Physiology & Behavior 167 (2016) 76–85

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

# Investigation of titania nanoparticles on behaviour and mechanosensory organ of *Drosophila melanogaster*

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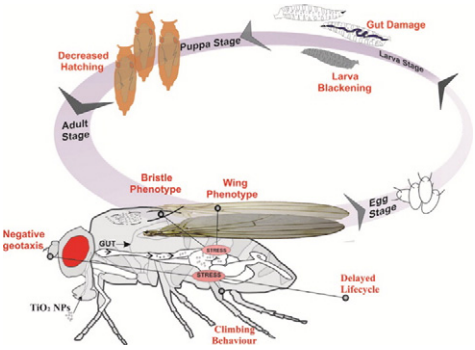
## HIGHLIGHTS

- Titania NPs exposure >200 mg·L$^{-1}$ delays life cycle and decrease pupation.
- Abnormal behaviour is observed in larval crawling and climbing behaviour assay at higher $TiO_2$ concentration.
- Titania NPs affects the development, mechanosensory bristles and wing venation.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 13 April 2016
Received in revised form 6 August 2016
Accepted 30 August 2016
Available online 5 September 2016

*Keywords:*
Titania nanoparticles
*Drosophila melanogaster*
Toxicity
Behaviour

## ABSTRACT

Titania nanoparticles are used in food, cosmetic, medicine, paint and many more domestic items. Its extensive use has raised the threat to the physiological system and thus the functioning of the body. In the current study, the toxicity of $TiO_2$ is checked by adding it in food and using *Drosophila melanogaster* as a model organism. Various concentrations of $TiO_2$ (50, 100, 200, 250 mg·L$^{-1}$) toxicity was assessed via oral route exposure. Survivability, life-cycle, mechanosensory behaviour and structure of various mechanosensory organs were monitored as a read out of nanoparticle toxicity. $TiO_2$ NPs generate reactive oxygen species which can modify multiple signalling pathways and thus can alter the development and behavioural pattern of the fly.



## 1. Introduction

The nanoparticles (NPs) have broad application and have extensive use in construction, aviation, health sector, robotics and domestic products. Its nano size (<100 nm) and acquired characteristic properties help to meet several challenges. The positive aspect of NP is in the growth, drug targeting and treatment of various plants or animals. Various other nanoparticles such as silver (Ag) and gold (Au) are used as antimicrobials, imaging and tumour treatment [1]. Similarly, magnetic NPs are used for targeted drug delivery [2] while zinc oxide, hydroxyapatite or silica NPs can be used as nano-fertilizers [3]. This also provides an opportunity for the NPs to enter into the food chain without being noticed or undetected due to its nano-form.

Among all the synthesised NPs, titania ($TiO_2$) has distinguished chemical properties producing white pigment due to high refractive index. It is highly stable and used in self-cleaning tiles, windows, textiles, and anti-fogging car mirrors. Its anticorrosive and photocatalytic

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http://dx.doi.org/10.1016/j.physbeh.2016.08.032
0031-9384/

property increases catalytic activity due to its increased surface area. [4]. In the field of nanomedicine, $TiO_2$ has application in photodynamic therapy (PDT), photosensitizers, imaging, nano-therapeutics, prosthetic implants of hip and knee [5–7].

Products like cake, candies, chewing gum, sweet and drugs contained a higher amount of $TiO_2$ [8,9]. For example, chewing gum on an average has 2.4–7.5 mg of $TiO_2$ NPs and the intake of $TiO_2$ increase in a time-dependent manner. In a chewing gum, the majority of $TiO_2$ NPs (~93%) is of size >200 nm and rest are with <100 nm range [10]. Similarly, several food product additives contain $TiO_2$ at a level of 0.02 to 9.0 mg/g and out of which 15% are <100 nm in size [9]. Although cosmetic and toothpaste contains some amount of $TiO_2$ NPs, so far its toxic effect on the body is not yet reported [11]. The toxicity in NP depends on the size, surface chemistry, route of administration and exposure route of NPs which is usually through inhalation, oral or dermal exposure. The food and drug administration (U.S.) have approved $TiO_2$ < 1% by body weight while Occupational Safety & Health Administration (OSHA) approved $TiO_2$ < 15 mg/m$^3$. Besides its wide application, its toxic effect classifies it to be as a group 2B carcinogen to humans [12]. The toxicity also depends on the grade of $TiO_2$ used [8]. The industrial grade shows toxicity up to 0.2 mg mL$^{-1}$ while food grade does not show toxicity up to 2 mg mL$^{-1}$. A recent report suggested the oral route intake of $TiO_2$, induced cytotoxicity on mid-gut, imaginal disc and increased DNA damage in *Drosophila* haemocytes [13]. The dietary intake of $TiO_2$ NPs increases the stress gene catalase and superoxide dismutase (SOD) activity [14]. However, dietary intake of (0.1–10 mM) anatase nano-$TiO_2$ did not induce genotoxicity in the wing spot test of *D. melanogaster* larvae [15] as it is well-studied in other model organisms [16,17]. In the rat model, $TiO_2$ NPs impair central nervous system when administered intranasally [18]. $TiO_2$ exposure produces free radical and damages the brain microglia, affects cell cycle of neurons, induces apoptosis in the neuronal cell [19]. The toxicity of $TiO_2$ on the various physiological systems including nervous system is alarming and warrants a model organism to investigate the molecular mechanism and its toxicity.

*Drosophila* serves to be an excellent model to study disease mechanism, drug testing, pathogen infection and also nanoparticle toxicity [20–23]. The advantage for selecting this model is due to its short lifespan, cost effectiveness, the whole genome sequenced, and 75% diseased genes in humans share homology with *Drosophila* [24–26]. The current study focuses on testing the toxicity of $TiO_2$ NPs (via oral route) in the *Drosophila* model by checking its behavioural, survival and phenotypical changes that the fly undergoes on exposure of $TiO_2$ NPs at various concentrations.

## 2. Materials and methods

### 2.1. Materials

Titanium isopropoxide ($Ti(OCH(CH_3)_2)_4$) (analytical grade) was obtained from Sigma-Aldrich. Methanol, trypan blue, agarose and molecular sieves were obtained from Hi-media. The chemicals were used as obtained and without any further purification.

### 2.2. Synthesis of $TiO_2$ nanoparticles

For the synthesis of $TiO_2$ NPs, 5 mL of methanol was taken in a beaker and 0.25 mL (5 vol.%) of water was added to it and stirred. 2.02 mL of titanium isopropoxide ($Ti(OCH(CH_3)_2)_4$) taken as the titanium precursor, was added to 5 mL of hot methanol freshly dried using molecular sieves. This was then added to the beaker immediately, and a gel was formed in 5–10 min. This was left to age overnight, followed by drying at a temperature of 150–200 °C for 1–2 h. The dried gel was then powdered using a mortar and pestle and calcined in air at a temperature of 550 °C for 3 h [27,28].

### 2.3. Characterisation of synthesised NP

The chemically synthesised NPs was characterised by using XRD, FESEM, TEM and FTIR techniques.

#### 2.3.1. X-ray diffractometer (XRD) analysis

The powdered sample was subjected to XRD analysis. The crystal structure of the synthesised nanoparticle was determined by X-ray diffractometer (XRD) of RIGAKU JAPAN/ULTIMA-IV with $CuK_\alpha$ radiation ($\lambda = 0.154$ nm), $2\theta$ in the range of 10°–80° and a scan rate of 2° per minute.

#### 2.3.2. Field emission scanning electron microscopy and transmission electron microscopy

The size, morphology and structure of the nanoparticle were investigated by field emission scanning electron microscopy (FESEM), performed in Nova NANOSEM/FEI. The sample was prepared by dispersing $TiO_2$ powder in methanol. Then one drop of the suspension was spared over ITO glass slides for analysis The Transmission Electron micrographs (TEM) of the $TiO_2$ NPs were recorded using PHILIPS CM 200 equipment using carbon coated copper grids. The HRTEM and SAED pattern of the NPs was also analysed.

#### 2.3.3. Zeta potential

The $TiO_2$ NPs were dispersed in Milli-Q water and sonicated for 20 min than the zeta potential was measured using Malvern NANO-ZS-90.

#### 2.3.4. FTIR analysis

The FT-IR spectroscopy (Perkin-Elmer) was used to determine the functional groups in $TiO_2$ NPs. The spectrum was scanned from 4000 cm$^{-1}$ to 400 cm$^{-1}$. Nearly 3–4 mg of the sample was mixed thoroughly with 30 mg of dried KBr and made into pallets. The pallets were stored in vacuum desiccators and exposed to IR lamp for 1 min before the IR measurement.

### 2.4. Fly stock

Oregon-R (OR) flies were obtained from the fly facility, C-CAMP Bangalore, India. The flies are grown on standard corn meal media with sucrose, agar agar type-I and yeast. The flies are kept in 25 °C, a 60% relative humidity (RH) incubator with 12 h of Light/Dark cycle.

### 2.5. Preparation of titanium dioxide stock solution

The 1000 mg L$^{-1}$ stock solution of Titanium dioxide ($TiO_2$) NP was prepared by mixing 50 milligrams (mg) of $TiO_2$ NPs in 50 mL of distilled water. The prepared mixture was sonicated using for 20–30 min under the ice to maintain a homogenised distribution of the NPs throughout the solution. The homogenised $TiO_2$ nanoparticles were mixed in the food to achieve a final concentration of 50 mg L$^{-1}$, 100 mg L$^{-1}$, 200 mg L$^{-1}$, and 250 mg L$^{-1}$. The food containing different concentrations of $TiO_2$ NPs were equally distributed for 3 sets of the replica. Later, an equal number of wild type (WT) Oregon-R male and female flies were transferred to each vial.

### 2.6. Survivability study (toxic effect on lifecycle)

The toxicity assay was measured as reported by Ales Panacek et al. [29]. The flies fed with different concentrations of $TiO_2$ NPs (50 mg·L$^{-1}$, 100 mg·L$^{-1}$, 200 mg·L$^{-1}$, 250 mg·L$^{-1}$) lay their eggs on food. The eggs developing to pupal stages were marked on each vial, and the number of flies hatching from each vial was recorded every day. The graph is plotted by the percentage of flies emerging from each concentration.