanosensory neurons after oral intake of NPs. Larve undergoes pupae where the metamorphosis occurs and the adult hatches. Various NPs often interfere with the metamorphosis and resulted an abnormal developmental cycle in *Drosophila*

(Lozinsky *et al.*, 2012, Chen *et al.*, 2015, Pappus and Mishra, 2018). A delay in larval and pupal developmental time point often resulted in defective generation of the fly.

How nanoparticles cause toxicity in *Drosophila*?

D. melanogaster was employed for the first time by Strawn et al to study the effect of NPs (Strawn *et al.*, 2006). Later, various studies used *Drosophila* to detect genotoxicity and mutagenicity of several metal and metal-oxide NPs (Demir *et al.*, 2011, Sabella *et al.*, 2011, Vecchio *et al.*, 2012, Demir *et al.*, 2013, Vales *et al.*, 2013, Alaraby *et al.*, 2015c, Carmona *et al.*, 2015a). NPs can interact with DNA to bring about harmful effects linked to cancer, infertility and genetic disorders over successive generations when germinal cells are affected (Singh *et al.*, 2009b). As mentioned earlier increased surface area of NPs help to penetrate into the skin more easily (Schneider *et al.*, 2009, Bolzinger *et al.*, 2011). NPs can cross cell membrane (Vasir and Labhasetwar, 2008), blood brain barrier (Geldenhuys *et al.*, 2011), bone marrow(Jing *et al.*, 2008), lymph nodes (Bhang *et al.*, 2009), heart (Stampfl *et al.*, 2011), lungs(Evans *et al.*, 2006) and central nervous system (Klyachko *et al.*, 2012). In pregnant mice NPs cross the placenta barrier and causes toxicity in the embryo (Saunders, 2009, Bai *et al.*, 2010, Chu *et al.*, 2010, Yamashita *et al.*, 2011). NPs, due to its small size, enter the nucleus through nuclear pore and interfere in the process of mitosis by disturbing DNA organized in chromatin or chromosome thereby causing genetic damage (Cheng *et al.*, 2013, Magdolenova *et al.*, 2014). Further NPs, attach to the surface of various macromolecules like lipids and interrupt the normal functioning of ion channels. Membrane channels are important for signal transduction, trans epithelial transportation, regulating ion concentration of cytoplasm as well as a vesicle, maintaining pH, and regulating the volume of the cell (Hübner and Jentsch, 2002). Altered function of ion channels such as Ca^{2+}, Na^{+}, K^{+}, Cl^{-} induce toxic effects along with endocytosis (Haney *et al.*, 2011, Shan *et al.*, 2011).

For existence or smooth conduction of cellular processes homeostasis is a required phenomenon which is often disturbed by stress. NPs can act as a stressor *in vitro* as well as *in vivo* models and alter the homeostasis. This is the most possible mechanism of NP toxicity (Green and Howman, 2005, Nel *et al.*, 2006, Monteiller *et al.*, 2007, Mocan *et al.*, 2010). Oxidative stress is mediated by reactive oxygen species(ROS). During oxidative phosphorylation in normal condition some electrons can skip the respiratory chain and bind to molecular oxygen forming superoxide ion radical (O_2^-), H_2O_2 (hydrogen peroxide) and subsequently hydroxyl radical (OH^-) (Boonstra and Post, 2004, AshaRani *et al.*, 2008). These free radicals (ROS) have a very high oxidizing power. Like toxic materials NPs causes ROS overproduction by losing or by accepting an electron from the respiratory chain and releasing it as molecular oxygen exclusive of disturbing the respiratory chain (Turrens, 2003). This process affects the ATP synthesis of mitochondria (You *et al.*, 2012). ROS produced by NPs causes' oxidative damage to DNA. ROS interacts with low molecular weight lipid and causes lipid peroxidation (Halliwell and Chirico, 1993, Ahamed *et al.*, 2010b). DNA damage causes chromosomal rearrangements like breaks of double-strand and dilapidation of strand breaks (AshaRani *et al.*, 2008). ROS over production further affects cascades like signal transduction, ubiquitination of protein, degradation of protein, upset of the cytoskeleton, protein damage, lipid peroxidation and apoptosis (Ryter *et al.*, 2007, Ahamed *et al.*, 2008, Li and Osborne, 2008, Posgai *et al.*, 2011, You *et al.*, 2012). ROS production for long period has a negative impact on development, viability alongwith various physiological systems defect which includes a reproductive system, circulatory system and immune system (Posgai *et al.*, 2011). Oxidative stress is often defended by the production of some antioxidant enzymes like SOD, catalase, peroxidase (Kohen and Nyska, 2002) which have the ability to convert reactive oxygen to less toxic H_2O_2 and successively to H_2O (Alaraby *et al.*, 2015a). Excessive production of ROS further suppresses the antioxidant activity of these enzymes.

Stress at cellular level resulted an enhanced expression level of heat shock proteins(HSP), activates the JNK pathway, and induces autophagy in *Drosophila* (Stronach and Perrimon, 1999, Scott *et al.*, 2004, Simonsen *et al.*, 2007, Singh *et al.*, 2009a). Janus Kinase (JNK) protein has been found to enhance tolerance to oxidative damage and longevity in both *Drosophila* and *C. elegans* model system (Wang *et al.*, 2003, Oh *et al.*, 2005). HSP or stress proteins are synthesized when induced by foreign inducers and their conservative property with ability to induced by a large number of environmental inducers make it a suitable molecule for tracing the effect of stressors (Lindquist, 1986, Aıt-Aıssa *et al.*, 2000, Mukhopadhyay *et al.*, 2003, Siddique *et al.*, 2008). Hsp70 is used as a biomarker in most of the NP toxicity studies (Bierkens, 2000). Heat and oxidative stress during *Drosophil*a development hamper synthesis of biogenic amines and hormones like dopamine, octopamine, juvenile hormone, ecdysteroids; which has a major role in reproduction (Rauschenbach *et al.*, 1995, Gruntenko *et al.*, 2003, Gruntenko *et al.*, 2005, Neckameyer and Weinstein, 2005, Gruntenko *et al.*, 2007, Rauschenbach *et al.*, 2007, Gruntenko and Rauschenbach, 2008, Bogomolova *et al.*, 2009, Gruntenko *et al.*, 2009, Panacek *et al.*, 2011). Dopamine and octopamine regulate gonadotropin secretion and also responsible for melanisation of cuticle (Walter *et al.*, 1996). Similarly, alteration in juvenile hormone (decrease in its degradation) and 20-hydroxyecdysone or 20HE (increased level due to change in ecdysteroid system) decreases the fertility in *Drosophila melanogaster* (Rauschenbach *et al.*, 1996, Hirashima *et al.*, 2000). Heat stress can also bring about oocyte maturation delay, delay in vitelline membrane degradation, inhibit genes expression level of follicle cells responsible for the formation of yolk protein, and increase in a number of mature oocytes (Gruntenko *et al.*, 2003). Various mechanisms through which NP toxicity can take place are summerised in figure (Fig . 3).

Nanoparticles as a behavioural teratogen

NPs alter the activity of nervous system a key regulator of an animal's behaviour. The behaviour of an organism connote the changes that it undergoes through different physiological processes either via external (environmental) or internal factors resulting change at the cellular or genetic level (Jaenike, 1982, Sokolowski, 2001). A defective physiological, metabolic, hormonal and neurological activity may alter the behavioural pattern of the animal (Gerdes *et al.*, 1999, Smoller *et al.*, 2005). Thus with the onset of any disease, changes may occur at the genetic, cellular morphology, molecular, proteome level, and finally alter the behaviour of the animal. Various methods to detect the behavioural defects of *Drosophila* were recently summerised (Mishra and Barik, 2018). NPs can enter into the neurons through ionic channels like Na^{+}, K^{+}, Ca^{2+}, Cl^{-} (Banerjee and Nimigean, 2011, Morgen *et al.*, 2011) via trans synaptic transportation (Praetorius *et al.*, 2007). NPs affect nerve growth factor, blood-brain barrier (BBB) and the voltage-gated channel of the hippocampus and thus cause neurodegeneration (Pisanic *et al.*, 2007, Tang *et al.*, 2008, Liu *et al.*, 2009b). NPs like silica, manganese, zinc oxide and ferrous can cause depletion of neurotransmitter like dopamine, aggravate heat stress, degenerate BBB, and lead to edema formation (Liu *et al.*, 2009b). Similarly, somatosensory neurons are affected upon exposure of copper NPs in the rat (Prabhu *et al.*, 2010). Various NPs is shown to affect larval mechanosensory neurons, cholinergic neurons and sub oesophageal ganglion (Sabat *et al.*, 2016, Mishra *et al.*, 2017, Pappus *et al.*, 2017). Aluminium NPs can alter the local interneuron activity of antennal lobe (Huang *et al.*, 2013), an important chordotonal organ analogous to olfactory bulbs of vertebrates which receives olfactory information from olfactory sensory neurons of the antenna (Silbering *et al.*, 2008). However, many of above alterations are yet to be explored in *Drosophila*.

Nanoparticle toxicity assessed using *Drosophila* model

In this section we have taken all the nanoparticles that uses Drosophila to check the toxic effect.

Silver nanoparticles (Ag NPs)

Silver NP (Ag NP) increases stress level, damage of DNA, apoptosis, and altered HSP after exposure to *Drosophila* (Ahamed *et al.*, 2010a, Key *et al.*, 2011, Pappus *et al.*, 2017). Larval ingestion of Ag NP increases the amount of malondialdehyde (MDA) and stress enzymes like catalase, superoxide dismutase (SOD), gluta thione, caspase-3 and caspase-9 within the hemolymph (Ahamed *et al.*, 2010a, Posgai *et al.*, 2011). Higher activity of p53 and p38 along with caspase-3 and caspase-9 were also observed (Ahamed *et al.*, 2010a). Ag NP resulted in spotty wings (Demir *et al.*, 2011). in *Drosophila.*

Ag NP uptake resulted in developmental delay and decreases developmental success (Ahamed *et al.*, 2010a, Gorth *et al.*, 2011, Philbrook *et al.*, 2011b). Ag NP exposure at larval stage reduces mating success (Posgai *et al.*, 2011). Flies hatched after Ag NP treatment has decreased body proportion (Panacek *et al.*, 2011). AgNP distress larva to pupa, pupa to adult transitions, and longitivity of flies in F1 generation (Key *et al.*, 2011). Ag NP exposure for 10 to 30 days in adult flies resulted in decreased egg laying capacity and retarded growth of ovary with very fewer numbers of ovarioles (Raj *et al.*, 2017). Flies exposed after AgNP treatment (F1 generation) have a transgenerational effect suggesting Ag NP acts as a teratogen. Phenotypic defect including depigmentation, and soft cuticle were also reported in the offspring(Gorth *et al.*, 2011, Key *et al.*, 2011, Posgai *et al.*, 2011). A similar phenotypic defect is seen in humans after silver exposure resulting an irreversible greyish discolouration of the skin called argyria (Chen and Schluesener, 2008, Kwon *et al.*, 2009).. Uptake of aerosolized coated and uncoated Ag NPs through respiratory system resulted in an increased Hsp70 expression (Posgai *et al.*, 2009). AgNPaltered copper homeostasis in adult *Drosophila* and affects the female fertility (Armstrong *et al.*, 2013). Cuticle depigmentation further alters

dopamine pathway due to heat and oxidative stress generated by Ag NP exposure (Key *et al.*, 2011, Panacek *et al.*, 2011). Silver NPs prepared from plants had a negative effect on larvae hatching and reduced larval longevity (Araj *et al.*, 2015). Ag NP alters the gut microbiota of *Drosophila* (Han *et al.*, 2014), a key player for regulating growth and mating behaviour (Charroux and Royet, 2012).

Gold nanoparticles (AuNPs)

AuNP is highly biocompatible thus can pass the cell membrane easily (Rosi and Mirkin, 2005, Rosi *et al.*, 2006, Dhar *et al.*, 2010). This property increases the wide use of AuNP in the field of medicine for drug delivery (Dykman and Khlebtsov, 2011). Besides its wide use *Drosophila* studies have shown DNA fragmentation, oxidative stress (detected by over expression of stress proteins) and a sign of apoptosis (p53 overexpression) after AuNP treatment (Pompa *et al.*, 2011, Vecchio *et al.*, 2012). AuNP affects the longevity and fertility of *Drosophila*. Signaling pathway like PI3K/Akt/mTOR altered after AuNP exposure as the regulation of energy and metabolism get affected (Wang *et al.*, 2012). In addition, phenotypic alterations like extreme eye deformities, damaged wing and thorax were found to be defective (Vecchio *et al.*, 2012).

Titanium oxide nanoparticles (TiO_2NPs)

Toxicity of TiO_2NP depends on grade (Weir *et al.*, 2012). In *Drosophila* oral intake of TiO_2NP causes cytotoxicity and DNA damage of mid-gut cells and imaginal disc (Patel and Champavat, 2014, Sabat *et al.*, 2016). The toxicity is caused due to overexpression of stress genes like catalase, glutathione and superoxide dismutase (Posgai *et al.*, 2011, Jovanović *et al.*, 2016). TiO_2NP affects the developmental stages and thus causes developmental delay in a dose dependent manner (Sabat *et al.*, 2016). This defect is further associated with alterations of oxidative stress due to increased ROS production (Lozinsky *et al.*, 2013, Shrivastava *et al.*,

2014). TiO_2NP fed larvae shows defective crawling behaviour suggesting impairment of nervous system (Sabat *et al.*, 2016). Reduced body weight, defective climbing behaviour, wing venation and bristle defects were reoprted after exposure of TiO_2 NP(Sabat *et al.*, 2016). Signalling pathways like notch, hedgehog and Bone Morphogenic Protein (BMP) regulate wing development in *Drosophila* (Vervoort *et al.*, 1999, de Celis, 2003). The homolog of these proteins is present in the vertebrate system. A defective wing further indicates the malformed functioning of signalling pathways. Fecundity rate is also affected by dietary intake of TiO_2 NP in *Drosophila* (Philbrook *et al.*, 2011b).

Magnetite nanoparticles (Fe_3O_4 NPs)

The toxic effect of magnetite NPs on *Drosophila* was studied by Chen *et al*. (Chen *et al.*, 2015). Fecundity was found to be affected by the exposure of magnetite NPs. Magnetite NPs disturb homeostasis of vital metals like Fe, Ca, Cu which are essential for development (Kambe *et al.*, 2008, Chen *et al.*, 2015). Disturbance of Ca level is responsible for defective embryogenesis whereas altered Fe level hampers the embryogenesis, larval growth and metamorphosis (Homa *et al.*, 1993, Mandilaras *et al.*, 2013, Uhrigshardt *et al.*, 2013). Likewise, Cu imbalance causes the decreased growth of embryo, foetus and adult (Turski and Thiele, 2007).

Zirconia nanoparticles (ZrO_2 NPs)

Exposure of zirconia NP causes numerous alterations in the development of both larva and adult *Drosophila* (Mishra *et al.*, 2017). It induces oxidative stress by producing ROS which in turn affect the structure of the larval gut as evidenced by DAPI staining. Malformed larva crawling behaviour is observed due to zirconia NP toxicity. Reduced pupal count and overall developmental delay have been observed. In treated adult flies, significantly decreased body weight, altered climbing behaviour and various phenotypic defects were

found. The phenotypic defects include defective eye (fused, misoriented, blistered ommatidia), bristle loss/ altered phenotype, defective abdomen development along with scattered black spots and segmental decolouration. Further wing venation pattern and trichome arrangement of the wing were severely affected. All these phenotypic transformations all together affect the behaviour as well as survivorship of the fly.

Hydroxyapatite nanoparticles (HApNPs)

Similar to zirconia, HApNPs affect larval neural activity (damage to sub oesophageal ganglion, mechanosensory neuron and brain) detected by defective larva crawling behaviour (Pappus *et al.*, 2017). Further oxidative damage by increased ROS production and nuclear damage to larval gut proves ROS production as a most common mode of NP toxicity. Developmental delay, reduced pupa percentage, defective wing (incomplete venation), bristle phenotype (lost or broken), eye deformities (rough eye with blisters) were the result of HAp NP toxicity at phenotypic level in *Drosophila*. Altered climbing behaviour was also seen. Bristles are involved in mechanosensation found to be affected after HapNP treatment.. Wing venation is found to be affected. Abnormality in wing hairs were observed due to defect in planner cell polarity, and the actin amount available in the hair cell (Fristrom *et al.*, 1993, Eaton *et al.*, 1996, Ren *et al.*, 2006). Decreased phosphorus and calcium level were also found within the gut after HApNP treatment (Pappus *et al.*, 2017).

Copper oxide and Copper nanoparticles (CuO and Cu NPs)

Cu NPs having myriads of usage (Hajipour *et al.*, 2012, Bondarenko *et al.*, 2013) can induce toxicity in *Drosophila* (Carmona *et al.*, 2015b). CuO NPs proved to be genotoxic to *Drosophila* as it causes DNA damage to larval haemocyte. CuO NPs causes mitotic recombination a potential mechanism to cause mutation. Malondialdehyde, a marker for oxidative stress was found to be increased after CuO NP exposure. The oxidative stress can

bring about genotoxicity in *Drosophila*. CuO NP further cause's developmental delay, which is associated either with genotoxicity or oxidative stress. Han et al., also reported that Cu NPs causes developmental delay, reduced adult longevity and sperm competition in *Drosophila* (Han *et al.*, 2014). CuO NPs resulted reduced larval growth, defective metamorphosis and delayed pupa to adult stage (Alaraby *et al.*, 2016). The toxicity is due to the copper ions released from the CuONPs. Genetic markers like *Dual oxidase (Duox), Hemolectin (Hml), Prophenoloxidase 2 (PPO2)* and *Unpaired 3 (Upd3)* in gut cells are downregulated due to effect of CuONP. Accumulation of CuO NPs in the gut lumen, gut cells and haemocytes (after translocation) decreased the microbiota population within the gut.

Silica nanoparticles (SiO_2 NPs)

Effect of silica NP using *Drosophila* model has been reported from various studies (Barandeh *et al.*, 2012, Pandey *et al.*, 2013, Demir *et al.*, 2015). Ingestion of nano sized synthetic amorphous silica (SAS) during larval stage affected the gut to a larger extent (Pandey *et al.*, 2013). Adverse effects include membrane destabilization, membrane potential loss of mitochondria, increased oxidative and apoptotic activity of gut cells. Nano SAS produced DNA damage due to oxidative stress in midgut cells and haemocytes by internalization assessed by comet assay (Demir *et al.*, 2015).

***Carbon nanomaterials* (*nanotubes and graphene*)**

Numerous attempts have been made to assess the toxicity level of various carbon nanotubes in *Drosophila* (Evans *et al.*, 2006, Leeuw *et al.*, 2007, Liu *et al.*, 2009a, Philbrook *et al.*, 2011a, Machado *et al.*, 2013, de Andrade *et al.*, 2014, Liu *et al.*, 2014, Vega-Alvarez *et al.*, 2014). All the studies suggest deposition of various size and shaped carbon nanotubes in different tissues without altering the development. Adult exposure of two types of carbon nanomaterials (carbon black and single walled nanotubes) initiated unnatural grooming

behaviour and affected the climbing behaviour (Liu *et al.*, 2009a). This behavioural defect is due to adherence of carbon nanotubes to sticky pads present in the foot (Liu *et al.*, 2009a). Again the physical mechanism of nanotoxicity suggests that carbon nanotubes can limit the oxygen diffusion and metabolism by partially blocking the spiracles, openings which regulate respiratory gas influx with respect to metabolism (Lehmann, 2001, Heymann and Lehmann, 2006, Liu *et al.*, 2009a). Due to increased grooming behaviour the carbon nanotubes can be transported into the body (Leeuw *et al.*, 2007). However, microtransfer of carbon nanotubes to *Drosophila* embryo induced mortality (Vega-Alvarez *et al.*, 2014). Similarly, various nanocomposites of graphene have a toxic effect on *Drosophila*. Larval exposure to graphene and Zn nanocomposite increased oxidative stress, apoptosis and DNA damage in a dose and time dependant manner (Siddique *et al.*, 2014). Further, it reduced the total protein content of *Drosophila*. Another nanocomposite of graphene and copper induces *hsp70* expression, oxidative stress, apoptosis, DNA damage along with altered total protein content and β-galactosidase activity (Siddique *et al.*, 2013). All these results provokes the need of a more systematic study to assess the carbon nanomaterial toxicity although it was one of the early NP to be used for toxicity assessment.

Zinc oxide nanoparticles (ZnO NPs)

ZnO NPs exposure induces stress and apoptotic response in *Drosophila* larva by altering Hsp70 expression, upregulating p53 gene and causing DNA damage in larval haemocytes (de Melo Reis *et al.*, 2015). Recently, ZnO NP toxicity was monitored for four successive generations (Anand *et al.*, 2017). ZnO NP exposure does not affect the F_0 generation flies. ZnO NP induce oxidative stress by significantly increasing the amount of ROS. ZnO NP exposure resulted in an increase in the percentage of DNA damage and apoptotic cells. A phenotypic defect like a single wing, deformed/segmented thorax and without ventral nerve cord were produced over successive generations. All these defects

suggest the mutagenic effect of ZnO NP in chronic treatment. Flies hatched after ZnO exposure has a developmental failure at the pupal stage with no progeny in the successive generation.

Cobalt nanoparticles (Co NPs) and Quantum Dots (QDs)

Exposure of larvae to cobalt NPs (Co NPs) induces somatic recombination in wing imaginal disc. In adult flies abnormal wing spots and bristle defect were observed (Vales *et al.*, 2013). Quantum Dots like CdSe–ZnS QDs, InP/ZnS QDs, CdSe QDs induced stress response by the production of ROS and overexpression of Hsp70 and Hsp83. Further they were genotoxic in nature confirmed by DNA damage. Upregulated p53 expression by QD exposure resulted in an enhanced apoptosis in larvae haemocytes (Galeone *et al.*, 2012, Brunetti *et al.*, 2013, Alaraby *et al.*, 2015b).

Microinjection of nanoparticles

Iron oxide or magnetite (Fe_3O_4), silver(Ag), gold(Au), titanium oxide(TiO_2) nanoparticles and carbon nanotubes (single walled and multi walled) were delivered to stage 15 *Drosophila* embryo by microtransfer method. NPs were delivered to specific tissue to assess target specific toxicity of nanomaterials (Vega-Alvarez *et al.*, 2014). The microtransfer of NPs affects the embryogenesis of *Drosophila* and thus the percentage of viability. Deleterious effects of various NPs with respect to size and physicochemical parameters are summarized in the table (Table 1).

Biomarkers to measure the teratogenic effects in *Drosophila*

Histological and molecular assays

We have summarised various NPs that checked the toxic effect using *Drosophila* as a model system in the above section. All the toxicity result proves that NP acts as a teratogen.

Numerous studies described above; suggests cellular stress as the most acclaimed mechanism for NP toxicity. Cellular stress is caused due to increased production of ROS. The easiest most accepted methods to measure overproduction of ROS are by NBT (nitroblue tetrazolium) reduction assay. The amount of ROS generated by NPs can be measured from haemolymph of *Drosophila* larva (Sabat *et al.*, 2016, Mishra *et al.*, 2017). Also, 6-carboxy-2,7'-dichlorodihydro-fluorescein diacetate (DCHF-DA) assay is used to measure intracellular ROS (Kalyanaraman *et al.*, 2012, Mishra *et al.*, 2017, Pappus *et al.*, 2017). DCHF-DA is cell permeable and is hydrolized to DCHF carboxylate anion. Oxidation of DCHF intracellularly forms a fluorescent product called dicholoroflluorescein (DCF) which can be further observed by techniques like fluorescence microscopy, confocal microscopy and flow cytometry (Kalyanaraman *et al.*, 2012). The increased amount of oxidative stress or cellular toxicity can cause damage to DNA (Love *et al.*, 2012). The amount of DNA damage can be easily accessed by comet assay or single cell gel electrophoresis assay (Love *et al.*, 2012). Comet assay reveals incomplete repair sites, damages like double strand DNA break, single-strand DNA breaks and alkali-labile sites (Tice *et al.*, 2000). However, the accuracy of comet assay can be enhanced by using an enzyme formamidopyrimidine DNA glycosylase (FPG) which can convert the damaged bases to breaks (Azqueta *et al.*, 2013). This technique is useful to detect the DNA damage at non genotoxic concentration where comet assay alone fails. FPG targets DNA damage like oxidized purines (8-oxoguanine) and ring-opened formamidopyrimidine bases (e.g.FapyGua) caused due to oxidative stress (Kain *et al.*, 2012). To check the effect of midgut after oral intake of NPs TUNEL assay was used (Terminal transferase dUTP nick-end-labeling) (Pompa *et al.*, 2011). Often if the damage is more it may affect the single layered gut epithelial cells. To check the arrangement of gut epithelial cells nuclei were stained with DAPI. Nuclei of gut cells were found to be blabbed at higher concentrations. In response to the toxic effect of NPs cells start destroying themselves by the

process of apoptosis or necrosis. Dead cells of *Drosophila* larval gut are stained with trypan blue exclusion test to distinguish the dead cells from living one (Pappus *et al.*, 2017). To check the apoptosis tissues were stained with annexin V/propidium iodide (Vecchio *et al.*, 2012).

Biochemical assay

NPs affect some of the proteins which are expressed under adverse conditions. Thus expression of those proteins or related genes is checked as an outcome of NP toxicity. The activity of antioxidant enzymes like SOD, catalase, glutathione assay is also used as a marker for detection of ROS production (Ahamed *et al.*, 2010a). Lipid peroxidation assay with the help of malondialdehyde (MDA) marker depicts the extent of oxidative damage to cells (Carmona *et al.*, 2015b). Further, upregulated heat shock protein activity mainly hsp70 is used as a marker. Overexpressed apoptotic protein levels like p53, p38, caspase-3, caspase-9 are assessed to confirm apoptotic activity in NP-treated *Drosophila* (Ahamed *et al.*, 2010a). Alteration in total amount of protein is also checked as a parameter to detect NP-induced stress.

Genotoxic assay

Wing spot assay is used for genotoxicity screenings where mutation resulted from NP toxicity give rise to wingspots on wings (Demir *et al.*, 2011). Wing spot assay can recognize somatic recombination along with point mutation, deletion and chromosomal aberrations (Graf *et al.*, 1984). Adult survivorship assay is used to detect the stress resistance ability of the animal. H_2O_2 is used as a stressor to check the stress tolerating ability of *Drosophila* by antioxidant production as an innate mechanism (Mishra *et al.*, 2017). Hsp70, Hsp83 were used to check genotoxicity as an alteration resulted DNA damage.

Phenotypic defect

Flies hatched after NP treatment has decreased body proportion (Panacek *et al.*, 2011). Reduced body weight, is observed after exposure to certain NPs. Phenotypic defect including depigmentation(Fig.4A), and soft cuticle were also reported in the offspring after AgNP treatment (Gorth *et al.*, 2011, Key *et al.*, 2011, Posgai *et al.*, 2011). Soft cuticle is further co related with reduced locomotory behaviour in various studies (Wright *et al.*, 1976, Walter *et al.*, 1991, Neckameyer *et al.*, 2001, Drapeau *et al.*, 2003, Suh and Jackson, 2007, Armstrong *et al.*, 2013). The depigmentation occurs either due to altered melanin synthesis or with increased hsp70 expressions (Denman *et al.*, 2008, Galván and Alonso-Alvarez, 2009, Glassman, 2011). Ag NP exposure further decreases the copper level which is a co factor for tyrosinase and Cu-Zn superoxide dismutase; subsequently production of these two enzymes is decreased. Tyrosinase is responsible for the synthesis of melanin; thus resulted demelanization (Wright, 1987). Enzymes involved in dopamine metabolism (a pathway involved in melanin synthesis) are encoded by genes like *pale*, *yellow*, *Dopa decarboxylase* (*Ddc*), and *ebony* (Sugumaran *et al.*, 1992, Qian *et al.*, 2002, Wittkopp *et al.*, 2003, Carroll, 2005, Sugumaran, 2009, Tang, 2009, Wittkopp and Beldade, 2009).

Phenotypic alterations like deformities in thorax (Fig.4B) over successive generations suggest the mutagenic property of various NPs (Vecchio *et al.*, 2012). Oral intake of NP causes cytotoxicity and DNA damage to mid-gut cells and imaginal disc (Patel and Champavat, 2014, Sabat *et al.*, 2016). The phenotypic defect in the gut and imaginal disc is caused due to overexpression of stress genes like catalase, glutathione and superoxide dismutase (Posgai *et al.*, 2011, Jovanović *et al.*, 2016). Bristles development is controlled by gene achaete-scute complex(Georgiev and Gerasimova, 1992) along with signal transduction pathways like EGFR and Notch (Furman and Bukharina, 2007). NPs are expected to alter the functioning of the above gene involved in this pathway and bring structural variation in bristle. Wing defects like small wing incomplete venation (Fig.4C,D)is a consequence of the

toxic effect of HAp NP on wing imaginal disc (de Celis, 2003). Abnormal wing venation pattern can be a result of a defect in posterior cross vein gene, bone morphogenetic protein (BMP) and Notch signalling pathways (de Celis, 2003). Wing spots within the wing are observed after NP treatment (Fig. 4E, F) Wingspot assay is a known marker to detect mutation (Demir *et al.*, 2011). Presence of wing spot indicates Co NPs acts as a mutagen. *multiple wing hairs* strain and flare-3 strain are taken as control for wing spot assay. Membrane destabilization, membrane potential loss of mitochondria, increased oxidative and apoptotic activity of gut cells are also used as the marker of gut. NP alter the eye imaginal disc and causes various pgenotypic defect in the eye (Fig. 5). Eye shape is reduced by decreasing the number of ommatidia from the periphery. Splitted eye is also observed in certain cases.

Developmental cycle

NP resulted alteration of developmental cycle (Fig.2) and decreases developmental success (Ahamed *et al.*, 2010a, Gorth *et al.*, 2011, Philbrook *et al.*, 2011b). Reproduction rate in *Drosophila* is affected due to reduced mating success after exposure upon NP (Posgai *et al.*, 2011). NP may alter various developmental stages as observed in AgNP. AgNP distress larva to pupa, pupa to adult transitions. Flies hatched after NP treatment may affect their longevity in F1 generation. Magnetite NP can cross the placenta and invade into ooplasm and vitelline membrane of *Drosophila* egg. Entry of NP alters oogenesis period, reduction in ovary size with increased defects, a decrease in nurse cells and abnormal egg chamber development which is the reason for the developmental delay and reduced fecundity. Magnetite NP can cross the placenta and invade into ooplasm and vitelline membrane of *Drosophila* egg(Chen *et al.*, 2015). Entry of NP alters oogenesis period, reduction in ovary size with increased defects, a decrease in nurse cells and abnormal egg chamber development which is the reason for the developmental delay and reduced fecundity. Adult longevity was

found to be affected in flies which were hatched after Ag NP treatment (Key *et al.*, 2011). NPs may affect the egg laying capacity and retarded growth of ovary with very fewer numbers of ovarioles. Similar results were observed after Ag NP exposure for 10 to 30 days in adult flies(Key et al., 2011). Defects may be seen in prepupae, pupae formation, adult eclosion, impaired adult longevity and ovarian development. Flies exposed after NP treatment (F1 generation) have a transgenerational effect. F2 generation flies, hatched from F1 generation (which were never exposed to Ag NP) also possess many structural defects in the body. Such defects were observed in AgNP treated flies suggesting Ag NP acts as a teratogen.

Behavioural assays

Behavioural assays are used as powerful tool to assess the endpoint of genetic and environmental factors on fly behaviour (Moore *et al.*, 1998, Nichols *et al.*, 2012). This test is done both on larvae and adult to check the numerous motor and sensory defects which are suitable for NP toxicity assays. In larvae, a behavioural test like foraging, phototaxis and chemotaxis is widely used. In adults behaviour like phototaxis, chemotaxis, geotaxis, aggression, grooming, courtship and mating are used to check the motor and sensory actions (Chifiriuc *et al.*, 2016). The complex interaction of various genes in different biological pathways is important for the development of body parts of required morphology (Joshi *et al.*, 2005). Thus, any structural defect may appear in the form of the functional defect and so is the behaviour of the animal.

Often the structure forms normal but then it undergoes degeneration. In such cases it can be detected by behaviour. Both defective phenotype and behaviour resulted from NP toxicity can affect the survivability of *Drosophila*. Larva crawling behaviour is done to check the extent of neuronal damage during early development and confirmed by abnormal crawling behaviour (twist, turn, sluggishness)(Sabat *et al.*, 2016). Effect of NPs on nervous

system mainly on mechanosensory neurons, cholinergic neurons, sub oesophageal ganglion can be studied indirectly by assaying larva crawling behaviour (Riedl and Louis, 2012). Unnatural grooming behaviour can act as a defective behavioural endpoint due to NP toxicity which can be assayed easily. Proper climbing behaviour is mediated by an antenna which helps in sensing the gravitational pull and thereby maintaining balance during flight (Bokolia and Mishra, 2015). This behaviour is affected due to NP exposure and can be assayed by simple methods (Mishra *et al.*, 2017)..

Nanoparticles as a potential teratogen

Results from various labs on different NPs suggest that NPs inducess toxicity in *Drosophila*. Once NPs surpass a certain threshold dose, it causes numerous physiological, developmental and behavioural defects in *Drosophila*. NP causes oxidative stress by generating ROS overproduction and increasing the activity of HSP protein. As a result of stress response various biochemical pathways are affected along with damage to the DNA. Genotoxic effects of NPs cannot be neglected as they can cause mutation over successive generations.

Due to the toxicity of NPs, the process of programmed cell death or apoptosis is initiated to destroy the affected cells. The process of apoptosis is regulated by activities of special proteins like p53, p38 and molecules like caspases. Proper functioning of various signalling pathways like Notch, BMP, Hedgehog, PI3K/Akt/mTOR, dopamine etc. are hampered due to nanotoxicity which is essential for proper development of an organism. Dysregulated hormonal system in NP treated flies shows their effect on biological pathways. Further toxicity may appear in the form of morphological anomalies resulting from defective phenotype in eye, wings, bristles, abdomen, thorax and cuticle. NPs also causes behavioural defect by disrupting the nervous system at various levels. Altered

crawling, climbing and grooming behaviour shows the neural flaws created by NPs on nervous system at any of the four developmental stages of *Drosophila*. Neural malfunctioning during embryogenesis may affect the whole process of neurogenesis and development of brain; which regulates the fly behaviour and hormonal system. Damage to the larval nervous system by NPs affects its motor and sensory behaviour like foraging for food, movement by neuromuscular coordination, phototaxis, chemotaxis etc. Drastic changes of a *Drosophila's* life cycle occur in the pupal stage where neuronal remodelling occurs to metamorphose the larval nervous architecture to an adult one. NPs effect at this stage affects the adult behaviour greatly. Imaginal discs, which is the precursor of various organs of *Drosophila* also disrupted by NPs and creates phenotypic alterations. Similarly, an adult nervous system with its regulator, brain is assigned to perform complex behavioural actions and to coordinate with other sensory organs. Intervention of NPs to these actions can have an effect on overall *Drosophila* survivorship. In addition, impairments such as developmental delay, reduced developmental success, fecundity and longevity are the result of NPs in *Drosophila*. These abnormalities by NPs at the cellular, molecular, genetic, phenotypic and behavioural level not only in one generation but also in subsequent generations foster the idea that NPs may act as a potential teratogen. Teratogenic effect of various chemicals had been studied in great detail starting back from the exposure of thalidomide (McBride, 1961, Taussig, 1962). The possibility of nanomaterials to act as a teratogen is miserably overlooked as compared to their uncontrolled implementation and rapid advancement. As the definition of teratogenesis suggests, teratogens can bring about anatomical, morphological, physiological, biochemical and behavioural abnormalities during the course of development (Coyle *et al.*, 1976). This statement holds true for NPs since NPs can bring about myriads of abnormalities during development in the model organism of the current review, *D.*

melanogaster. Compiling all the supportive data available we propose that NPs act as a teratogen during the course of development in *Drosophila*. This questions the huge application of NPs in day to day life of modern society. From all the toxicity studies carried out on NPs insist us to ask a question; is it correct to consider NPs as a boon to modern human society or bane? What would be the consequences of uncontrolled use of NPs on our future generations? NPs have numerous applications in today's world without any doubt, but we must be concerned about its risk of toxicity. We must ask questions about their safety and efficacy before application and rules and regulations should be followed strictly to curb their uncontrolled production as well as usage (Rycroft *et al.*, 2018). Thus, to prevent such situations NP toxicity assessment should be done in a systematic way in various *in vivo*, *in vitro* model systems prior to their use. We have to understand the way of interaction of NPs within biological system and accordingly we have to design or reshape the properties of different nano materials.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

Fig.1. Mechanism of nanoparticle absorption taken through oral route in vertebrate and *Drosophila* gut. (A) In vertebrate system three possible pathways have been suggested such as (a) paracellular pathway (b) transenterocytic pathway (c) M-cell mediated pathway. Among three transenterocytic pathway is responsible for most of the nanoparticle transport. It

involves enterocytes, which are abundant in gut lining increasing the surface area for absoption. Tight junctions, involved in the paracellular pathway, in fully opened condition have space less than 20 nm and normally NP transport is restricted. M-cell mediated pathway also helps in absorption of large sized NPs. M-cells are having decreased protease activity and less glycocalyx which can be suitable for NP absoption; at the same time M-cell population is very less in gut lining and these cells are associated with dendritic cells and macrophages which can engulf the NPs. (B) In *Drosophila* transport of NPs through enterocytes is found to be a most exact mechanism of NPs absorption.

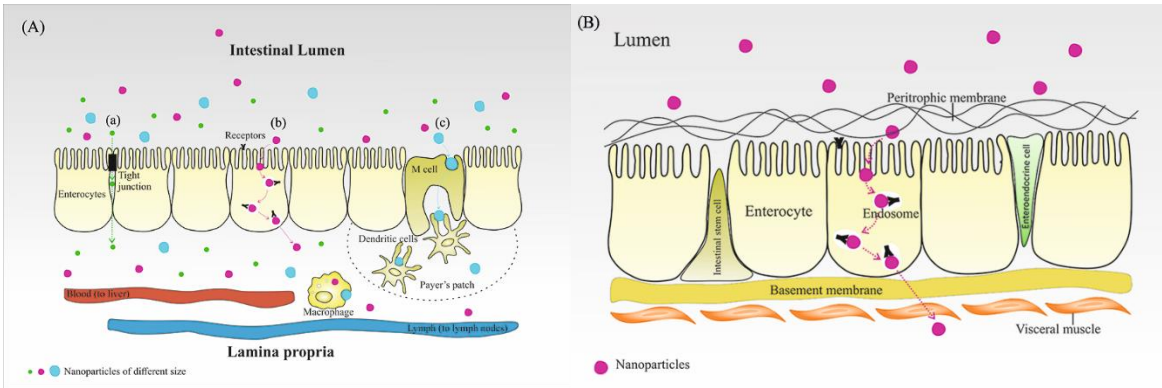


Fig.2. Life cycle of Drosophila depicting various developmental stages

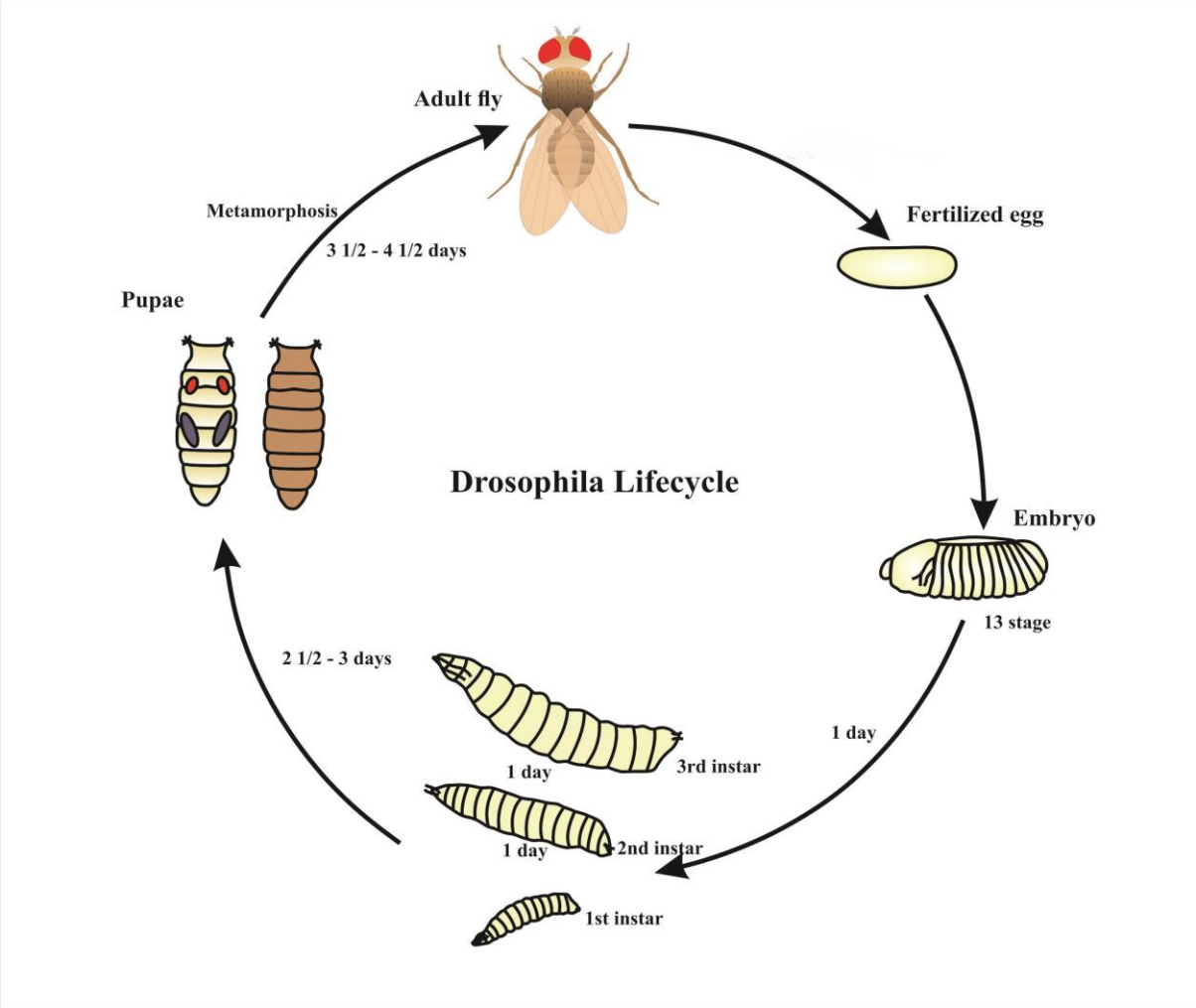


Fig.3 Mechanisms of NP toxicity

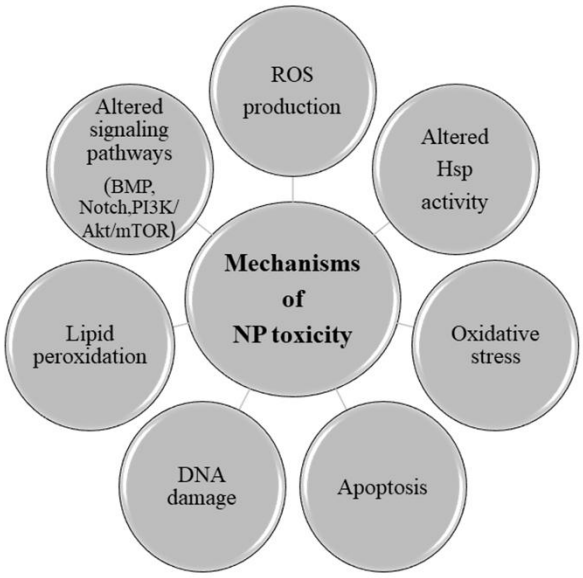


Fig. . Defective body, thorax, wing, bristle phenotype. (A) Loss of body pigmentation (b) when flies were treated with silver nanoparticle (a: control). (B) Deformed thorax by zinc oxide and gold nanoparticle treatment. Single wing phenotype upon exposure to zinc oxide nanoparticles. Bristle loss in flies exposed to zirconia, hydroxyapatite and titania nanoparticles indicated by a circle. (C,D) Wing deformities were seen in gold, titania nanoparticle exposure. (E) Incomplete venation in hydroxyapatite and titania nanoparticle treated flies. Brown patches due to thick wing hairs on hydroxyapaptite treatment indicated by a top circle. Trichomes may get fused or absent in zirconia nanoparticle treated flies due to defective planar polarity. (F) Genotoxicity checking by wing spot assay in Drosophila. Silver, copper oxide and cobalt nanoparticle increase the frequency of mutant spots in Drosophila.

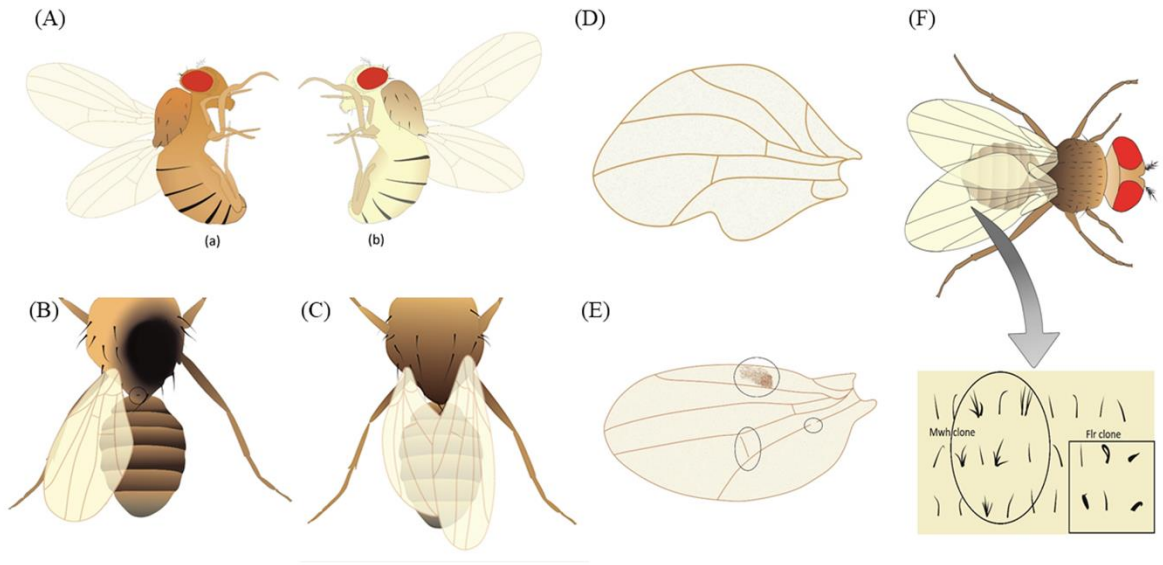


Fig.5. Defective eye phenotype in *Drosophila* upon exposure of different nanoparticles. (A) Rough eye phenotype with the formation of blister due to hydroxyapatite, zirconia nanoparticles indicated by the arrow. (B) Marginal ommatidia loss by hydroxyapatite, zirconia nanoparticles exposure indicated by the arrow. (C) Small eye phenotype in hydroxyapatite treated flies. (D) Bisected eye upon gold nanoparticle exposure.

Fig.6. Behavioural defects. (A) Drosophila exposed to carbon nanomaterials showing increased grooming behaviour. (B) Defective larva crawling behaviour upon exposure of titania, hydroxyapatite and zirconia nanoparticles which shows neuronal damage in the early stage of development. (C) Defective adult climbing behaviour checks the functioning of the antenna where adult flies are made to cover a marked distance in a given time. Flies which are unable to cover the marked line are regarded as abnormal. Nanoparticles like zirconia, hydroxyapatite, titania, carbon are able to affect the climbing behaviour in Drosophila

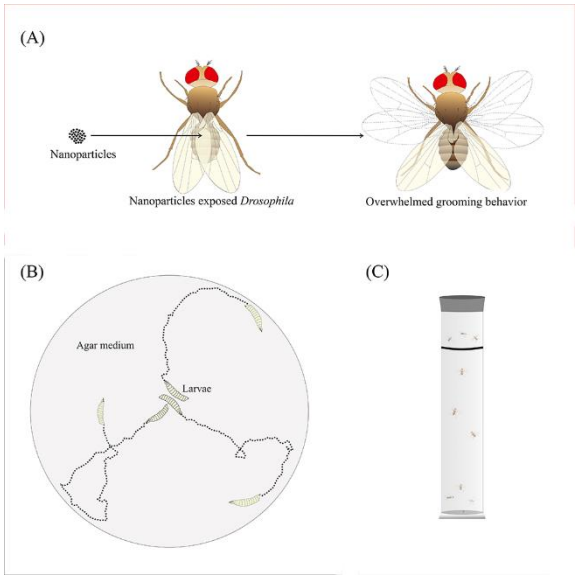
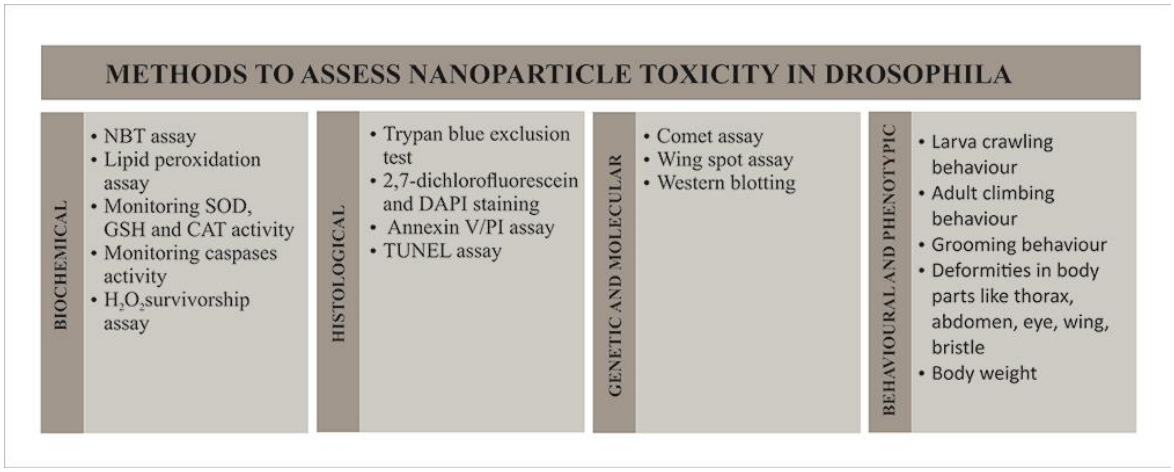


Fig.7. Methods to assess NP toxicity. Numerous methods used to detect NP toxicity in *Drosophila melanogaster.*



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