Evaluation of antioxidants in amelioration of toxic effects of engineered nanoparticles using *Caenorhabditis elegans* as a basic *in-vivo* alternative model

A Thesis Submitted to
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in

Biochemistry

by

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May, 2018

Dedicated to my Family members for

their unconditional love,

endless support and

encouragement

Date:

CERTIFICATE

This is to certify that the thesis entitled **"Evaluation of antioxidants in amelioration of toxic effects of engineered nanoparticles using *Caenorhabditis elegans* as a basic *in-vivo* alternative model"** submitted by **Ms. Madhavi Sonane** for the award of Doctor of Philosophy in Biochemistry to Babu Banarasi Das University, Lucknow is a record of authentic work carried out by her under my supervision at Ecotoxicology Division, Council of Scientific & Industrial Research-Indian Institute of Toxicology Research, Lucknow. To the best of our knowledge, the matter embodied in this thesis is an original research work done by candidate and has not been submitted elsewhere for the award of any degree or diploma.

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DECLARATION

I, hereby, declare that the work presented in my thesis, entitled **"Evaluation of antioxidants in amelioration of toxic effects of engineered nanoparticles using *Caenorhabditis elegans* as a basic *in-vivo* alternative model"** in fulfillment of the requirements for the award of the Doctor of Philosophy in Biochemistry by Babu Banarasi Das University, Lucknow. The research work has been carried out under the supervision of **Dr. Aruna Satish** at Ecotoxicology Division, Council of Scientific & Industrial Research-Indian Institute of Toxicology Research, Lucknow**.** I also declare that the work embodied in the thesis is original research and has not been submitted anywhere for degree or diploma.

(Madhavi Sonane)

CSIR- SRF

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PREFACE

The present thesis entitled **"Evaluation of antioxidants in amelioration of toxic effects of engineered nanoparticles using *Caenorhabditis elegans* as a basic *in-vivo* alternative model"** is the outcome of the research carried out by me under the supervision of Dr. Aruna Satish, Scientist, CSIR-IITR, Lucknow.

The thesis has been divided into five chapters. The **Chapter 1** deals with the descriptive background, history, scope, problems and future aspects of compounds such as titanium di-oxide nanoparticles (nano-TiO_2), zinc oxide nanoparticles (nano-ZnO), ascorbic acid and curcumin. The reports concerning the NPs applications as well as their toxicity are documented in this chapter, covering the recent reports. Further, studies on the protective efficacy of antioxidants (curcumin and ascorbic acid) against various diseases, toxicity and adverse conditions are reported in this chapter. **Chapter 2** describes the lethal and the toxic effects of nano-TiO_2 and nano-ZnO along with bulk in *Caenorhabditis elegans* (*C. elegans*) at acute and chronic exposure. Further, high ROS generation in *C. elegans* on exposure to nano/bulk TiO_2 and ZnO is reported in this chapter. The ameliorating properties of ascorbic acid and curcumin against nanoparticle induced ROS and lethality at acute and chronic exposure is also discussed. The results in this chapter clearly describes that in presence of antioxidants, the lethal effects of nano/bulk TiO_2 and ZnO is mitigate even at high exposure concentrations in *in-vivo* models system such as, *C. elegans*. In the **Chapter 3**, we describe the role of the major stress response (Insuline/insulin-like growth factor realted signaling) and immune response (p38 and JNK-1) signaling pathways against the oxidative stress induced by nano/bulk TiO_2 and ZnO exposure. Further presence of antioxidants during exposure to nano/bulk TiO_2 and ZnO is shown to protect the worms from particle induced alteration in the gene and protetin expresion. Thus, the protective efficacy of antioxidants at gene expression level is

described in this chapter. Both the antioxidants ascorbic acid and curcumin protect the organism from gene and protein alteration against nano/bulk TiO_2 and ZnO exposure. In the C**hapter 4,** the functional validation of oxidative damage and antioxidant enzyme regulation when organism exposed to nano/bulk TiO_2 and ZnO is discussed. Exposure to nano/bulk particles enhances the free radical generation leading to disturbance in the oxidant-antioxidative enzyme/molecules equilibrium, which inturn damages the macro-molecules such as protein, lipid and DNA and ultimately leads to cytotoxicity in the organism. However, co-exposure of antioxidants ascorbic acid and curcumin protect the worm from oxidative damage and maintain the normal biochemical equilibrium. This section of the study clearly describe, that antioxidants co-exposure along with nano/bulk exposure provide protection from nano/bulk TiO_2 and ZnO induces oxidative damage to worms. Finally, in the **Chapter 5**, the functional validation of the protective role of antioxidants ascorbic acid and curcumin against nano-TiO_2 and nano-ZnO exposure on the physiology of *C. elegans* is reported. Growth, reproduction, behavior and the life span of the organism is adversely affected by nano/bulk TiO_2 and ZnO while, antioxidants provide protection against the same. Thus, ascorbic acid and curcumin are efficient to ameliorate the adverse effects of nano/bulk TiO_2 and ZnO.

Wide applicability of both the antioxidant gave us the vision to attenuate the toxicity of nano and bulk TiO_2/ZnO by combining antioxidant along with nanoparticles exposure. Majority of the studies regarding the protective efficacy of antioxidant towards different cell lines and organism are reported against various compounds, chemical and metals but in this study the novelty is with regard to the ameliorating nature of antioxidant at high exposure concentration of nanoparticles at molecular, biochemical and physiological level. The involvement of IIS-signaling and immune response in the nanoparticles induced

oxidative stress and apoptosis is observed, and the capability of antioxidants to protect the organism from the oxidative damage is also reported.

A systematic study of ameliorating effects of antioxidant against nano/bulk TiO_2 and ZnO induces lethality and toxicity at acute and chronic exposure described in **Chapter 2** is published as

1. **The role of antioxidants in attenuation of *Caenorhabditis elegans* lethality on the exposure to TiO_2 and ZnO nanoparticles.** **Madhavi Sonane**, Nida Moin and Aruna Satish (2017). ***Chemosphere***, 187 (2017), 240-247. **IF. 4.506**

TABLE OF CONTENTS

CHAPTER: 2 DETERMINING THE TOXIC POTENTIAL OF TIO_2 AND ZNO NANOPARTICLES AND EVALUATING THE EFFICIENCY OF ANTIOXIDANTS AGAINST THE LETHAL EFFECTS OF TIO_2 AND ZNO NANOPARTICLES

ABBREVIATIONS

nm	**Nanometer**
µg	**Microgram**
mg	**Milligram**
g	**Gram**
µl	**Microlitre**
ml	**Millilitre**
l	**Litre**
µM	**Micromolar**
mM	**Millimolar**
M	**Molar**
s	**second**
min	**Minutes**
h	**Hours**
˚C	**Degree centigrade**
cDNA	**Complementary DNA**
DW	**Distilled water**
DMSO	**Dimethyl sulfoxide**
Cur	**Curcumin**
H_2O_2	**Hydrogen peroxide**
NaN_3	**Sodium azide**
NPs	**Nanoparticles**
AO	**Antioxidants**
RT	**Room temperature**

List of Figures

List of Table

Chapter 1

INTRODUCUTION

&

REVIEW OF LITRATURE

1.1. INTRODUCTION

Nanotechnology (NT) is an immense combination of science and technology that play an essential role to solve real-life problems, boosting the economic development of the country. NT implies engineering an organic and inorganic matter at the atomic or molecular level yielding the nanometer scale (>100 nm), fundamentally new and controlled molecular organization with unique properties called as nanoparticles (NPs) or engineered nanoparticles (ENPs) (Khan et al., 2017). NPs are ultrafine microscopic units with at least one dimension less than 100 nm. NPs are defined as "*A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm*" (European Commission, 2011).

Apart from the small size, high surface reactivity, tunable chemical and physical properties [particle shape, surface structure, chemical composition (purity, oxidation states)] have made ENPs a central component in an array of emerging technologies (Babu, 2016; Choi et al., 2016). The novel attributes of NPs has revolutionized various fields such as electronics, therapeutics, cosmetics and a wide range of consumer products (Bui et al., 2017; Colombo al., 2017; Mendes et al., 2017; Truppi et al., 2017; Babu, 2016; Choi et al., 2016). NPs are even used for environment remediation (Khan et al., 2017; Snousy and Zawrah, 2017). NT also offer novel functionalities to existing technology and product to improve the performance such as lightweight alternatives of sports goods, transportation and construction, high-quality packaging materials,

scratch, and heat resistant UV shield, etc. (Choi et al., 2016; Ramachandraiah et al., 2015; Stark et al., 2015). At present more than 1500 consumer products categorized in health and fitness (788 products), cosmetics (154 products), clothing (187 products), personal care (292 products), sporting goods (119 products), sunscreen (40 products) are available in the market (Vance et al., 2015). Moreover, the global investment in nanotechnology research and development ($3 trillion by 2020, Roco et al., 2011) is rising worldwide, in turn giving employment to more than 6 billion people. Some of the nanomaterials along with their estimated requirment or production are listed in table 1.1.

Table 1.1 Estimated requirment or production of ENPs

Nanomaterial (NMs)/ nanoparticles (NPs)	**Estimated requirment/ production (2012-2023)**	**Application**	**References**
Carbon nano tube	**$1.1 billion (12,800 Metric Tons in 2016)**	**Cathode ray tubes, electrostatic discharge, electrical-shielding applications, racquets, golf clubs, surfboards, ice hockey sticks, mass transportation fuel system components, battery electrode additives, plastics additives and master batches**	**https://www.nanowerk.com/spotlight/spotid=23118.php**

Graphene	**$195 million** **(921.50 Tons in 2018)** **$1.3 billion by 2023**	**Energy, electronics, composite, coatings, biomedical, sensors, automobile, aerospace**	**https://www.bccresearch.com/market-research/advanced-materials/graphene-technologies-applications-markets-report-avm075d.html**
Nano-Metal/metal oxides	**270, 041-1663, 168 tons** **(2012-2020)**	**Consumer goods, electronics, personal care, automotive, medical, electronics, and military markets**	**https://www.prnewswire.com/news-releases/the-global-market-for-metal-oxide-nanoparticles-to-2020-210803631.html**
Nanocomposites	**$4.2 billion** **(5,84,984 Metric Tons** **by 2019)**	**Packaging, Automotive, Aerospace & Defense, Electronics & Semiconductor, Energy, Coatings and others**	**https://www.bccresearch.com/market-research/nanotechnology/nanocomposites-market-nan021f.html**
Nano-ceramic	**$12.1 billion in 2018**	**Energy supply and storage, communication, transportation systems, construction and medical technology**	**https://www.bccresearch.com/market-research/nanotechnology/advanced-ceramics-nanoceramic-powders-nan015g.html**

Engineered nanoparticles (ENPs):

Changes in particle size from bulk to nano are associated with changes in properties, providing many advantages over bulk material such as;

1. **Mechanical properties:** Mechanical properties depend upon the bonds (covalent, valet, ionic) between the atoms but as the size reduces up to the nano level, the elasticity of material reduces dramatically. Hence the hardness of the material is also related to the size. For example, cutting tools made by NPs such as tungsten carbide, tantalum carbide is much stronger, erosion resistant than their conventional counterparts (Sahijpaul, 2015).

2. **Structural properties:** Size, shape, and surface area of the particles affects various properties of the material such as small size of particles makes NPs easy to penetrate as compared to the bulk. Round shape particles have more absorption efficiency compared to rod-shaped or fibrous NPs and high surface area to volume ratio increase chemical reactivity of the material (Albanese et al., 2012).

3. **Quantum effect:** Material often has unexpected visible properties once they are in nano-form because they are small enough to confine their electrons and produce quantum effects. Such as gold NPs appear deep red to black in solution, depending on their size. At the nano-scale, the quantum effects rule the behavior and properties of particles. Hence the quantum effect of NPs is responsible for its wide application spectrum in optics and electronics (Cho and Park, 2016).

NPs exist in the natural world such as volcanic ash, physical and chemical weathering of rocks, glacial ice cores, oceans, surface waters, groundwater, atmospheric water, treated drinking water, diesel exhaust, electroplating, and welding etc. (Strambeanu et al., 2014) and also are created by human activities such as ENPs. Manipulation of the matter with desired properties and utility at atomic, molecular or sub-molecular level generate ENPs.

1.1.1 Classification of ENPs:

There are many ways to classify NPs by their composition, morphology, dimension, origin, uniformity, etc. By morphology, nanoparticles can be categories as nanorods, nanoparticles, nanowires, nanotube, nanofibers. They can also be classified by dimension as one dimension (films), two dimensions (fibers), and three dimensions (particles). Further coated, encapsulated and mixed depending on the composition. By origin NPs are classified as natural and artificial nanoparticles.

There are four categories of ENPs based upon their chemical composition

i. **Carbon-based nanoparticles (CNPs):** CNPs are mostly composed of carbon or in form of hybrids with other NPs (Ti-Ni-C, Ti-Fe-C). CNPs can be in the form of hollow spheres, ellipsoids, or tubes. Spherical and ellipsoidal carbon nanomaterials termed as fullerenes, while cylindrical carbon NPs termed as carbon nanotubes (CNTs) are the most widely used form of CNPs. Nanotubes can be categorized as single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), nanodiamonds, and graphene. Excellent mechanical strength, electrical and thermal conductivity, and optical properties make these CNPs more valuable for various applications, such as high-strength composite materials and electronics (Yuan et al., 2016).

ii. **Dendrimers:** Dendrimers are nanosized polymers made by the repetitive addition of branching groups which in turn are branched molecules. Dendrimers have three components: a central core, an interior dendritic structure (the branches), and an exterior surface with functional surface groups. Different combination of these components gives products of different shapes and sizes. Dendrimers are

monodispersed, usually symmetric and their properties are mainly controlled by the functional groups on the surface. Dendrimers are used in drug delivery, gene transfection, catalysis, energy harvesting, photo activity, molecular weight and size determination (Undre et al., 2016).

iii. **Composites:** Composites are the multiphase solid materials such as combination of NPs with other NPs or bulk materials, in that at least one of the phases has one, two or three dimensions in nanoscale such as colloids, gel, copolymers. Nanosized clays like NPs are incorporated into various products such as packaging materials, to enhance mechanical and/or thermal barrier, and flame-retardant properties (Ozbakkaloglu et al., 2016).

iv. **Metal-based materials:** Metal is the main component of these particles which includes nanogold, nanosilver and metal oxides, such as titanium dioxide, copper oxides, aluminum oxides, chromium oxides and closely packed semiconductor like quantum dots. Biomedical and pharmaceutical industries are mainly focusing on the metal-based nanomaterials due to the diverse and interdisciplinary properties. Ability to be joined chemically with antibodies or pharmaceutical compounds/chemicals offers use of metal-based nanoparticles in biochemistry, catalysis as well as biology as a sensor. In the field of medicine, NPs are used in drug delivery and drug designing (Bui et al., 2017; Colombo et al., 2017; Mendes et al., 2017; Truppi et al., 2017; Yan et al., 2016; Titus et al., 2016).

1.1.2 Adverse effect of nanotechnology/nanoparticles:

ENPs are associated with the economic development of the country but as a coin have two sides; the very properties of nanostructured materials that make them so attractive

could potentially lead to unforeseen health or environmental hazards. Concerns regarding the potential risk to human and environment associated with the exposure of NPs are raised. At present advancement and large-scale production of ENPs increases the intentional or accidental release of ENPs into the environment during all the stages of production, recycling and disposal (Lai and Alsudir, 2017; Bossa et al., 2017; Dobias and Bernier-Latmani, 2013). Thus there is urgent need to draw guidelines for proper handling and safe utilization of NPs (Maynard, 2012). From the environment, NPs can enter in organism through various routes such as dermal, oral and respiratory tract and from there to other parts of the body (Fig. 1.1). NPs are capable of crossing the skin barrier, blood-brain barrier as well as placental barrier (Song et al., 2016, Song et al., 2015). It has been reported that inhalation could be the most frequent route of exposure to NPs present in the environment. Aerosols containing nanometer size metallic particles (e.g., Zinc oxide, tin) enter the bloodstream after passing through the respiratory system and distributed in the target organs such as liver, spleen, lung, kidney, heart (Antonini, 2003). These ultrafine particles can cause major pulmonary and cardiac diseases.

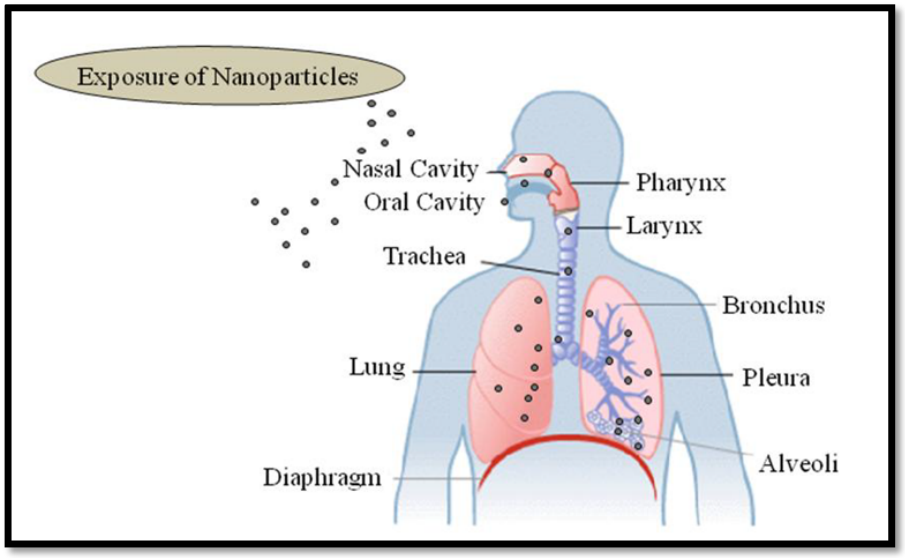

Figure 1.1 Routes of exposure to nanoparticles. Nanaoparticles can enter through dermal route, oral route and respiratory tract to other parts of the body (Source: Roy et al., 2014)

A review published on nanomaterial consumer products inventory listed 1814 nano-enabled consumer products from 622 companies in 32 countries (Vance et al., 2015) and also states that majority of the commercial products do not present sufficient information about the toxicological impact of NPs. Further, a European database claimed the presence of 2300 nano-based consumer products contains biocidal property (Mackevica et al., 2016). An estimate of 260,000-309,000 tonnes of global production of ENPs in 2010 ended up into the landfills (63-91%), soils (8-28 %), water bodies (0.4-7 %), and the atmosphere (0.1-1.5 %) (Keller et al., 2013). In the ecosystem, the particle has the potential of interacting with the biotic and abiotic components in unexpected ways (Krzyżewska et al., 2016). The toxic potential of ENPs has been tested in various lower and higher model systems as the representative

of the different ecosystem. The main aim of toxicity assessment studies is to protect human beings whereas ecotoxicity addresses different trophic levels and intended to protect populations and ecosystem. Presently existing nano-ecotoxicological data includes studies mostly (approximately one third) on crustaceans such as water flea *Daphnia magna* (337 entries), followed by bacterium *Escherichia coli* (120 entries), unicellular alga *Pseudokirchneriella subcapitata* (107 entries), fish *Danio rerio* (66 entries), naturally luminescent bacterium *Vibrio fischeri* (44 entries), and nematode *Caenorhabditis elegans* (41 entries) (Juganson et al., 2015). Some of the studies are listed in table 1.2. The eco-toxicology of ENPs shows that toxicity in lower organism occurs at very low concentration mg/l and physiology of the lower organism was found to be most affected (Hyseni, 2016; Khare et al., 2015; Exbrayat et al., 2015). The *in-vitro* studies on different cell line to trace the possible mechanisms of NPs toxicity indicated an increase in oxidative stress, cytotoxicity, decreased mitochondrial potential, intracellular release of [Ca^{2+}], mutagenic effects and inflammatory responses (interleukin production) (Petrarca et al., 2015; Dissanayake et al., 2015) as mentioned in table 1.2. Even in the vertebrates exposure to NPs has revealed injury to epithelial tissue, fibrosis through inflammation, oxidative stress response, etc. (Zhu et al., 2012; Lindberg et al., 2009).

1.1.3 Adverse effects of Metal-based ENPs:

Among the wide variety of ENPs (e.g. carbon-based materials, metals, metal oxides and biopolymers), metal-based nanoparticles [such as silver (Ag NPs), titanium dioxide (TiO_2 NPs), zinc oxide (ZnO NPs), cadmium selenide quantum dots (CdSe QD)] are being predominantly used by several industries (Bui et al., 2017; Truppi et

al., 2017; Colombo et al., 2017; Yan et al., 2016; Titus et al., 2016). Metal oxides NPs are important in the field of physics, chemistry, and material sciences. They have application in the fabrication of microelectronic circuits, sensors, piezoelectric devices, fuel cells, coatings for the passivation of surfaces against corrosion and as catalysts (Truppi et al., 2017; Yan et al., 2016; Titus et al., 2016; Falcaro et al., 2016). Usage in applied science such as medicine, information technology, catalysis, energy storage and sensing have driven massive production of these nanostructures (Bui et al., 2017; Truppi et al., 2017; Falcaro et al., 2016; Yan et al., 2016; Titus et al., 2016; Kaushik and Moores, 2016). The global market report 2013, estimates hike in the production of metal oxide NPs from 2,70,041 tons to 16,63,168 tons in 2012-2020, which represent a class of NPs with highest global production. Metal oxide NPs attracts considerable interest concerning the environment, health and safety issues due to their potential toxic impact. Silver, gold, copper, TiO_2 and ZnO NPs are extensively utilized in clothing, disinfection, cosmetics, etc. The nano metal and metal oxides such as TiO_2, gold, and silver, were considered to be non-toxic due to the lack of toxicity of the respective bulk material.

Metal oxides NPs exposure causes a broad range of adverse health effects in humans and animals. Invertebrates and human (workplace exposure) metal oxides NPs toxicity are associated with pulmonary, renal, hepatic, neuronal and haematologic dysfunctions. However, in a lower organism, metal oxides NPs exposure has an adverse effect on the physiology (growth, reproduction, and lifespan), neuronal function and biochemical endpoints (behavior, enzyme activity and its intracellular concentration). Additionally, metal oxides NPs at very low concentration were found

toxic to lower model organisms (Khare et al., 2011; Gaiser et al., 2011). Metal oxide NPs can also accumulate in plants and have a negative effect on the growth, seed germination, metabolism, chlorophyll content and induces oxidative stress (Faisal et al., 2016; Deng, 2014; Oukarroum et al., 2013; Larue et al., 2012). When illuminated, metal oxides NPs are capable of being oxidized or dissolved in water and can release metal ions, leading to metal toxicity. Although there is a number of different routes by which metal oxides NPs cause toxicity but the underlying basic mechanisms can be summarized as ROS mediated oxidative stress which further leads for other consequences such as inflammatory responses, apoptosis, neuronal damage, chronic diseases etc. The toxicity reported for various metal oxides NPs, are listed in table 1.2

Table 1.2 Adverse effects of metal/metal oxide NPs on different model system

NMs/NPs	Target	Effect	References
Carbon NPs/nanotubes/ fullerence/ qantum dots	**Fetal lung fibroblast MRC-5 cells**	**↑OS, distorted cell membrane, cell damage and cell death**	**Shindea and Tsai, 2015**
	Dunaliella tertiolecta	**Inhibition of growth and photosynthesis**	**Thakkar et al., 2016**
	C. elegans	**↓ body length, ↓ survival, alteration in metabolic response**	**Walczynska et al., 2018**
	Wistar Rat	**Induced biochemical alterations and histopathological abnormalities (severe alveolar edema, hemorrhage in lungs and myocytolysis in heart)**	**Zayerzadeh et al., 2016**
	C. elegans	**↓ body length, ↓ survival, alteration in metabolic response**	**Walczynska et al., 2018**
	Rapeseed and Wheat	**↑Accumulation at peripheral areas of leaves; more accumulated in newly developed leaves**	**Larue et al., 2012**
Cerium	**Freshwater alga**	**↓ Photosynthesis, ↓carbon fixation,**	**Taylor et al.,**

NPs	***Chlamydomonas reinhardtii***	**↓energy metabolism**	**2016**
	C. elegans	**↑ ROS, ↑ OS, ↓ lifespan**	**Zhang et al., 2011**
	Mice	**↑ ROS, ↑ OS, ↑lifespan, ↑DNA damage**	**Nemmar et al., 2017**
	Rat	**↑ Cytotoxicity, ↑ OS, ↑ inflammation**	**Srinivas et al., 2011**
Silica NPs	**A549, HepG2 epithelial cells and NIH/3T3 fibroblasts cells**	**↑ Cytotoxicity, ↑membrane disruption, ↑ OS**	**kim et al., 2015**
	Dunaliella tertiolecta	**Growth alteration, cell entrapment and agglomeration of NPs**	**Manzo et al., 2015**
	Albino rats	**↑ Body weight, ↑ AST, ↑ ALT**	**Shalaby et al., 2018**
	Mice	**↑Airway inflammation, ↑pathological changes, and ↑ cytokine levels (IL-5, IL-13, IL-1β, and IFN-γ)**	**Han et al., 2016**
Cobalt NPs	**Human alveolar (A549), and bronchial (BEAS-2B) cells**	**Cyto-genotoxic and inflammatory effects**	**Cavallo et al., 2015**
	Liver cells (BRL-3A cells)	**↑ Cell membrane damage, ↑ OS, ↑ immune inflammation and ↑ DNA damage**	**Liu et al., 2016**
	Eggplant	**↓ Seed germination, ↓ root growth, ↑DNA damage, ↑ MD, ↑OS, ↑cell death**	**Faisal et al., 2016**
Gold NPs	**Human pulmonary fibroblasts**	**↑ Cytotoxicity, ↑ OS**	**Avalos et al., 2015**
	Mice	**↓ Body weight, ↓ spleen index, ↓ red blood cells**	**Chen et al., 2013**
	Tobacco	**Accumulated within leaf mid rib near petiole, concentration ranges between 2.2 and 53.5 mg/kg in aerial tissues**	**Deng, 2014**
Silver NPs	**Primary human umbilical vein**	**↑ ROS, disruption of endothelial layer, ↑ inflammation in liver,**	**Guo et al., 2016**

	endothelial cells	**kidney and lung**	
	Human pulmonary fibroblasts	**Size dependent cytotoxicity and OS**	**Avalos et al., 2015**
	Oreochromis niloticus and Tilapia zillii	**↑ OS, ↑ AEA**	**Afif et al., 2016**
	Rat	**Alteration in biochemical and hematological parameter (red blood cell count, platelet count, white blood cell count and AST)**	**Qin et al., 2017**
	Aquatic plant *Lemna gibba*	**↑ OS**	**Oukarroum et al., 2013**
Copper NPs	**Primary hepatocytes of *Epinephelus coioides***	**↑ Cytotoxicity, ↑ ROS, ↑ OS, ↑ cell apoptosis and necrosis,**	**Wang et al., 2016 1**
	Epinephelus coioides	**Growth inhibition, accumulation of particles in tissue, ↑ lipid damage**	**Wang et al., 2014**
	Rat	**Damage to red blood cells, thymus, spleen, liver, and kidney**	**Lee et al., 2016**
	Albino rats	**↑ Body weight, ↑ AST, ↑ ALT**	**Shalaby et al., 2018**
	Rat	**Agglomeration in intestine, stimulate sex related difference, morphological changes in the liver, kidneys, and spleen**	**Lee et al., 2016**
Iron NPs	**Human normal fibroblasts and fibrosarcoma cells**	**↑Cytotoxicity, ↑ genotoxicity**	**Yang et al., 2013**
	Chlorella pyrenoidosa	**↓ Growth, ↑ MDA content**	**Lei et al., 2016**
	Chlorella vulgaris	**EC_{50} 76 mg/l, cell count 90 mg/l**	**Ko et al., 2018**
	Zebrafish (*Danio rerio*)	**Developmental toxicity, embryos mortality, hatching delay, and malformation**	**Zhu et al., 2012**
Aluminum NPs	**Freshwater algal isolate *Chlorella ellipsoids***	**↑ Cytotoxicity, ↑ROS, morphological changes and cell wall damage**	**Pakrashi et al., 2013**
	Ceriodaphnia	**↑ OS, ↑ acute toxicity**	**Pakrashi et**

	dubia		**al., 2013**
	C. elegans	**↓ body length, ↓ survival, alteration in metabolic response**	**Walczynska et al., 2018**
	C. elegans	**↑ ROS, altered locomotion**	**Li et al., 2012**

↑ = Increased level, ↓= Decreased level, OS= Oxidative stress, MD= Mitochondrial dysfunction, AEA= Antioxidative enzymes activity, ADM= Antioxidant defense mechanism, ROS= Reactive Oxygen Species, MDA= Malondialdehyde, ALT= Alanine aminotransferase, AST= Aspartate aminotransferase

1.1.4 Adverse effects of TiO_2 NPs and ZnO NPs:

World revenues for TiO_2 and ZnO metal oxide NPs were approximately $6,150 million in 2009, concerning this estimated raise in 2017 was $14,550 million (Research and Market, 2011). While the annual production of nano form of TiO_2 in 2025 is predicted to reach 2.5 million tons (Lin et al., 2014). TiO_2 NPs are transparent, can absorb and reflect UV light and one of the top five ENPs used in consumer products while ZnO NPs appears as the white powder and have remarkable optical, physical, and antimicrobial properties (Srivastava et al., 2015). The European Commission of Cosmetics Regulation has permitted the use of TiO_2 NPs in sunscreens; however not zinc oxide NPs (Vance et al., 2015). Both the NPs (TiO_2 and ZnO NPs) have been used in a wide array of applications such as cosmetics, paints, paper, food additives and personal care products (Uikey and Vishwakarma, 2016; Weir et al., 2012).

Nanoparticles, their aggregates, and ions with such a high production and utilization may get released into the environment in high amounts during all the stages of

processing. Thus, TiO_2 and ZnO NPs would leach into the environment, increasing the critical issues of addressing the safety of health and environment. Estimated concentrations of TiO_2 and ZnO NP in the sewage treatment plant are 950 and 200 mg/kg respectively, which increases by 10 mg/kg yearly and it is predicted to increase by 1-2 μg/kg per year in urban soil (Gottschalk et al., 2013). Similarly, the concentration of TiO_2 and ZnO NPs in the air is estimated to increase by 0.001-0.004 $\mu g/m^3$ (Sun et al., 2014). Further, in 10% of river stretch in Europe, about 150 ng/l concentration of ZnO nanoparticles is estimated (Dumont et al., 2015). These studies highlight the presence of ENPs in the environment and their unpredictable consequences on ecosystem and organism.

Published literature indicates that nanomaterials are toxic to a wide range of organisms such as bacteria, algae, crustacea, fish, *Daphnia*, fairy shrimp and amphibians such as *Xenopus laevis* and mammals (Kosyan et al., 2016; Hong and Zhang, 2016; Chakraborty et al., 2016; Yang et al., 2015). Much of the literature is available on the toxicity of TiO_2 and ZnO NPs ranging from the organism of different biota to varying properties of particles (different size or structure). TiO_2 and ZnO NPs cause DNA damage, chromosomal abbreviation, ROS generation, cytotoxicity, activation of inflammatory response and apoptosis (Hong and Zhang, 2016; Saliani et al., 2016; Dubey et al., 2015). A study reported the IC_{50} of TiO_2 (25.29±0.12, 34.99±0.09, 35.06±0.09 mg/l) and ZnO NPs (5.716±0.1, 3.160±0.1, 5.57±0.12 mg/l) depending on different cytotoxicity assays MTT assay, neutral red uptake assay, and lactate dehydrogenase assay respectively, on WAG cells. Further, dose-dependent damage to macromolecules such as DNA, lipid, protein, and alteration in total

antioxidant capacity was observed in WAG cell line (Dubey et al., 2015). Another study reports the toxicity of TiO_2 NPs with disodium laureth sulfosuccinate (surfactant) on digestive cell and lysosomal response of the cell. TiO_2 NPs accumulation in endosomes, lysosomes and residual bodies of digestive cells and disturb membrane integrity (Jimeno-Romero et al., 2016). There are reports about anatase/rutile samples of TiO_2 NPs to have higher toxicity than the pure anatase NPs on human intestinal Caco-2 cells. Further, it is studied that specific surface area and crystalline structure of NPs are the important aspects for determining the toxicity of TiO_2 NPs in intestinal cells (Gerloff et al., 2012). TiO_2 NPs induces genotoxic and cytotoxic effects at two trophic levels: plant (*Allium cepa* and *Nicotiana tabacum*) and human lymphocytes. In plant, TiO_2 NPs inhibits the root growth and induces lipid peroxidation, leading to DNA damage (4 mM) while in cell line TiO_2 NPs at the very low concentration (0.25 mM) induces cytotoxicity and genotoxicity (Ghosh et al., 2010). In liver cell lines (A549, V79) exposure to TiO_2 NPs induces internalization, oxidative stress, pro-inflammatory (LDH, Interleukin-8) and genotoxic response (Petrarca et al., 2015; Wang and Fan, 2014). However, TiO_2 NPs in neuronal cell line affect the cell structure, proliferation, changes the release and metabolism of neurotransmitters and the tendency of the exposed cell toward the neurons (Song et al., 2015; Ma et al., 2010). Most of the dermal exposure studies have reported TiO_2 NPs to have low penetration efficiency for stratum corneum of the dermal barrier while high concentration and long-time exposure of NPs induce cytotoxicity, inflammatory response, and oxidative stress (Crosera et al., 2015). ZnO NPs and TiO_2 NPs were classified as most toxic and harmful for all species on the basis of risk

assessment of 7 engineered NPs (TiO_2, ZnO, CuO, Ag, SWCNTs, MWCNs, and C60-fullerenes) on bacteria, algae, crustaceans, ciliates, fish, yeasts and nematodes (Kahru et al., 2010). Ecotoxicity study of TiO_2 NPs on microalgae *Scenedesmus* species and *Chlorella* species have reported a decrease in the chlorophyll content in the treated group (Sadiq et al., 2011). A comparative study on TiO_2 NPs in light and dark condition on *Bacillus licheniformis* has indicated increased ROS generation, reduced LDH level and high lethality in presence of light (Dalai et al., 2012). The *in-vivo* toxicity evaluation at sub-acute exposure (100 and 200 mg/l concentration) of TiO_2 NPs in juvenile carp (*Cyprinus carpio*) induces a significant decrease in antioxidant enzyme activity, increase in lipid peroxidation and histopathological changes indicating that liver is the most susceptible organ (Linhua et al., 2009). TiO_2 NPs have a negative effect on body length, hatching rate, reproductive ability and antioxidative defense system of *Daphnia magna* and zebra fish (Liu et al., 2014). In *C. elegans*, analysis of the effect of TiO_2 NPs in light and dark conditions indicated, that the presence of light caused significant oxidative stress and reduction in the reproductive ability of the worm (Angelstorf et al., 2014). In absence of a light significant alteration in expression of genes such as *cyp35a2, sod-3, ced-3, ced-4*, and genes involved in insulin signaling pathway has been reported (Khare et al., 2015; Roh et al., 2010). Further, metabolomics studies have indicated disruption of TCA cycle and neuronal toxicity in response to TiO_2 NPs exposure in *C. elegans* (Ratnasekhar et al., 2015). Through microarray followed by reproductive test, anatase particle was observed to be more toxic on metabolic pathways while rutile particle has shown greater toxicity on the developmental process (Rocheleau et al., 2014). Toxicity of

different shapes of TiO_2 NPs (rod, bipyramidal and quasispherical) revealed that rod-shaped NPs affect the pharyngeal pumping, reproduction, and development of *C. elegans* and found more toxic than bipyramidal and quasispherical NPs (Iannarelli et al., 2016). Chronic (7 days) exposure of TiO_2 NPs in earthworm *Lumbricus terrestris* has been reported to induce apoptosis in the cuticle, intestinal epithelium, and chloragogenous tissue (Lapied et al., 2011). Further TiO_2 NPs induces oxidative stress, genotoxicity and moderate inflammatory response in mice (Shi et al., 2013). Exposure of ultrafine TiO_2 particles at high concentration for long time induced lung cancer in rat (Trouiller et al., 2009). Therefore, The International Agency for Research on Cancer (IARC) classified TiO_2 as a Group 2B carcinogen and possibly carcinogenic to humans. In humans, TiO_2 NPs on reaching the circulatory system was found to adversely affect liver, spleen, kidney, and brain (Chang et al., 2013). National Institute for Occupational Safety and Health issued a report on the "Occupational Exposure to Titanium Dioxide" in 2011 and emphasized that TiO_2 NPs are able to induce lung tumor, lung cancer and other adverse effect (http://www.cdc.gov/niosh/docs/2011-160/pdfs/2011-160.pdf).

Among the *in-vitro* toxicity assessment, the primary culture of rat alveolar epithelial cell monolayers showed mitochondrial dysfunction and intracellular ROS production on exposure to ZnO NPs in dose and time-dependent manner (Kim et al., 2010). Similarly at sub-cellular level isolated mitochondria from ZnO NPs exposed Wistar rat liver showed alteration in mitochondrial membrane potential, the permeability of H^+ and K^+ ions, release of cytochrome C and generation of ROS (Li et al., 2012). The human alveolar epithelial-like type-II cell line A549 on exposure to ZnO NPs revealed

dose-dependent increase in cellular toxicity in terms of decrease in cell number, size, and viability by ROS generation and genotoxicity (Heim et al., 2015), Similarly the human lung epithelial cells (L-132) showed that ZnO NPs induces ROS generation and depletion in GSH level, cell shrinking, nuclear condensation, formation of apoptotic bodies and DNA fragmentation (Sahu et al., 2013). In primary astrocytes, ZnO NPs induced dose and time-dependent cytotoxicity (by MTT), oxidative stress (by LDH release, high ROS generation, caspase-3 activation), and apoptosis (by nuclear condensation and poly(ADP-ribose) polymerase-1 cleavage) by the activation of JNK pathway (Wang et al., 2014). The human bronchial epithelial cells (BEAS-2B) which were already under the oxidative stress of H_2O_2 showed higher toxicity in response to the low concentration of ZnO NPs compared to unstressed cells (Heng et al., 2010). Acute toxicity of ZnO NPs have been estimated in crustaceans (*Daphnia magna* and *Thamnocephalus platyurus*) and protozoan (*Tetrahymena thermophila*) with the L(E)C_{50} value of 1.1 and 16 mg/l, respectively (Blinova et al., 2010). In *C. elegans* the LC_{50} value for 10 nm ZnO NPs was found to be >0.7 g/l, that of <25 nm and <100 nm ZnO NPs were 0.32 mg/l and 2 mg/l respectively (Khare et al., 2011). Acute exposure (24 h) of ZnO NPs in *C. elegans*, induces reproductive toxicity and significant expression of stress-responsive gene such as *mtl-1* and *sod-1* (Khare et al 2015; Gupta et al., 2015). Chronic exposure (48 h and 72 h) of ZnO NPs (50 and 500 µg/l) on *C. elegans* led to increases ROS production and significantly reduced body bends as well as ATP levels thus, exert higher metabolic and locomotive toxicity compared to $ZnCl_2$ (Huang et al., 2017). In *C. elegans* exposure to ZnO NPs (61.4 µM) led to altered gene expression related to apoptotic pathways and induced more

apoptosis compared to $ZnCl_2$ (O'Donnell et al., 2017). Additionally, in plant-feeding nematodes *Xiphinema vuittenezi* the uptake and toxicity of nano-ZnO were higher than bulk-ZnO and $ZnSO_4$. Further, the toxicity of NPs was the combined effect of nano-specific and dissolved Zn in nematode (Sávoly et al., 2016). 14 days exposure of ZnO NPs in earth worm *Eisenia fetida* was acute and it showed a negative impact on reproduction (Canas et al., 2011). While, 7 days exposure of ZnO NPs in earth worm *Eisenia fetida* caused bioaccumulation, mitochondrial damage, alteration in antioxidative enzymes level and DNA damage (Hu et al., 2010). In marine brine shrimp (*Artemia salina*) larvae exposure to ZnO NPs caused significant increase in the lipid peroxidation (Ates et al., 2013). Exposure of ZnO NPs in murine macrophages (*in-vitro*) induces intracellular ROS generation and expression of NF-kB transcription factor. While, ZnO NPs exposure in male ICR mice (*in-vivo*) induces a gain of body weight, reduction in organ weight, dose-dependent alteration in organs histopathology and in biochemical parameters (alanine aminotransferase) (Hong et al., 2013). Moreover, mammalian toxicity studies showed that exposure of ZnO NPs induced inflammatory responses, alteration in enzyme activity and genotoxicity on mice, rat and human respectively (Bahadar et al., 2016; Slama et al., 2015; Rim et al., 2013).

The toxicity of TiO_2 and ZnO NPs depend on their size, structure, morphology, concentration, exposure dose/duration/medium or the test model used for the experiment. The adverse effects of NPs have been well studied in both *in-vivo* and *in-vitro* system (table 1.3). The studies in this field so far has established that both TiO_2 and ZnO NPs induced toxicity by either getting deposited on the cell membrane or by penetrating into the subcellular organelles thereby inducing ROS generation and

oxidative stress signaling cascades (Saliani et al., 2016; Dubey et al., 2015; Sabella et al., 2014; Manke et al., 2013), followed by genotoxicity, cytotoxicity, apoptosis, and inflammatory response (Shah et al., 2017; Saliani et al., 2016; Khare et al., 2015; Dubey et al., 2015; Wang et al., 2014; Kim et al., 2010). Since the ROS generation is the major cause behind the NPs mediated toxicity, the intracellular antioxidative defense system tries to cope up with the toxicity. If the system is not able to maintain the balance between the free radical generation and its removal, an antioxidant supplement can be used as prevention. It has been suggested that fruits, vegetables, and plants are the main source of antioxidant in the diet. Natural antioxidants may have free-radical scavengers, reducing agents, complexes of pro-oxidant metals, quenchers of singlet oxygen etc. In Asian history, as a natural remedies turmeric, honey, lemon, ginger, basil, curry leaves, fenugreek seeds, Indian malabar leaves, red silk cotton tree leaves, cowitch leaves, holy fruit tree leaves, and black mustard seeds etc were used against oxidative stress, toxicity, infection, inflammation, as an anti-ageing agent, food preservatives etc. Nowadays, the use of natural products/antioxidants in oxidative stress or in diseases condition is gaining lots of scientific attention because they are affordable, economic and easily available with minimum side effects.

Table 1.3 Adverse effects of TiO_2 NPs and ZnO NPs

NPs	Target	Effect	Reference
TiO_2 NPs	**Human glial Cells C6 and U373**	**↑ OS, ↑ MD**	**Huerta-Garcia et al., 2014**
	WAG cell line	**Acute toxicity, ↑ DNA**	**Dubey et al.,**

	(from gill tissue of *Wallago attu*)	**damage, ↑ OS**	**2015**
	Chinese hamster lung fibroblast cells	**↑ Cytotoxicity, ↑ apoptosis/necrosis**	**Hamzeh et al., 2013**
	Whole human blood Model	**Thromboinflammation, generation of bradykinin (fuel for chronic inflammation)**	**Ekstrand-Hammarstro m et al., 2015**
	Blood plasma	**↑ Cytotoxicity, ↑ ROS**	**Ganapathi et al., 2015**
	Escherichia coli	**↑ OS, ↑ DNA damage, ↑ cytotoxicity**	**Kumar et al., 2011**
	Fungus *Pichia pastoris*	**↑ Cytoxicity, ↑ OS**	**Yu et al., 2015**
	Raphidocelis subcapitata	**↑ MDA, ↑ mortality, ↑ membrane deformation**	**Ozkaleli and Erdem, 2018**
	Dunaliella tertiolecta	**Growth alteration, cell entrapment and agglomeration of NPs**	**Manzo et al., 2015**
	Daphnia magna	**↑ Acute toxicity, ↑ agglomeration**	**Seitz et al., 2015**
	C. elegans	**LC_{50} of 77 mg/l**	**Khare et al., 2011**
	C. elegans	**Altered physiology (lethality, growth, reproduction, and locomotion behavior)**	**Wu et al., 2013**
	C. elegans	**↑ ROS, ↓ reproduction, altered metabolites level, neuronal damage**	**Ratnasekhar et al., 2015**
	Juvenile Carp (*Cyprinus carpio*)	**↑ OS, ↓ AEA, ↑ hepatotoxicity**	**Linhua et al., 2009**
	Earthworm *Eisenia fetida*	**↑ Bioaccumulation, ↑ MD**	**Hu et al., 2010**
	Zebra fish (*Danio rerio*)	**↓ Growth, ↓ liver weight ratio, Accumulation in gills, livers, brains and heart tissues**	**Chen et al., 2011**
	Mice	**↑ ROS, ↑ glucose level**	**Hu et al., 2015**
	Mice	**↑ Chromosomal aberration, ↓ ADM**	**Rizk et al., 2017**
	Male Wistar rats	**↑ Hepatic and renal damage**	**Vasantharaja et al., 2015**
	Wistar rats	**Alteration in hepatic tissue,**	**Younes et al.,**

		accumulation, alter the neurobehavioral performance	**2015**
	Tomato	**↓ Growth, ↓photosynhetic ability**	**Tiwari et al., 2017**
	Wheat	**↓ Biomass of wheat, significant changes in soil enzyme activities**	**Du et al., 2011**
ZnO NPs	**WAG cell line (from gill tissue of *Wallago attu*)**	**↑ Acute toxicity, ↑ DNA damage, ↑ OS**	**Dubey et al., 2015**
	***Pseudomonas sp*., human promyelocytic leukemia cells, and peripheral blood mononuclear cells**	**↑ Cytoxicity, ↑ OS, ↑ genotoxic**	**Soni et al., 2017**
	MRC5 Human lung fibrobasts and *Drosophila melanogaster*	**↑ Cytoxicity, ↑ OS, ↑ genotoxic**	**Ng et al., 2017**
	Escherichia coli	**↑ OS, ↑ DNA damage, ↑ cytotoxicity**	**Kumar et al., 2011**
	Microalgae *pseudokirchneriella subcapitata*	**72 h, EC_{50} =0.04 mg/l**	**Aruoja et al., 2009**
	Daphnia magna	**48 h, LC_{50} =1.02 mg/l, feeding inhibition, ↓ reproduction**	**Lopes et al., 2013**
	C. elegans	**LC_{50} of 0.32 mg/l, ↑ cytotoxic, ↑ genotoxic, ↑ OS**	**Khare et al., 2015; Khare et al., 2011**
	C. elegans	**Affect survival in size dependent manner, gene expression of *mtl-1* and *sod-1***	**Gupta et al., 2015**
	C. elegans	**↓ Body bends, ↓ ATP level**	**Huang et al., 2017**
	Earthworm *Eisenia fetida*	**Bioaccumulation , ↑ MD**	**Hu et al., 2010**
	***Cyprinus carpio* carp**	**↓ ADM, ↑ OS**	**Hao et al., 2012**
	Nile tilapia	**↑ OS, ↓ SOD, ↓ CTL, ↓GPx**	**Abdelazim et al., 2018**
	Wistar rats	**↑ AST, ↑ ALT, ↑ morphological changes in rat tissues**	**Ben-Slama et al., 2015**
	Male Wistar rats	**Significant changes in liver enzymes, liver and renal tissue damage, sperm quality and**	**Abbasalipour kabir et al., 2015**

		quantity, ↑OS	
	Rice Seed *Oryza sativa* L	**Stunt roots length,reduce number of roots, phytotoxicity**	**Boonyanitipong et al., 2011**
	Wheat	**Reduced biomass of wheat, significant changes in soil enzyme activities**	**Du et al., 2011**

↑ = Increased level, ↓= Decreased level, OS= Oxidative stress, MD= Mitochondrial dysfunction, AEA= Antioxidative enzymes activity, ADM= Antioxidant defense mechanism, ROS= Reactive Oxygen Species, MDA= Malondialdehyde, ALT= Alanine aminotransferase, AST= Aspartate aminotransferase

1.2 ANTIOXIDANTS

All living organism utilizes antioxidants, either synthesized by them or supplemented through diet, to prevent cell damage from free radicals. Free radicals are the most reactive form of the chemical, mainly derived from oxygen, nitrogen and sulfur molecules such as hydrogen peroxide, hydroxyl radical, superoxide anion, nitric oxide, singlet oxygen, hypochlorite radical, lipid peroxides etc. Predominantly generated as a byproduct of mitochondrial electron transport chain in normal physiological condition and play a significant role in biological process such as cell signaling, apoptosis, gene expression, ion transportation (Zhang et al., 2016; Sewelam et al., 2016; Lu et al., 2010). Free radicals with an unpaired electron are highly reactive and either damage bio-molecules or passes unpaired electron to another recipient molecule turning recipient in to a free radical. Antioxidant defense system includes antioxidant enzyme such as super oxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, which help in maintaining the equilibrium between the production and clearance of ROS inside the organism. Antioxidants neutralize the unpaired condition of

free radical by accepting or donating the electron, thus maintaining the balance. In adverse conditions animal is unable to manage this balance. Uncontrolled ROS production is considered to be deleterious for cell organelles for it disturbs cell signaling or cellular redox and causes oxidative stress culminating in apoptotic conditions. In stress condition cell try to defend against the stress and activates antioxidative defense pathways which includes antioxidant enzyme such as super oxide dismutase (SOD), catalase (CTL), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and low molecular weight antioxidant molecules such as ascorbic acid, glutathione, tocopherols, carotenoids, and flavonoids etc. Antioxidative enzyme SOD react with super-oxides/lipid-oxides and convert it into hydrogen peroxides (H_2O_2) which is further converted in to water and oxygen by CTL. Similarly, reduced glutathione (GSH) donates its reducing equivalent ($H^+ + e^-$) to free radicals and neutralizes them by oxidizing itself. This reaction is catalysed by GPx enzyme. Oxidized GSSG is further converted in to GSH in the presence of glutathione reductase enzyme. Antioxidant supplement in stress condition is beneficial and help to minimize free radical generation as well as its concentration in cells. Antioxidants are classified based on their solubility, occurrence, reactive groups, essential or nonessential etc.

Types of antioxidants: antioxidants can be classified into three major groups phytochemical, vitamins and enzymes.

(i) **Antioxidant enzyme:** Some protein/enzymes have antioxidant properties. Human body can majorly synthesize these enzymes and others are taken up in diet. They are superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and catalases.

(ii) **Antioxidant vitamin:** Antioxidant vitamins are essential obtained from dietary sources, since human cannot produce them. Common antioxidant vitamins are folic acid, vitamins A, C, E, and beta-carotene.

(iii) **Antioxidant phytochemicals:** Phytochemicals are naturally found in plants, such as carotenoids, flavonoids, allyl sulfides, polyphenols.

Application of antioxidants:

In intracellular and extracellular environment antioxidants detoxify ROS and work as a free radical scavenger, hydrogen/electron donor, peroxide decomposer, singlet oxygen quencher, and metal-chelating agents (Das and Roychoudhury, 2014; Mates, 2000). High ROS generation or low functionality of antioxidants is the marker of oxidative stress condition which can lead to diseases conditions. Hence use of natural and synthetic antioxidants in such condition has been extensively studied. The potential application of antioxidants in various field have been reviewed in many studies (Mut-Salud et al., 2016; Szymanska et al., 2016).

(i) **Antioxidants in food:** Antioxidants have wide application spectrum. During the food processing or storage process naturally occurring antioxidants are lost. Thus, intentionally antioxidants or precursor of antioxidant are added to retain the product stability (Sani, 2016). Antioxidants are used as additives in oil and fat to prevent food from spoilage (Tagliafierro et al., 2015). Studies have indicated that spices and some herbs are good sources of antioxidants hence they or their extract are added to the products to increase their qualities such as aroma, taste, appearance and shelf-life (Kumar et al., 2015). Some synthetic and natural phenolic antioxidants [butylated hydroxyanisole (BHA), butylated

hydroxytoluene (BHT), and propyl gallate] behave as a chelating agent or reducing agent thereby inhibiting oxidation reaction (Shahidi and Zhong, 2010).

(ii) Medical application: Numerous studies have focused on the therapeutic properties of antioxidants (Mut-Salud et al., 2016; Szymanska et al., 2016). Compounds such as lanthanides (a coordinated compound with antitumour activity), selenium (an essential cofactor for antioxidant enzyme), flavonoids (plants polyphenols), lycopene and glutathione (intracellular antioxidant enzyme) have anticancerous properties (Sharma et al., 2016; Bhuvaneswari et al., 2014). Clinical trials performed on hepatocellular carcinoma patients have confirmed the protective role of antioxidants such as vitamin C, E etc in hepatic injury (Singal et al., 2011). The cerebellum which controls various motor activities in body is highly sensitive to ROS resulting in neuronal degeneration, hence antioxidant play an important role in delaying the progression of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and amylotrophic lateral sclerosis (Sharma et al., 2016; Bhuvaneswari et al., 2014). Oxidative stress can damage the normal physiological balance of body and induce chronic diseases such as rheumatoid, arthritis, cardiovascular disorders, ulcerogenesis and immunodeficiency. Thus, antioxidanthave been tried by many researchers for the control/treatment of various diseases (Sharma et al., 2016; San Miguel et al., 2013; Singal et al., 2011).

(iii) Antioxidants in cosmetics: Due to increased outdoor activities human skin is damaged by ultra violet (UV) rays, air pollutants and smoke, which leads to the generation of ROS, oxidative stress and ageing. It has been shown that

combination of organic, inorganic formulation with vitamin derivatives such as retinyl palmitate, ascorbyl tetraisopalmitate, tocopheryl acetate and plant extracts such as *ginkgo biloba*, Brazilian *Araucaria angustifolia* and *phorphyra umbilicalis* provide increased protection against UV radiation (da Mota et al., 2014; Gianeti and Maia Campos, 2014). Hence antioxidants are used in cosmetics for its protective effects against ageing (Bogdan Allemann and Baumann, 2008).

Adverse effects of antioxidants: antioxidants confer many health benefits but at the same time they can be dangerous too (Villanueva and Kross, 2012; Kizhakekuttu et al., 2010). A study investigated that high dose of vitamin C reduces the efficiency of some anti-cancerous drugs (methotrexate, doxorubicin and imatinib) and in turn promote survival of cancerous cell (Heaney et al., 2008). A review by Bjelakovic comprises about 80 studies on the clinical trial of antioxidants (vitamin A, C, E, beta-carotene and selenium) where high dose of vitamin E, beta-carotene or vitamin A, than recommended dietary dose (700 µg for women and 900 µg for men), increased mortality rate and thus, was found to be harmful for both healthy as well as diseased human (Bjelakovic et al., 2012). National center for complementary and integrative health have highlighted some studies on the adverse effect of antioxidant supplement such as high dose beta-carotene increases the risk of cancer in smokers while high dose of vitamin E increases risk of hemorrhagic stroke and prostate cancer, vitamin E caused bleeding in patients on anticoagulant medication (https://nccih.nih.gov/health/antioxidants/introduction.htm#ususe).

1.2.1 Antioxidants against metal toxicity:

Metals are ubiquitous in our environment and essential for biological functioning in all living organism in trace amount. Toxic effects of metals in certain forms and dose have been proven as a major threat associated with several heath risks. Metal exposure in human causes renal, lung, kidney diseases, also induces cancer and physiological damage (Ihmed, 2016; Zhai et al., 2015). Metals such as Fe, Co, Cr, Cu, etc. disturbs redox cycling and produces reactive free radicals leading to oxidative stress in the biological system. Various studies have indicated that ROS production and mitochondrial dysfunction are the major consequences of metal induced toxicity which further causes disturbance in antioxidant defense system, leading to activation of transcriptional factors, DNA damage, lipid peroxidation, immune responses, genotoxicity and in some cases induces cancer (Mazdeh et al., 2016; Sabella et al., 2014). Therefore, to retrieve the toxic effects of metals researchers have employed antioxidant supplements.

In *in-vitro,* the therapeutic properties of pure garlic acid were evaluated in human embryonic kidney cells (HEK 293) against cadmium toxicity (Boonpeng et al., 2014). Antioxidant mixture of resveratrol, ferulic acid, phloretin and tetrahydrocurcuminoids ameliorate adverse effects of Cu, Ni and Zn exposure on oral fibroblasts by increasing cell viability, DNA synthesis and decreasing ROS generation (San Miguel et al., 2013). Mexidol, carnosine, N-acetyl cysteine were found to mitigate lead, cadmium, cobalt, and molybdenum induced cell death in human neuroblastoma SH-SY5Y cells (Kulikova et al., 2016). Similarly, in the presence of thymoquinone, spirulina, gallic acid and vitamin C supplement, significant prevention against lead and arsenic induced oxidative stress has been reported in rat liver, kidney, blood and plasma

(Firdaus et al., 2016; Reckziegel et al., 2016; Zhai et al., 2015; El-Tantawy, 2015). Further, vitamin C and quercetin were identified for their protective efficacy against nicotine induced toxicity in the blood of Wistar rat (Paunovic et al., 2016). Reno-protective effect of zinc and/or vitamin E against lithium-induced nephrotoxicity was evidenced by histopathological and biochemical parameter in male rat (Omar et al., 2016). by *Pistacia lentiscus* oil (a shrub) was found to ameliorate sodium arsenate induced oxidative damage and liver dysfunction in Wistar rat (Klibet et al., 2016). Silver nitrate induced oxidative stress and alteration in biochemical parameters were mitigated on co-administration of selenium and/or vitamin E in rats (Gueroui and Kechrid, 2016). Protective effects of kolaviron and gallic acid were identified against cobalt chloride induced oxidative stress, biochemical and structural damage in heart and kidney of rat (Akinrinde et al., 2016). Quercetin and α-tocopherol showed a significant reversal of manganese and copper induced reproductive toxicity in Wistar male rat (Adedara et al., 2017; Mandil et al., 2016). Similarly, in mice preventive mechanism of vitamin E and C has been established on the basis of biochemical parameter and histological structures of kidney and testis against metal mixture (Pb, Hg, Cd and Cu) toxicity (Al-Attar, 2011; Sharma and Bhattacharya, 2010). Antioxidants (green tea, garlic and vitamin C) and arsenic co-exposed mice were observed for significant decline in arsenic induced liver and kidney damage (Amer et al., 2016). Hepatic and renal toxicity induced by the exposure of lead in rabbit was mitigated by the pretreatment of rosemary extract (Mohamed et al., 2016).

The protective efficacy of essential metals, vitamins, edible plants, phytochemicals, probiotics and dietary supplements against Cd and Pb toxicity is reviewed by Zhai et

al., 2015. This review recommended the intake of essential elements and vitamins in daily diet as preventive measures against heavy metal toxicity. Further, dietary supplements with the great health advantages were found to have fewer side effects than chelating therapy. Another review explained that arsenic interacts with sulphydryl groups, and formation of free radical leads to oxidative stress. Chronic exposure of arsenic at high concentration in drinking water to human increases the risk of skin lesions, peripheral vascular disease, hypertension, blackfoot disease and high risk of cancer. However, chelating agent is known for the treatment of arsenic toxicity but have their own side effects thus, antioxidant supplement (N-Acetyl cysteine, taurine, melatonin, α-Lipoic acid, vitamin-A, E) along with chelating agent (British anti-lewisite, 2, 3-dimercaptopropane 1-sulfonate and meso 2, 3-dimercaptosuccinic acid) may provide a better option for optimal effects (Flora et al., 2007). Additionally, 34 medicinal plants and 14 natural products were found potentially useful against arsenic toxicity in preclinical and clinical trials in human (Bhattacharya, 2017). Heavy metals such as arsenic, lead, mercury, and cadmium were found to induce oxidative stress which in turn casued hepatotoxicity, neurotoxicity, genotoxicity, nephrotoxicity. Natural and synthetic antioxidant was suggested as a possible remedy for metal induced oxidative stress and adverse effects (Sharma et al., 2014).

1.2.2 Antioxidants against nano-metal oxide toxicity:

Nano form of metal oxides is representative of new generation of material science. With enourmous applications of metal oxide nanoparticles (MONPs), their synthesis

as well as possible adverse effects are widely studied. In majority of MONPs exposed organism, oxidative stress was the hallmark (Mazdeh et al., 2016; Manke et al., 2013) which led to further damage at the physiological and biochemical level. Hence, to overcome oxidative stress generated due to MONPs, some natural antioxidants/compounds have been screened. Antioxidants are generally reducing agent which inhibits the oxidation of other bio-molecule and are categorized as hydrophilic and lipophilic based on their solubility. Antioxidants are necessary to overcome the redox misbalance induced by variety of the toxicant. Amelioration of petrol exhaust NPs adverse effects such as lipid per-oxidation and increased anti-oxidant level has been witnessed in erythrocyte by pre-treament of fenugreek leaf extract and quercetin (Durga et al., 2015). Similarly, cyto-protective effects of quercetin (50 µmol/l) against cyto-toxicity and apoptosis induced by Fe_2O_3 NPs (250 µg/ml) in murine hepatocytes was also noticed (Sarkar and Sil, 2014). Protective effect of vitamin E (0.01-2 mM) against the adverse effects by single-walled carbon nanotubes (SWCNTs) has been reported in neuronal cells PC12 cells, where presence of antioxidants led to increased cell viability and decreased oxidative stress and apoptosis (Wang et al., 2012). Polyphenol antioxidant danshensu (isolated from Chinese herb) is reported to reduce the cytotoxicity and oxidative stress induced by non-coated gold particle by quenching free radicals in mouse blood cells (Du et al., 2013). Dietary supplement with 5 mM N-acetyl-l-cysteine or over expression of SOD-3 retrieved Al_2O_3-NPs chronic exposure adverse effect of reduces locomotion and oxidative stress in *C. elegans* (Li et al., 2012).

Neurotoxic effect of silver oxide NPs on rat was ameliorated by the protective effect of vitamin E as evaluated by significant loss in body weight and destruction of the granular layer of the cerebellum (Yin et al., 2015). Green tea exhibit neuro-protective effects in rats against MnO_2 NPs (Sarkozi et al., 2017). Glycyrrhizic acid a sweet constituent of *Glycyrrhiza glabra* (liquorice) root has hepatoprotective and antioxidant effect against TiO_2 NPs induces hepatic injury and oxidative stress in rats (Khorsandi et al., 2015). Idebenone, carnosine and vitamin E administration either individually or in combination was found to efficiently ameliorate the alteration in biomarkers and histopathological changes in the liver on exposure to TiO_2 NPs (Sanna et al., 2015), in addition to this, the antioxidants significantly reduces inflammatory response and activation of apoptosis in male albino mice (Azim et al., 2015). Co-administration of quercetin or L-arginine with ZnO NPs alleviates the biochemical marker of hepatic toxicity, pro-inflammatory markers, tissue damage, DNA damage and metabolic disorder in Wistar albino rats (Baky et al., 2013). Similarly, the protective effects of quercetin and arginine against ZnO NPs-induced nephrotoxicity in Wistar albino rats has also been reported based on parameters such as serum inflammatory markers, serum urea and creatinine levels, reduced glutathione (GSH), histopathological alteration in kidney (Faddah et al., 2012). ZnO NPs induced renal toxicity in Wistar albino rats were ameliorated on co-administration of vitamin E and α-lipoic acid based on parameters such as level of endothelium growth factor, nitric oxide, inflammatory marker, blood glucose, serum urea, and creatinine in serum and levels of GSH in renal tissue (Rasheed et al., 2012). Co-administration of B-vitamins (B3, B6 and B12) along with ZnO NPs ameliorates hepatotoxic effect of ZnO NPs.

B-vitamins maintain the level of various biomarkers such as alanine aminotransferase, aspartate aminotransferase, lactate dehdrogenase, glucose level, malondialdehyde, glutathione peroxidase, apoptotic marker and inflammatory markers, near to control (Yousef and Mohamed, 2015). Green tea extract rescue rats from copper nanoparticles induce hepatotoxicity, oxidative stress, genotoxicity and apoptosis (Ibrahim et al., 2015). Pretreatment of vitamin E (200 mg/l) mitigates of Al_2O_3-NPs induces toxicity by reducing oxidative stress and intestinal permeability of NPs thus preventing translocation of NPs, neuronal and behavior damage in *C. elegans* (Yu et al., 2015). Similarly, anti-amyloid compounds protect against silica NPs induced protein homeostasis, protein aggregation and inhibition of serotonin neurotransmission in *C. elegans* (Scharf et al., 2016). Natural organic matter such as fulvic acids from Suwannee river or Pony lake is reported to reduces silver NPs induced toxicity in *C. elegans* by reducing its intracellular uptake (Yang et al., 2014). Accumulation and translocation of graphene oxide NPs from primary organ (intestine and pharynx) to secondary organs (reproductive system, tail) affect locomotion, reproduction, life span and microRNAs expression (regulatory genes of oxidative stress) of *C. elegans*. While, pretreatment of glycyrrhizic acid, an active compound of glycyrrhizae radix prevent graphene oxide NPs induced alteration in *C. elegans* (Zhao et al., 2016).

1.2.3 Curcumin:

Curcumin is a yellow colour, hydrophobic, active phenolic pigment of turmeric (curcuma longa linn or Jiang Huang), found abundantly (~80%) in the class of curcuminoids. Curcumin chemically known as *Diferuloylmethane,* is widely used in

spice, health care, food additives and preservation of food in India and other parts of Asia. Curcumin was first identified by Lampe and Milobedzka in 1913 and in Ayurveda curcumin has long history as a therapeutic agent in diseases conditions.

Curcumin has many functional groups such as β-diketo group, carbon–carbon double bonds and phenyl rings having many hydroxyl and methoxy substituents. Curcumin has an excellent free radical scavenger and chain breaking antioxidant ability. The phenolic OH group and the CH_2 group of the β-diketone moiety provide free radical scavenging activity to curcumin. The phenolic OH group is majorly credited for the antioxidant activity of curcumin. While the meta-methoxy groups were suggested to further increase its antioxidant activity. The reaction of curcumin with the molecular oxygen (O_2) abstract hydrogen atom from one of the phenolic hydroxyl groups thus initiating auto-oxidation of curcumin and generating phenoxyl radical. This phenoxyl radical is then reduced by removing a hydrogen atom from another curcumin molecule to form hydroperoxide. Subsequently, the hydroperoxide rearranges into the spiro-epoxide after losing a water molecule. Epoxide molecule hydrolysed by the hydroxyl group resulted in the formation of a stable product bicyclopentadione (Nimse and Pal, 2015). Curcumin is a potent inhibitor of free radicals, lipid peroxidation, DNA damage (single and double strand breakage) and nitrite induced oxidation of hemoglobin (Asouri et al., 2013, Unnikrishnan and Rao, 1995). Further curcumin is found to be more effective antioxidant than beta-carotene, vitamin-E, lipoic acid (Guzior et al., 2015; Khalil and Ali, 2011). β-carotene and curcumin co-adminstration is shown to protects scrotal hyperthermia and associated oxidative stress and apoptosis in spermatogenic cells of male mice (Lin et al., 2016).

High ROS production can modulate the expression of the nuclear factor-κβ (NF-κβ) and tumor necrosis factor alpha (TNF-α) pathways which are majorly involved in the inflammatory response and thus lead to chronic inflammation and diseases. Curcumin can down regulate the oxidative stress and the subsequent inflammatory response by the Nrf2 pathway. Curcumin can block TNFα production, NF-κβ signaling (which is the primary transcription factor involved in the initiation of the inflammatory response) alter the enzymes activity (cyclooxygenase-2, lipoxygenase, nitric oxide synthase) and inflammatory cytokines (interleukins (ILs), chemokines) (Fadus et al., 2016). ROS and inflammation are related to carcinogenesis processes and act as initiator of cancer. Pro-inflammatory responses are linked with tumor formation and other neurological disorder (Parkinson's disease). Further, preclinical research on curcumin has showed remarkable anticarcinogenic, anti-neurodegenerative properties (Tizabi et al., 2014; Lin et al., 2007). Promising results were obtained when curcumin was used for the treatment of cancer (Salem et al., 2014; Gupta et al., 2010). Curcumin is also reported to enhance the efficacy of cancer drugs against ovarian cancer and breast cancer (Chirnomas et al., 2006), lung cancer (Chanvorachote et al., 2009) and bladder cancer (Kamat et al., 2007). Curcumin is a highly pleiotropic molecule with many molecular targets such as transcription factors, inflammatory cytokines, kinases, growth factors and antioxidant system. Thus, curcumin can be defined as a potent antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, antigenotoxic agent. Additionally, curcumin was found protective against rheumatoid arthritis, neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases (Aggarwal et al., 2009; Mishra and Palanivelu, 2008). Curcumin was also found to be helpful in

improving overall functioning of β-cells in type 2 diabetes mellitus patients (Chuengsamarn et al., 2012). Similarly, pre-treatment of curcumin has been reported to ameliorate fructose and streptozotocin induces metabolic syndrome and diabetes in rats (Bulboaca et al., 2016).

1.2.3.1 Attenuation of oxidative stress by curcumin:

Curcumin has been reported to ameliorate toxicity against chemical/compound [H_2O_2, zearalenone, quinocetone (antimicrobial food additive in China)] induced oxidative stress related toxicity in *in-vitro* models, such as astrocytes, human airway epithelial cells, porcine granulosa cells, Human SK-N-MC cell line (Daverey and Agrawal, 2016; Qin et al., 2015). In *in-vivo*, the protective role of curcumin has been shown against hepatotoxicity induced by heavy metals (arsenic, cadmium, chromium, copper, lead, mercury, iron), zinc oxide NPs, diethyl nitrosamine, gentamicin, acetaminophen, tartrazine; neurotoxicity induced by monosodium-glutamate, carbofuran, mancozeb; renotoxicity induced by gentamicin, cadmium; genotoxicity and inflammation induced by lead ; reproductive toxicity induced by hexavalent chromium ; gasoline and dermal lesion induced by cypermethrin in rats (Akinyemi et al., 2017; EL-Desoky et al., 2017; Azab et al., 2016; Khorsandi et al., 2016; Habibian et al., 2016; Liu et al., 2016; Lee et al., 2016; Khalil et al., 2016; Jaiswal et al., 2016; Saber and El-Aziz, 2016; Ismail and Salem, 2016; Elsayed et al., 2015; Badria et al., 2015; Elhalwagy et al., 2015; Kadasa et al., 2015; Garcia-Nino and Pedraza-Chaverri, 2014; Mahmoud et al., 2014). Similarly in mice, curcumin is shown to attenuate the cellular toxicity and genotoxicity induced by sodium fluoride, chromium, 3 Gy γ-rays (Sharma et al., 2014; AL-Harbi et al., 2014; Tawfik et al., 2013; Devi and Raju,

2012). Additionally, curcumin provides protection against pneumonia infection caused by *Staphylococcus aureus* to mice (Wang et al., 2016). In *C. elegans*, curcumin has been shown to have anti-virulence, antibacterial, antipathogenic effects against *P. aeruginosa, B. pseudomallei*, tauopathies infection (Miyasaka et al., 2016; Eng and Nathan, 2015; Rudrappa and Bais, 2008). Further, curcumin exhibit therapeutic properties against arsenite and juglone (5-hydroxy-1, 4-naphthoquinone) induces neurodegenerative diseases and protect proteins from oxidative damage. Additionally curcumin extends the life span of organism (Yu et al., 2014; Yu and Liao, 2014; Alavez et al., 2011). In aquatic organism, the protective activity of curcumin was evaluated against chromium trioxide in fish *Channa punctatus* (Prasad et al., 2017). Further, dietary supplement of curcumin protects the antioxidant status and protein content in *Anabas testudineus* (Bloch) during long-term feeding (Manju et al., 2012). Additionally, beneficial effect of curcumin was reviewed against cardiovascular dysfunction and cancer (Kukongviriyapan et al., 2016; Pavan et al., 2016; Trujillo et al., 2013). Locomotion impairment in zebrafish and *Drosophila melanogaster* was recovered when curcumin was co-exposed with rotenone (Khatri and Juvekar, 2016). Similarly, co-exposure of curcumin and rosemary efficiently ameliorates gentamicin induced hepatotoxicity in guinea pigs (Azab et al., 2016).

Thus, curcumin is extensively studied and documented for its beneficiary effect but some contradictory reports are also available which states that curcumin induces cytotoxicity in healthy cells in non-targeted manner but toxicity of curcumin depend on the dose and type of cells lines in cancerous leukemia cells (Ravindran et al., 2009; Lantto et al., 2009). Some studies also highlighted the phototoxic effect of curcumin

(Atsumi et al., 2007). Protective effect of curcumin against various toxicants are listed in table 1.4.

1.2.3.2 Attenuation effect of curcumin against ENPs induced oxidative stress:

Recently, few studies have indicated curcumin ameliorates NPs induced toxicity. In human airway epithelial (HEp-2) and human breast cancer (MCF-7) cells, curcumin supplementation was found to efficiently abolish NiO_2 NPs induced ROS generation, oxidative stress, DNA fragmentation and apoptosis (Siddiqui et al., 2012). Similarly curcumin was found to mitigate oxidative stress mediated toxic effect of long term cadmium quantum dots (luminescent nanoparticles used in *in-vivo* imaging) exposure in *C. elegans*, noticed via the expression of ROS, SOD, GST and heat shock protein HSP-16.2 (Srivastava et al., 2016). Antiviral activity of curcumin modified silver nanoparticles (cAgNPs) was significantly higher than silver nanoparticles (Yang et al., 2016), against the respiratory syncytial virus. Additionally, hepatotoxicity induced by ZnO NPs in rat was found to be mitigated by the pre-treatment of curcumin at 200 mg/kg concentration (Khorsandi et al., 2016).

1.2.4 Ascorbic acid:

Ascorbic acid or vitamin C is a potent water soluble, essential vitamin and most widely used along with all other vitamins (Padayatty et al., 2003). The Recommended Dietary Allowances (RDA) for ascorbic acid is 90 mg/day for adult men and 75 mg/day for adult women (Naidu, 2003). Humans lack L-gulacolactone oxidase enzymes necessary for synthesis of ascorbic acid hence it is mainly obtained through diet. Ascorbic acid plays an essential role in growth, wound

repairing/healing/regeneration (bones, cartilage, teeth etc), produces collagen (a part of cartilage) and some principle protein which are involve in the formation of ligaments, blood vessels, tendons and skin (Naidu, 2003). Ascorbic acid is reported for its various beneficial and therapeutic effects by many researchers. Ascorbic acid is required for the synthesis of muscles carnitine (β-hydroxy butyric acid) which is important in fatty acids transfer and transportation into mitochondria (Nelson et al., 1981). Ascorbic acid act as a cofactor for enzymes such as ferrous [Fe(II)] and 2-oxoglutarate dependent dioxygenases (collagen synthesis), dopamine-β-hydroxylase (catalyze the conversion of dopamine neurotransmitter to norepinephrine) and hormones such as oxytocin, vasopressin, cholecystokinin and alpha-melanotripin (Lykkesfeldt et al, 2014). Ascorbic acid is reported to have anti-cytotoxic and anticancerous properties (Subramani et al., 2014; Mamede et al., 2012). Ascorbic acid is known to neutralize free radicals thus preventing DNA damage and tumor growth, it induces collagen synthesis which prevent tumor invasions to other tissues, further it also leads to inhibition of cell proliferation by interfering in cell cycle and insulin-like growth factor 1-receptor mediated apoptosis in cancer cells (van der Reest and Gottlieb, 2016; Naidu et al., 2001).

Ascorbic acid is a known sequester of free radical which maintains the antioxidant enzyme level in the body. Ascorbic acid in aqueous phase ($AscH^-$) donates a hydrogen atom ($H\cdot$ or H^+ + e-) directly to oxidizing radicals such as hydroxyl, alkoxyl and lipid peroxyl ($ROO\cdot$) to neutralize them and form H_2O, alcohol and lipid hydroperoxides. Further ascorbic acid itself produces the resonance-stabilized tricarbonyl ascorbate free radical ($Asc^{-\bullet}$) which is comparatively more stable and do

not cause cellular damage. Even the oxidized form of ascorbic acid such as semidehydroascorbate, dehydroascorbate etc are relatively stable. Ascorbate free radical reacts with NADH or NADPH-dependent reductases to regenerate ascorbic acid.

1.2.4.1 Attenuation effect of ascorbic acid against oxidative stress: Ascorbic acid ameliorates H_2O_2 induced oxidative stress in human chondrocytes (Chang et al., 2015), protects HL-60 cells (glutathione independent cells to transport or reduce dehydroascorbic acid) by generating dehydroascorbic acid (Guaiqui et al., 2001). Vitamin C supplement recues HL-7702 cells from dichlorodiphenoxytrichloroethane toxicity, which is known to affect viability of cells by inducing ROS generation, disrupting mitochondrial membrane potential and cytochrome C (Jin et al., 2014). Ascorbic acid mediated protective effect was identified against doxorubicin induced genotoxicity (mitotic recombination) in the somatic cells of *Drosophila melanogaster* (Fragiorge et al., 2007).

Ascorbic acid significantly attenuates butylated hydroxyanisole induced oxidative stress in colonic rats determined by level of reduced glutathione, total antioxidant activity, malondialdehyde, nitric oxide and validated by histopathological assay (Khalil and Ali, 2011). The changes in ROS production in the presence of various anticancer drugs at different temperatures in prostate cancer cells was significantly ameliorated by the ascorbic acid (Fukumura et al., 2012). Ascorbic acid was found to maintain the antioxidant enzyme levels, and nullify the morphological changes that occur in lungs and brain of adult albino rat, in response to chronic $CdCl_2$ exposure

(El-Sokkary et al., 2011). Imidacloprid insecticide belongs to the class of neonicotinoides act as an insect neurotoxin induces ROS generation and disturbs antioxidant enzyme regulation in mice. Pretreatment of vitamin C mitigate imidacloprid toxicity by reducing lipid peroxidation and maintaining the antioxidant defense system (EL-Gendy et al., 2010). Potassium bromated exposure in adult male albino rat induced cardiac muscles damage which was found to be ameliorated on administration of vitamin C (El-Deeb et al., 2015). Ascorbic acid has many health benefits as an antioxidant in biological system against oxidative stress (Chakraborthy et al., 2016; Nimse and Pal, 2015; Lu et al., 2010) and they are listed in table 1.4.

Ascorbic acid has low toxicity and does not cause serious damage. However, high dose of ascorbic acid increases excretion of urinary oxalate and uric acid which might have some role in kidney stone formation, especially in patients of renal disorder (Assimos, 2004). Further, vitamin C enhances the availability and absorption of iron from non-heme iron sources like in plants where iron is not attached to heme proteins: this could lead to iron overload and tissue damage in individuals with hereditary hemochromatosis (Jacob et al., 2002).

1.2.4.2 Attenuation effect of ascorbic acid against ENPs induced oxidative stress:

In primary rat hepatocytes the ameliorating effect of 22 antioxidants was evident against ZnO NPs. All the antioxidant showed hepatoprotective effect against ZnO NPs at different extent but propolis, boric acid and ascorbic acid were observed to have maximum protection (Turkez et al., 2016). In addition, oral intake of ascorbic acid reduces the acute pulmonary oxidative stress and inflammation induced by

intratracheal exposure to ZnO NPs in rat lungs (Fukui et al., 2015). Similarly, co-administration of ascorbic acid along with oral treatment of ZnO NPs significantly attenuates histopathological changes and hepatic oxidative damage in rat (Nemenqani, 2015; Nemenqani et al., 2015). In Wistar rat, ZnO NPs exposure showed dose dependent rise in liver enzyme concentration (AST, ALT, ALP) and disruption of liver cell membrane, which was potentially minimized in presence of ascorbic acid (Somayeh and Mohammad, 2014). Exposure of fine particulate matter (aerodynamic diameter=2.5 μm) induced oxidative stress, inflammation and inhibition of mitochondrial gene expression in 16HBE cells (Human bronchial epithelial cells), where in addition of ascorbic acid cured cells from respiratory oxidative damage (Jin et al., 2015). Ascorbate (vitamin C) and N-acetyl-l-cysteine were shown to efficiently reduce ROS level and boost antioxidant defense system, further it recovers the locomotion behavior defects in *C. elegans* caused by chronic Al_2O_3 NPs exposure (Li et al., 2012).

Table 1.4 Antioxidative effects of curcumin and ascorbic acid against xenobiotic oxidative stress

	Compound/Chemical/Metals	**Cells/Organism**	**Biomarkers**	**References**
Curcumin	**Hydrogenperoxide**	**SK-N-MC cells**	**↑ ROS generation, ↑ LPO, ↑ protein peroxidation**	**Kamarehei et al., 2014**
	Zearalenone	**Porcine granulosa cells**	**↑ OS, ↑ ROS levels, ↓ AEA**	**Qin et al., 2015**
	Juglone (5-hydroxy -1,4-	***C. elegans***	**↑ ROS generation, ↑ gene expression of**	**Yu et al., 2014**

	naphthoquinone)		***gst-4* , *sod-3*, and *hsp-16.2***	
	Paraquat	***Drosophila melanogaster***	**↑ OS, altered life span,**	**Xiang et al., 2011**
	Cadmium and chromium	***Cyprinus carpio carpio L***	**↓ SOD, ↓ GPx, ↓ GSH, altered level of GSSG**	**Karaytug et al., 2014**
	Chromium trioxide	***Channa punctatus***	**↑ Micronuclei, genotoxic**	**Prasad et al., 2017**
	Cadmium quantum dots	***C. elegans***	**↑ ROS, ↑SOD, ↑GST, ↑ HSP 16.2**	**Srivastava et al., 2016**
	Ethanol	**Adult male mice**	**↓ Expression of detoxifying genes, alteration in blood biomarkers, histopathology, AEA and liver peroxidation**	**Xiong et al., 2015**
	Lindane	**Adult male Wistar rat**	**↑ LPO, ↓ AEA**	**Singh and Sharma, 2011**
	cis-Diammineplatinum (II) dichloride	**Male Wistar rat**	**↑ MDA , ↑ ALT, ↑AST, ↓ SOD, ↓ CTL activity**	**Palipoch et al., 2014**
	Tartrazine	**Male Wistar albino rat**	**↑ MDA , ↑SOD, ↑ CTL, ↑GPx, ↑ GSH**	**EL-Desoky et al 2017**
	Gentamicin	**Rat**	**↑ Serum TNF-α, ↓ GSH, ↓ GPx, ↓ SOD**	**Mahmoud et al., 2014**
	$CdCl_2$	**Rat**	**↑ Adenosine deaminase, ↑ arginase, ↑LPO,**	**Akinyemi et al 2016**
	Cu(II)	**Rat**	**↑ OS**	**Huang et al., 2011**
	Monosodium glutamate	**Male Wistar rat**	**↑Acetyle choline esterase**	**Khalila et al 2016**
	Mercury	**Rat**	**↑OS, ↑MDA, ↑apoptosis, ↑serum latate dehydrogenase**	**Liu et al., 2017**
	Mercury	**Rat**	**↑ LPO, ↓ SOD, ↓ CTL, ↓ GSH, ↓ AEA**	**Agarwal et al., 2010**

Ascorbic acid	**Hydrogen peroxide and diethyl maleate**	**Porcine small intestinal epithelial cell line IPEC-J2**	**↑ Intracellular OS, ↓ membrane integrity, ↓ viability, ↓ wound healing capacity**	**Vergauwen et al., 2015**
	Sodium arsenite	**Human lymphocytes**	**↑ DNA damage, genotoxicity**	**Roy et al., 2014**
	ZnO NPs	**Male rats of Sprague-Dawley strain**	**↑ROS, ↑cytotoxicity, ↑OS**	**Türkez et al 2016**
	ZnO NPs	**Nile tilapia**	**↑ OS, ↓ SOD, ↓ CTL, ↓GPx**	**Abdelazim et al., 2018**
	Dimethoate	***Clarias batrachus***	**↑ GOT, ↑ GPT, ↑ ACP, ↑ ALP, ↑ renal protein**	**Dubey et al., 2015**
	Fenvalerate	**Male albino Wister rats**	**↑ ALT, ↑ AST, ↑ MDA, ↓ CTL, ↓ SOD, hepatorenal toxicity**	**Hussein et al., 2012**
	Formaldehyde	**Male albino Rat**	**↑ MDA, hepatotoxicity, inflammatory responses**	**Abdulqader and Mustafa, 2014**
	Formaldehyde	**Male rat**	**↑Urea, ↑creatinine**	**Kasnaviyeh et al., 2017**
	Carbon tetrachloride	**Rat**	**↑LDH, ↑ alkaline phosphatase, ↑totl bilirubin**	**Rahmouni et al., 2017**
	Dimethoate	**Rat**	**↑ MDA, ↓ SOD, ↓ CTL, ↓ GPx, ↑ OS in spermatozoa, ↓ sperm mobility, viability**	**Ben Abdallah et al., 2012**
	Fluoride and Chlorpyrifos	**Rat**	**↓ SOD, ↓ GSH, ↓ GPx, ↓ CTL, ↑ MDA,**	**Baba et al., 2013**
	Nickel and Lead	**Rat**	**↑ LPO, ↓ GSH, ↓ GPx, ↓ CTL, ↑ NO**	**Das and Saha, 2010**
	Chlorpyriphos and Lead acetate	**Rat**	**↑ OS, ↑ LPO, ↓ GSH, ↓ CTL, ↓ SOD, ↓ GPx, ↓ GST**	**Nisar et al., 2013**
	Cadmium	**Rice (*Oryza***	**↑ LPO, ↓ chlorophyll**	**Chao et al.,**

		***sativa*)**		**2010**
	Oxytetracycline	**Male white new zealand rabbits**	**↑ LPO, ↓ GSH, ↓ CTL, ↓ SOD, ↓ TAC, induces hepatonephrotoxicity**	**Abdel-Daim and Ghazy, 2015**

↑ = Increased level, ↓= Decreased level, OS= Oxidative stress, MD= Mitochondrial dysfunction, AEA= Antioxidative enzymes activity, LPO= Lipid peroxidation, ROS= Reactive Oxygen Species, NO= Nitric oxide, GSH= Reduced Glutathione, GPx= Glutathion peroxidase, GSSG= Oxidized glutathione, GST= Glutathione-S-transferase, MDA= Malondialdehyde, ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, SOD= Super Oxide Dismutase, CTL= Catalase, GST= Glutathione transferase, HSP= Heat shock protein, TNF-α = Tumor necrosis factor alpha, TAC= total antioxidant capacity, GOT= Glutamate Oxaloacetate Transaminase, GPT= Glutamate Pyruvate Transaminase, ACP= Acid phosphatase, ALP= Alkaline phosphatase, JNK= c-Jun N-terminal kinases

Curcumin and ascorbic acid are naturally occuring organic compound with excellent antioxdant property. Traditinally these antioxidants are included in diet becaause of their beneficial effect and high reducing power. The most remarkable feature of curcumin and ascorbic acid is that they have multiple beneficial functions with minimum probability of side effects and hence used as a therapeutic agent (Gupta et al., 2013; Ohno et al., 2009). Both the antioxidants are in clinical trials for the treatment of cancer (Fadus et al., 2016; Mastrangelo et al., 2016). Several reports states that these antioxdants scavenge free radicals and rescue biological system from heavy metal and metal oxides like arsenic oxide, cadmium oxide, zinc oxide, lead oxdie, titanium di-oxide induced toxicity (García-Niño and Pedraza-Chaverrí, 2014;

Gupta et al., 2013; Al-Attar, 2011). Therefore with the aim of determining easy and economical protection against NPs exposure, the amelioration effect of the antioxidants (curcumin and ascorbic acid) against the nano-TiO_2 and nano-ZnO induced adverse effects were investigated in an *in-vivo* model organism *Caenorhabditis elegans*.

1.3 *Caenorhabditis elegans*:

Caenorhabditis elegans is a free-living, microscopic, soil dwelling transparent nematode (round worm) belonging to nematoda phylum. Sydney Brenner was a pioneer in the area of neuronal development (Brenner, 1974) employing *C. elegans*. Research using *C. elegans* has helped researchers (Sydney Brenner, John Sulston, Robert Horvitz, Andrew Fire, Craig Mello and Martin Chalfie) bag Nobel prizes in Physiology and Medicine in the year 2002and 2006 and Nobel prizes in Chemistry in the year 2008.

1.3a Biology of *C. elegans:*

C. elegans is free living, unsegmented, bilaterally symmetrical, transparent nematode, about 1mm in length, and has three weeks of life span. *C. elegans* has 3-4 days of reproductive period and by self fertilization produces approximately 200-250 progeny in its life span. It has five pair of autosomes (I-V) and a pair of sex chromosomes (XX) and if the 6^{th} chromosomes is in XX combination produces hermaphrodite or in XO combination produces male *C. elegans* (Hunt, 2017; Maglioni et al., 2016; Kaletta and Hengartner 2006)

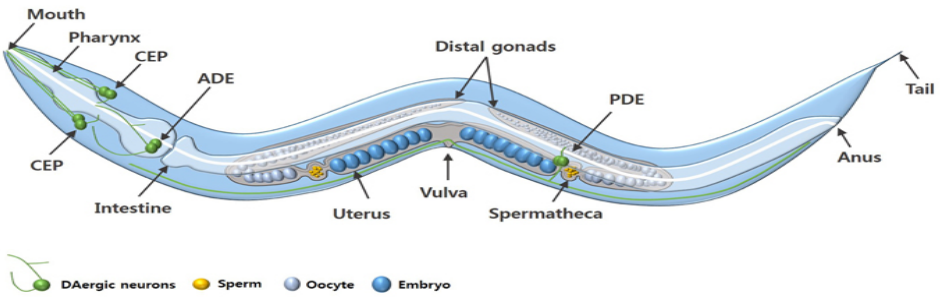

Figure 1.2 The anatomy of an adult hermaphrodite *Caenorhabditis elegans* worm (Source: Chege and Mccoll, 2014)

The simple anatomy of *C. elegans* comprises mouth, pharynx, intestine, gonad, and collagenous cuticle. Once food is taken up by mouth, pharynx (worked as muscular food pump in head region) pump the food into the intestine where food gets digested and it is finally excreted through anus. Cylindrical body of *C. elegans* is divided into outer tube and inner tube by pseudocoelomic space. The outer tube is made of cuticle, hypodermis, excretory system, neurons, and muscles, while the inner tube is made of pharynx, intestine and gonad. At L4 stage sperm maturation occurs in gonad region and in adult stage gonad triggers egg production and oocyte maturation. Mature oocytes encourage sperm movement into the spermatheca. In spermatheca sperm fertilizes the oocytes, finally the fertilized egg start to develop inside the body. Once embryo reaches 30-cell stage it is laid out of the body through vulval opening from the uterus. Embryogenesis in worm is completed in two steps (i) proliferation (ii) organogenesis/morphogenesis. Embryonic development of worms takes about 14 h and post-embryonic development takes about 43 h. An egg contains 558 cells fully formed larva in a 3-fold pretzel position inside the eggshell just before the hatching. After hatching the L1 molt successively and passes L2, L2-L3, L3-L4 and L4-Adult

(Figure 1.3). Under unfavorable conditions (lack of food, overcrowding and environmental stress), the larvae gets arrested in the dauer stage, in which it can survive up to several months and revive once conditions are favorable.

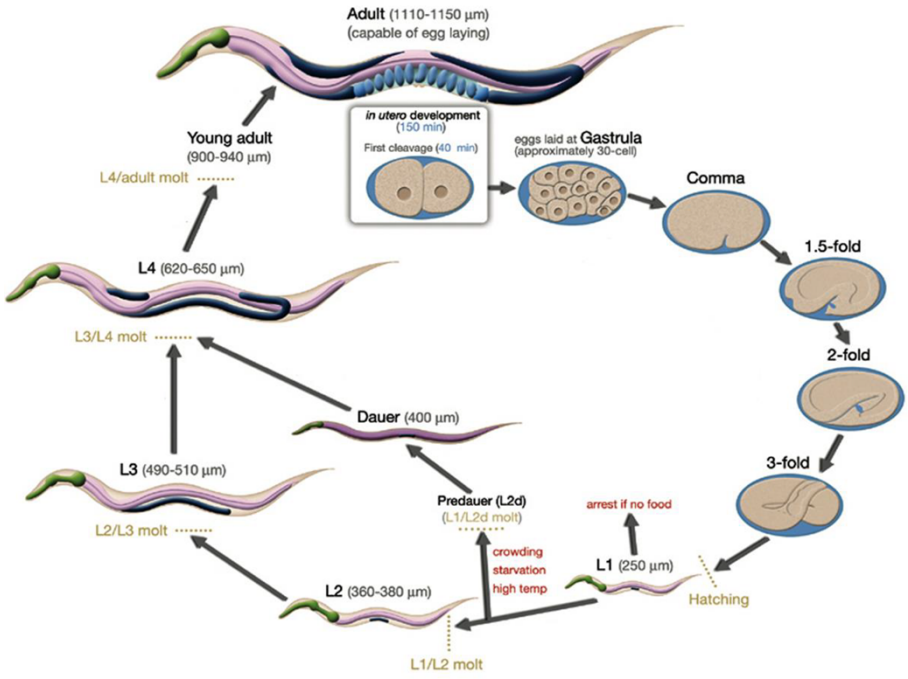

Figure 1.3 Life cycle of *Caenorhabditis elegans* at 20º C

(Source: Altun and Hall, 2009)

1.3b *C. elegans* as a model organism:

Caenorhabditis elegans has several advantages over the conventional model for first tier compound screening. First, the worms have a relatively low cost of cultivation because of their small size, rapid life cycle, and short life span (Maglioni et al., 2016; Kaletta and Hengartner 2006), which allow screening of thousands of animals on microtiter plates (O'Reilly et al., 2014). Second, 60–80% of human genes and 40% of human diseases gene have an ortholog in *C. elegans* (Maglioni et al., 2016; Chen et

al., 2013). Third, there are highly conserved biochemical pathways between worms and humans (Shaye and Greenwald, 2011). Fourth, the simple structure of the organism along with the ease of genetic manipulations, has led to the availability of mutants and RNA interference (RNAi) knockdown (nearly 90% of genome), which is an added advantage for mechanistic studies (Watson et al., 2013; Ceron et al., 2008). Further, in comparison to *in-vitro* models, (Fifth) *C. elegans* harbors a large repertoire of scorable phenotypes (O'Reilly et al., 2014), Sixth the multi-cellular and multi-organ system complexity existing in a whole organism improves the chances of identifying compounds that will ultimately be more efficacious in more complex multicellular organisms such as humans (Charao et al., 2015). In fact, several natural compounds have been screened in *C. elegans* to understand their effect on extension of the worms' life span, stress response protein (HSP-16.2), age-related behavioral declines and muscle degeneration (Cao et al., 2007; Brown et al., 2006). Further, *C. elegans* is also used for toxicity testing of chemical/compound/metal/nanoparticles (Hunt, 2017). Thus, we are using *C. elegans* to determining the amelioration effects of antioxidants against nanoparticles toxicity.

We are screening mitigation of TiO_2 and ZnO NPs because in literature, both TiO_2 and ZnO NPs have been used as model compounds to understand the possible toxicological implications of exposure to metal oxide NPs (Ratnasekhar et al., 2015; IARC 2010; Huang et al., 2010; EPA 2010). The affect of TiO_2 and ZnO NPs exposure has been studied in various *in-vitro* model of mice and human such as WAG cell line (Dubey et al., 2015), cell line A549 (Heim et al., 2015), mouse bone marrow mesenchymal stem cells (Syama et al., 2014), brain microglia and neurons

(Saquib et al., 2012), human amnion epithelial cells (Saquib et al., 2012), human epidermal cells (Shukla et al., 2011), human lymphocytes (Ghosh et al., 2010) as well as *in-vivo* rat model such as male Wistar rats (Vasantharaja et al., 2015), male rats (Slama et al., 2015), mice (Xie et al., 2012). In *C. elegans* (including our lab studies) the affect of TiO_2 and ZnO NPs exposure noticed, are similar to those found in the previous studies. Such as, oxidative stress mediated genotoxicity and cytotoxicity (Ratnashekar et al., 2015; Khare et al., 2015; Xiong et al., 2011; Huang et al., 2010), behavioral disruption (Khare et al., 2015; Kim et al., 2014) and mis-regulation of genes related to stress response and apoptosis, as indicated by biochemical and microarray studies (Khare et al., 2015; Huang et al., 2010). Majority of studies have implicated **R**eactive **O**xygen **S**pecies (ROS) generation as the major mechanism behind NPs toxicity (Ratnashekar et al., 2015; Cochran et al., 2013; Huang et al., 2010; Ramsden et al., 2009,). **Hence, we hypothesized that antioxidants might help in mitigation of NPs toxicity.**

We choose curcumin and ascorbic acid as antioxidants because they are economical and traditionally used as a food ingredient in India and other countries. Both, curcumin and ascorbic acid have efficiently shown their protective effect against various diseases/toxicity (reviewed in He et al., 2015; Harrison et al., 2014; Harrison, 2012). Extensive clinical trials over the past quarter century have addressed the pharmacokinetics, safety, and efficacy of curcumin as nutraceutical against numerous diseases in humans (reviewed in Gupta et al., 2013). Curcumin has already cleared Phase I of clinical trial for Cancer treatment. On the other hand, ascorbic acid or ascorbate is most commonly found water soluble antioxidant in nature. Ascorbate-

Glutahione cycle is major mitochondrial non-enzymatic antioxidant cycle in aerobic organism. Ascorbic acid is also involved in reduction of oxidized vitamin-E (α-tocopherol), another major fat-soluble antioxidant. Therefore, the present study is focused on elucidating the possible protective effects of anti-oxidants such as curcumin and ascorbic acid against adverse effects of NPs in a simple *in-vivo* model system *Caenorhabditis elegans* [ASTM, 2001]. Such common and well recognized antioxidants can provide easy and economically viable options for protecting environmental and occupational health.

Objectives

1. To evaluate the protective role of selected antioxidants against adverse affects of TiO_2 and ZnO nanoparticles.
2. Evaluate the molecular mechanism underlying antioxidant mediated protection against nanoparticles.

This may have application to cover wider groups of nano metal oxides and may provide insights for developing interventional/therapeutic strategies for higher organisms, including mammals.

Objective 1

Chapter 2

To evaluate the protective role of selected antioxidants against adverse affects of TiO_2 and ZnO nanoparticles

2.1 Introduction

Titanium dioxide NPs (nano-TiO_2) and zinc oxide NPs (nano-ZnO) are enormously used in field of cosmetics, medicine, food products, plastic, paints, paper, sunscreen, textiles, wastewater treatment, UV resistant material, as antibacterials etc. (Bui et al., 2017; Truppi et al., 2017; Colombo et al., 2017; Mendez-Medrano et al., 2016). Excessive use/application of these NPs enhances their release in to the environment. Studies show the predicted environmental relevant concentration (ERC) for nano-TiO_2 in surface water is 21 ng/l, in sewage treatment effluent is 4 µg/l (Wu et al., 2014) and 0.7 mg/kg in sediment which represents a main reservoir for the NPs (Praetorius et al., 2012). The predicted ERC for nano-ZnO in surface water is 0.01 µg/l (10% of river stretches in Europe 150 ng/l), in sewage treatment effluent is 0.432 µg/l, in sediments is 2.9 µg/l and in sludge-treated soil is 3.25 µg/l (Dumont et al., 2015; Gottschalk et al., 2009).Therefore, the risk of nanoparticles (NPs) exposure is high. In this context, it is crucial to determine the adverse effect of NPs on the environment and human and also to determine the possible ways of minimizing the impact of NPs.

Nano-TiO_2 is known to adversely affect bacteria [*Escherichia coli* (3 h; LC_{50} 17 µg/ml), *Vibrio fischeri* (18 h; EC_{50} 100 µg/ml; microbial assay for risk assessment)], Rotifers [*Brachionus plicatilis* (48 h; EC_{50} 5.37 µg/ml; less than 10% lethality was observed)], crustaceans [*Daphnia magna* (72 h; EC_{50} 3.8 µg/ml and 96 h; EC_{50} 0.73 µg/ml; immobilization test) , zebrafish (96 h; LC_{50} of 124.5 µg/ml), *C. elegans* (24 h; LC_{50} 77 µg/ml) (Lin et al., 2014; Minetto et al., 2014; Xiong et al., 2011; Dabrunz et al., 2011; Khare et al., 2011). Similarly, nano-ZnO were also found to cause lethality in bacteria [*Bacillus*

subtilis (LC_{50} 0.3-0.5 µg/ml) *Escherichia coli* (LC_{50} 15-43 µg/ml)], crustaceans [*Daphnia magna* (48 h mortality; LC_{50} 3.2 µg/ml), *Thamnocephalus platyurus* (24 h mortality; LC_{50} 0.18 µg/ml)], zebrafish embryo (5days exposure; LC_{50} 3.5-9.1 µg/ml), *C. elegans* (24 h; LC_{50} 0.45 µg/ml), earthworm *Eisenia fetida* (96 h; LC_{50} 50 µg/ml) (Wehmas et al., 2015; Khare et al., 2015; Li et al., 2011; Heinlaan et al., 2008). Both the *in-vivo* and *in-vitro* studies have shown, nano-TiO_2 and nano-ZnO induces strong oxidative stress, inflammatory response, alteration in antioxidative enzymes level, organelle dysfunction and provoke cell death (Kosyan et al., 2016; Chakraborty et al., 2016; Saptarshi et al., 2015; Pandurangan and Kim, 2015; Sha et al., 2015; Shi et al., 2013). Majority of these studies have emphasizes on reactive free radical generation (reactive oxygen species, reactive nitrogen species and reactive sulfur species) as the primary means for adverse effect of NPs (Saliani et al., 2016; Mazdeh et al., 2016; Khare et al., 2015; Saptarshi et al., 2015; Pandurangan and Kim, 2015; Sha et al., 2015). In the normal course, free radical acts as a messenger for basic physiological and biochemical phenomenon, while in an adverse condition they play a vital role in generating oxidative stress which in turn leads to inflammation, genotoxicity, apoptosis, and cytotoxicity (Zijno et al., 2015; Ivask et al., 2010).

Various natural compounds are known to neutralize the free radicals by exchange of electron(s), thereby reducing the damage (Turkez et al., 2016; Nemenqani, 2015; Al-Rasheed et al., 2015; Fukui et al., 2015; Yousef and Mohamed, 2015; Siddiqui et al., 2012). Further, it has been reported that co-exposure of curcumin with nano-nickel oxide rescue cultured human airway epithelial (HEp-2) and human breast cancer (MCF-7) cells from nanoparticle induced cytotoxicity and oxidative stress (Siddiqui et al., 2012). Similarly, ascorbic acid was successfully employed to reduce nano-ZnO induced oxidative stress in the

rat (Fukui et al., 2015; Nemenqani et al., 2015). Few other studies have revealed the efficient use of natural compounds/chemicals or antioxidants such as quercetin, L-arginine, vitamin B and C against nano-iron oxide-mediated oxidative stress and cell death in murine hepatocytes cell (Sarkar and Sill, 2014) as well as against nano-ZnO induced oxidative damage in rat (Nemenqani, 2015; Baky et al., 2013). Boric acid, propolis, and ascorbic acid were found to be hepatoprotective against ZnO NPs induced primary rat hepatocytes (Türkez et al., 2016). A majority of the studies focused on the co-administration of antioxidant with a particular dose of toxin (not lethal dose) and are limited only up to the specific tissue or biochemical marker determination. Recently, curcumin was shown to protect worms from cadmium quantum dots induced acute toxicity (Srivatsava et al., 2016). However, there has been a lacuna regarding the understanding of the therapeutic value of the antioxidant during accidental high dose exposure of nanoparticles or altogether preventing the adverse effects by incorporating antioxidants in the everyday diet. Comprehensive information about the attenuation ability of antioxidants against nano-induced chronic toxicity has not been reported so far. Thus, the present study was carried out with the aim of determining the extent of protection that antioxidant (curcumin and ascorbic acid) can confer against nano/bulk TiO_2/ZnO, chronic/acute exposure using *Caenorhabditis elegans*.

Our preliminary studies have also shown lethality and adverse toxicity of nano/bulk TiO_2 and ZnO in *Caenorhabditis elegans* (Ratnasekhar et al., 2015; Khare et al., 2015; Khare et al., 2011) which were similar to those noticed in higher model systems. Therefore, in the present study, pre-, post- or along with-antioxidant supplement were given as a measure against nano/bulk TiO_2 or ZnO, acute/chronic exposure, and both survival, as well as ROS

generation, was determined in all cases. This study was carried out with an aim to identify an easy and economical way to reduce the toxic potential of the NPs.

2.2 Material & methods

2.2.1 Test organism:

2.2.1.1 Growth medium and solutions:

2.2.1.1.1 Nematode Growth Media (NGM) Agar:

The constituents of NGM are 34.22 mM NaCl, 2% Bactoagar, 0.59% Bactotryptone, 6 mM Tris-HCl (pH 7.04). The media was autoclaved to sterilize and allowed to cool. At ~55ºC, 0.02 µM cholesterol was added and mixed well. The media was then poured into the petri dish in laminar flow and allowed to solidify at RT. In laboratory, *C. elegans* were propagate and maintained on the Nematode Growth Medium (NGM) agar plates with lawns of nonpathogenic *Escherichia coli* strain OP50 as a food source (Brenner, 1974).

2.2.1.1.2 Luria Bertani (LB) broth/Agar media:

2.5 g of LB broth (Himedia) was dissolved in 100 ml DW and autoclaved. Further for LB plates 4 g of LB agar (Himedia) was mixed in 100 ml DW and autoclaved. Sterile LB agar was poured in 90 mm petri plates. Allow to dry for 2-3 h then covered with seran wrap/plastic wrap and stored at RT for further use.

2.2.1.1.3 Bleach (0.5%) solution for egg isolation:

10 N NaOH, household bleach (4% solution of sodium hypochlorite) and autoclaved water was mixed in ratio 1:1:8. Due to the tendency of the sodium hypochlorite to

degrade over time, the bleaching solution was prepared fresh before every egg isolation process.

2.2.1.1.4 Phosphate Buffer (PB) (pH 7.0±0.2):

1 N KH_2PO_4 was titrated with 1 N K_2HPO_4 until the pH 7.0 was reached. The solution was autoclaved and stored at RT.

2.2.1.2 Culture and Maintenance:

2.2.1.2.1 *Escherichia coli*:

A single colony of *E. coli* OP50 bacteria was streaked on LB agar plate then allowed to grow overnight at 37ºC. From newly streaked plate a single colony of OP50 *E. coli* was inoculated in LB broth and incubated at 37 ºC at 200 rpm, till the culture reaches log phase at OD600 =0.8.

2.2.1.2.2 *Caenorhabditis elegans* embryo isolation and maintenance:

C. elegans culture was maintained on the NGM agar plates seeded with *Escherichia coli* OP50, at 20°C in an incubator for 3 days. These plates were harvested by rinsing with PBS and collected in a 15 ml centrifuge tube. After centrifugation at 300 X g for 5 min, the supernatant was discarded; the worm pellet was treated with freshly prepared 0.5% bleach solution and vortexed for 8-10 min. The suspension was again centrifuged at 300 X g for 5 min and the supernatant was discarded. Bleach treatment kills all life stages of worms except the eggs. The egg pellet was washed and centrifuged thrice with PBS and subsequently loaded on fresh NGM agar plates seeded with *E. coli* OP50. After 15-16 h, synchronized L1 and 48 h L4 stage worms were harvested by rinsing with water into a 15 ml centrifuge tube, centrifuged at 300 X g for 5 min and the supernatant was discarded (Donkin and Williams, 1995). Pellet was washed 3 more times with water

which removes all the bacteria. Thus, pellet obtained contain synchronized L1 and L4 stage worms and were used for the experiments.

2.2.2 Test material:

Titanium-(IV)-oxide anatase (TiO_2) (<21nm and bulk) and zinc oxide (ZnO) (<50nm and bulk) along antioxidants (curcumin and ascorbic acid) were purchased from Sigma-Aldrich, USA.

2.2.2.1 Characterization of nanoparticles:

The stock solutions of 200 µg/ml for TiO_2 and 80 µg/ml for ZnO were prepared in ultrapure Milli-Q water (Millipore, India) and sonicated for 5 min with 45/15 sec on/off pulse (Sonics & Material Inc, Newtown, U.S.A) to obtain a homogeneous suspension. The particle size distribution, as well as zeta potential, was analyzed by Zetasizer (Malvern Instrument Ltd., UK) and pH was determined by pH meter (Thermo Fisher Scientific, USA), for stock suspension. Particle stability in suspension was monitored for 0 h, 24 h, and 72 h. The size and compositional analysis of both the particles were determined by transmission electron microscopy (TEM, TECHNAI G2, FEI, Netherland) and scanning electron microscopy (SEM with EDAX-ApolloXL, FEI Company, Netherland), respectively.

2.2.3 Lethality assay:

2.2.3.1 Chronic toxicity: Synchronized L1 stage worms were exposed to five-seven concentrations (0-80 µg/ml for TiO_2 and 0.1-2.5 µg/ml for ZnO) of NPs and bulk for 72 h, in the presence of food in ultrapure MilliQ water. To minimize the agglomeration of NPs, treatment was given in the ultrapure water with continuous shaking. The experiment was repeated thrice. Worms were counted as live and dead by visual

inspection under dissecting microscope by gentle probing with Pt-wire (Sonane et al., 2017). LC_{50} was calculated by EPA probit analysis.

2.2.3.2 Acute toxicity: Synchronized L4 stage worm 20 (±1) were exposed to five-six concentrations (0-500 µg/ml for TiO_2 and 0.1-5 µg/ml for ZnO) of NPs and bulk for 24 h at 20°C without food source in ultrapure MilliQ water (Sonane et al., 2017; Khare et al., 2011). Worms were scored as mentioned in 2.2.3.1.

2.2.4 Recovery assay:

2.2.4.1 Antioxidant supplement and chronic toxicity:

Amelioration from chronic toxicity by antioxidant supplement was assessed by exposing age synchronized worms to the LC_{50} of nano/bulk particles for 72 h. The antioxidants were given along with food/nanoparticles and based on antioxidant supplement they are categorized into three groups:

a. 48 h antioxidant supplement: L1 worms exposed to the nano/bulk particles and after 24 h the dead worms were scored and curcumin/ascorbic acid (concentration range: 20 µM-140 µM) were added, again after 48 h worms were scored for live and dead (total 72 h NPs exposure)

b. 24 h antioxidant supplement: L1 worms exposed to the nano/bulk particles and after 48 h the dead worms were scored and curcumin/ascorbic acid (concentration range: 20 µM-140 µM) were added, again after 24 h worms were scored for live and dead (total 72 h NPs exposure)

c. No antioxidant: L1 worms exposed to nano/bulk particles and after 72 h the live/dead worms were scored.

2.2.4.2 Antioxidant supplement and acute toxicity:

Amelioration from acute toxicity by antioxidant supplement was assessed in two sets of the experiment:

a. 48 h antioxidant supplement: Age synchronized L1 worm, were allowed to attain young adult stage, in presence of food along with antioxidants (10 µM-180 µM curcumin/ascorbic acid). The pre-treated young adults were exposed to the LC_{50} concentration of nano/bulk (TiO_2 and ZnO) for 24 h in the presence (10 µM-180 µM curcumin/ascorbic acid) and absence of antioxidants. Live and dead worms were scored as mentioned in 2.2.3.1.

b. 24 h NPs exposure in presence of antioxidant: Age synchronized L4 stage worms were exposed to the LC_{50} concentrations of nano/bulk at the different concentrations of antioxidants (ranging from 20 µM-180 µM curcumin/ascorbic acid). Live and dead worms were scored as mentioned in 2.2.3.1.

2.2.5 ROS assay:

Generation of reactive oxygen species (ROS) among nano/bulk exposed worms (with or without antioxidant) was determined by 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA). This is a cell-permeable non-fluorescent dye. Worms exposed to nonlethal concentrations of rotenone (Sigma-Aldrich, MO) were used as positive controls both in acute and chronic experiments. After treatment about 1000 worms from control and treated groups were separately transferred into each well of a transparent bottom black 96-well plate in triplicates to which 0.05 mM H_2DCF-DA dye (Sigma, U.S.A) was added and incubated for 30 min on an orbital shaker. Dye enters into a cell and gets de-esterified intracellularly. Nonfluorescent dye turns into highly fluorescent

2′,7′-dichlorofluorescein upon oxidation. ROS was measured at 485/535 nm in Spectrophotometer Spectramax (Molecular Devices, UK) (Ratnasekhar et al., 2015).

2.2.6 Statistical analysis:

Dose-response curve was plotted based on percent mortality (Microsoft office excel, 2007) and LC_{50} was calculated by EPA Probit Analysis Program (Version 1.5). Percent survival was determined and survival graph was plotted (Microsoft office excel, 2007). Further based on the comparison of % survival of worms in treatment solutions in absence/presence of antioxidant, recovery was calculated, assuming 0% recovery for treatment group (without supplement). One way-ANOVA and student t-test was carried out to compare the differences between groups. Differences were considered statistically significant when $p<0.05$, with Bonferroni posthoc corrections. For ROS fold-change was determined and plotted using Excel. Fold change was expressed as mean±SEM, fold change of ±0.5 were considered significant.

2.3 Results

2.3.1 Characterization of nano/bulk TiO_2 and ZnO:

The average particle sizes determined by TEM of nano-TiO_2, bulk-TiO_2, nano-ZnO, and bulk-ZnO were 11 nm, 124 nm, 21 nm and 242 nm respectively (Figure 2.1). SEM-EDAX analysis revealed the percent of the chemical composition, for nano-TiO_2 (Titanium 47.5%, Oxygen 23%, and Carbon 13%) and nano-ZnO (Zinc 65.5%, Oxygen 23%, and Aluminum 2.9%). The hydrodynamic size of the particle as obtained through DLS is greater than the TEM size because it is the size of the particle along with the solvent layer attached to it, as the particle moves under the influence of Brownian motion. The hydrodynamic size for

nano-TiO_2, bulk-TiO_2, nano-ZnO, and bulk-ZnO were 240±10 nm, 346±1.7 nm, 153±0.6 nm and 1589±213 nm and zeta-potential were found to be -14±0.7 mV,-21±1.6 mV, 22±0.6 mV and -19±0.9 mV, respectively. The particle size distribution in exposure medium was found to be constant for 0 h, 24 h and 72 h in the absence/presence of antioxidants (table 2.1, 2.2).

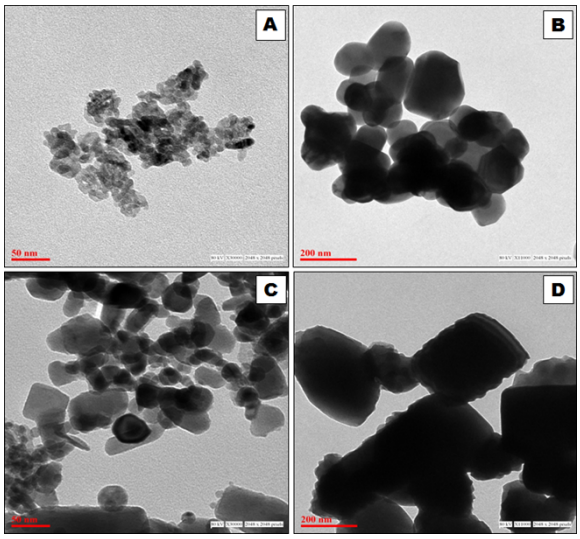

Figure 2.1 Transmission-electron micrographs of (A) <25 nm nano-TiO_2 (B) bulk-TiO_2 (C) <50 nm nano-ZnO and (D) bulk-ZnO

Table 2.1 Characterization of nano/bulk TiO_2 and ZnO particles

Physio-chemical parameters	Duration in exposure medium	Nano-TiO_2 <25 nm	Bulk-TiO_2	Nano-ZnO <50 nm	Bulk-ZnO
Average particle size[1]	-	11 nm	124 nm	21 nm	242 nm
Chemical composition[2]	-	Ti, O, C	Ti, O, C	Zn, O, Al	Zn, O, Al
Percentage of chemical composition[2]	-	47.5, 22.6, 13.1	50.9, 27.1, 21.9	65.5, 23, 2.9	75.2, 16.6, 1.14
Hydrodynamic size[3]	0 h	240±10	346±1.7	153±0.6	1589±213
	24 h	338±37	342±1.9	347±1.4	2283±167
	72 h	215±15	345±3.3	345±1.2	1787±158
Poly dispersity index[3]	0 h	0.18	0.17	0.15	0.37
	24 h	0.26	0.17	0.155	1
	72 h	0.16	0.17	0.16	0.85
Surface charge[3]	0 h	-14±0.7	-21±1.6	22±0.6	-19±0.9
	24 h	-14±0.1	-18±1.9	24±2.1	-14±0.4
	72 h	-15±0.1	-18±1.5	20±0.04	-14±3.1
pH	0 h	7±0.03	6±0.14	7±0.2	6±0.1
	24 h	7±0.4	7±0.14	7±0.1	7±0.2
	72 h	7±0.07	7±0.07	7±0.07	7±0

[1]As measured by transmission electron microscopy (TEM)

[2]As measured in scanning electron microscope-EDAX (SEM-EDAX)

[3]Determined through Zeta Sizer through Dynamic light scattering (DLS) and Zeta potential

Table 2.2 Characterization of nano/bulk TiO_2 and ZnO in the presence of antioxidants (100 µM)

Antioxidants		Ascorbic acid				Curcumin			
Physio-chemical parameters	**Duration in exposure medium**	**Nano-TiO_2 <25 nm**	**Bulk-TiO_2**	**Nano-ZnO <50 nm**	**Bulk-ZnO**	**Nano-TiO_2 <25 nm**	**Bulk-TiO_2**	**Nano-ZnO <50 nm**	**Bulk-ZnO**
Hydrodynamic size[1]	**0 h**	**341±6.3**	**435±2.3**	**341±3.3**	**732±83**	**333±1.4**	**446±9.4**	**132±3**	**916±90**
	24 h	**309±3.2**	**385±17**	**304±3**	**3880±82**	**304±8.4**	**661±73**	**122±4.4**	**689±65**
	72 h	**262±1.8**	**416±13**	**262±1.5**	**2651±15**	**244±3.7**	**652±8.9**	**115±2.1**	**977±107**
Surface charge[1]	**0 h**	**-23±0.4**	**13±0.05**	**-27±0.3**	**-28±0.7**	**-19±0.3**	**11±0.14**	**-27±2.3**	**-17±0.02**
	24 h	**-26±0.8**	**14±0.2**	**-27±1.2**	**-9±1.1**	**-24±1.2**	**9±0.72**	**-24±0.6**	**-19±1**
	72 h	**-22±0.2**	**14±0.2**	**-20±4.3**	**-10±1.9**	**-20±0.1**	**11±4.5**	**-19±1.3**	**-19±0.4**
pH	**0 h**	**7±0.07**	**6±0.07**	**6±0.07**	**6±0.2**	**6±0.1**	**7±0.1**	**7±0.1**	**7±0.3**
	24 h	**7±0.2**	**7±00**	**7±0.2**	**7±0.14**	**7±0.2**	**7±0.35**	**7±0.4**	**7±0.4**
	72 h	**6±0.07**	**7±0.14**	**7±0.0**	**6±0.07**	**7±0.4**	**7±0.42**	**7±0.01**	**7±0.3**

[1]Determined through Zeta Sizer through Dynamic light scattering (DLS) and Zeta potential

2.3.2 Dose response:

The LC_{50} values (lethal concentration at which 50% worms were dead) for chronic exposure (72 h, L1 to L4) of nano-TiO_2, nano-ZnO, bulk-ZnO were found to be 54.2 µg/ml, 0.18 µg/ml and 0.93 µg/ml respectively, while bulk-TiO_2 was found non-lethal up to 200 µg/ml. Similarly, the LC_{50} values for acute exposure (24 h) in young adult were found to be 172 µg/ml, 1.125 µg/ml and 4.64 µg/ml for nano-TiO_2, nano-ZnO and bulk-ZnO. However, bulk-TiO_2 was found non-lethal even at the 500 µg/ml (Figure 2.2).

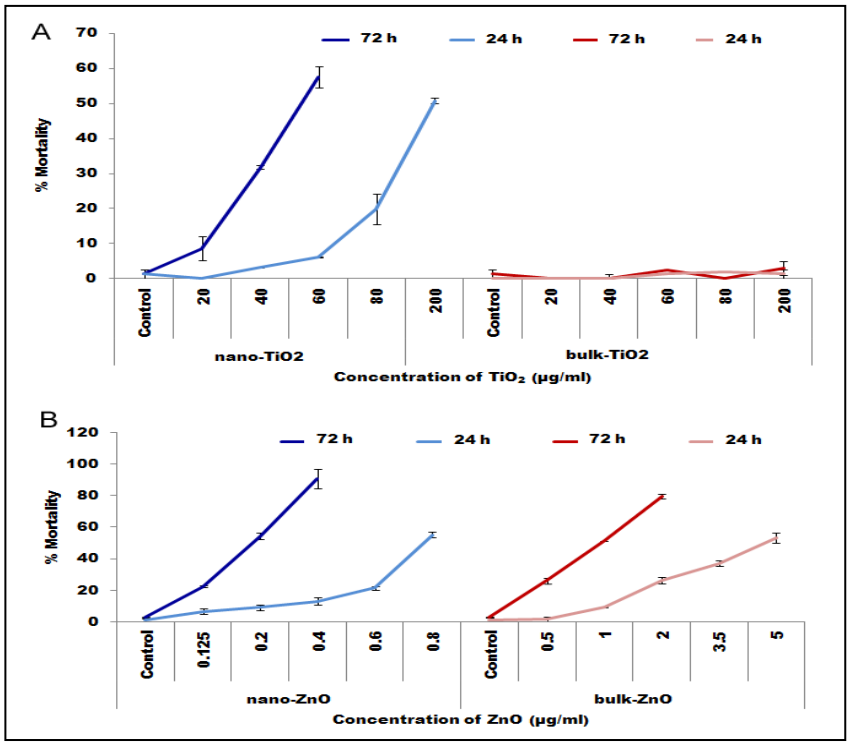

Figure 2.2 Dose response curve of 72 h and 24 h exposure for (A) nano/bulk TiO_2 and (B) nano/bulk ZnO

2.3.3 Amelioration effect of antioxidants from NPs induced lethality in *C. elegans*:

Recovery assay for nano-TiO_2, nano-ZnO, and bulk-ZnO was carried out at both acute as well as, chronic level in absence or presence of an antioxidant. Bulk-TiO_2 being non-lethal was not employed in this experiment.

Pre-antioxidant supplement of 48 h (L1-L4 stage) to worms followed by the acute treatment of nano/bulk (24 h) in absence or presence of antioxidant(s) did not lead to any significant mortality at 20 μM and higher concentrations of curcumin/ascorbic acid. However, in worms which recieved10 μM pre-antioxidant supplement followed by nano/bulk treatment in presence/absence of antioxidants, the percent survival is 56-69% in the absence and 61-70% in the presence of curcumin, while the same is 60-73% in the absence and 63-90% in the presence of ascorbic acid compared to respective controls (Figure 2.3A).

In worms, exposed to particles in presence of antioxidants for 24 h, mortality was not noticed at 100 μM and above concentration. Whereas at 60 μM concentrations of antioxidants significant mortality ($p<0.05$) was observed with only 40-60% recovery in the presence of curcumin as well as ascorbic acid (Figure 2.3B).

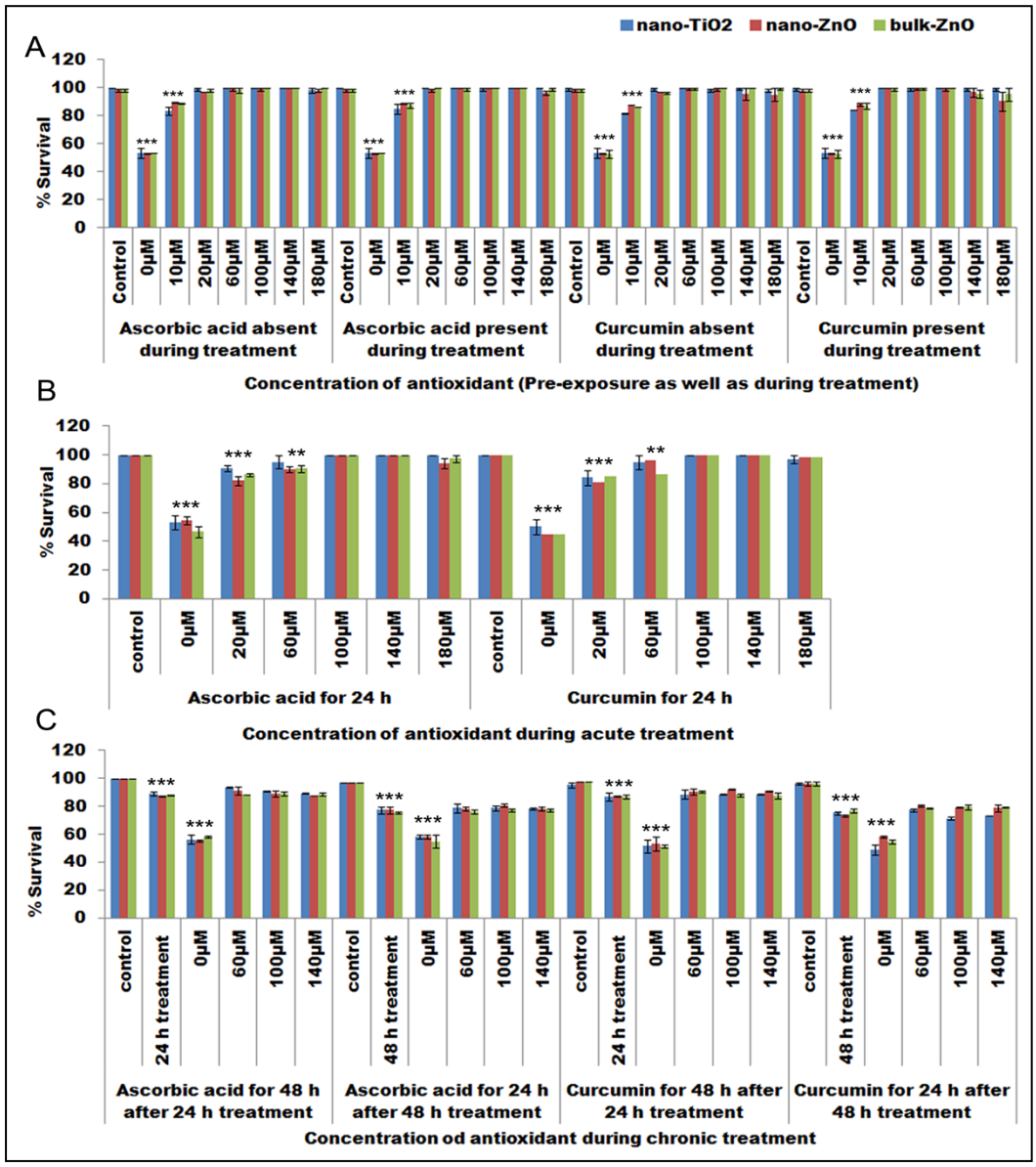

Figure 2.3 Amelioration of nano-TiO_2 and nano/bulk ZnO induced lethality (at LC_{50} concentration) in presence of antioxidants (AO). A. Worms pre-exposed to AO for 48 h followed by 24 h treatment in absence/presence of AO; B. Worms were exposed to nano-TiO_2 and nano/bulk ZnO for 24 h in presence of AO; C. AO added to the worms at different time duration of chronic exposure (72 h) to nano-TiO_2 and nano/bulk ZnO. Bars represent mean±SE; Bonferroni corrected *p<0.05

In the chronic exposure, worms (L1) exposed to LC_{50} concentration, 12-12.5% mortality was scored after 24 h (11.9%, 12.6 % and 12.4% for nano-TiO_2, nano-ZnO and bulk-ZnO particles respectively) and on 48 h exposure 23.5-24.5% mortality (23.6%, 24.4% and 23.6% for nano-TiO_2, nano-ZnO and bulk-ZnO particles respectively) was recorded, finally after 72 h 46-50% death (49.7%, 45.8% and 47.3% for nano-TiO_2, nano-ZnO and bulk-ZnO particles respectively) was noticed. Further mortality was not noticed for any of the treatment (after 24 h or 48 h) on the addition of 60 µM and above concentrations of antioxidants (curcumin/ascorbic acid) (Figure 2.3C). On the addition of a 40 µM antioxidant, the recovery was hardly 8-13% (curcumin) or 10-20% (ascorbic acid), when added after 24 h or 48 h**.**

2.3.4 Amelioration effect of antioxidants from NPs induced ROS generation:

ROS production in organisms exposed to particle significantly increased in comparison to control. Acute exposure to nano/bulk TiO_2 and nano/bulk ZnO caused a 6.3-/4.4- fold and 7.4-/5.4- fold increase in ROS generation at LC_{50} concentration, respectively (Figure 4). Exposure of worms to a nonlethal concentration of bulk-TiO_2 or 0.25 µM rotenone [(+) ve control] also showed significant ROS production. Pre-treatment as well as along with treatment of antioxidants (ascorbic acid/curcumin) at 20 µM concentration was found to completely attenuate ROS generation in case of nano/bulk particle but not for rotenone treatment (Figure 2.4A; Control=1).

The acute exposure (24 h) of nano/bulk TiO_2 and nano/bulk ZnO at L4 stage worm caused a 6.9-/5.3- fold and 7.3-/5.5- fold increase in ROS generation at LC_{50} concentration compared to control (Figure 2.4B; Control=1). Particle exposure in

presence of antioxidants lead to mitigation of ROS production in worms however, it was not complete. ROS production was 4/1.88-fold and 5.1/2.7-fold high when ascorbic acid (100 μM) was present along with nano/bulk TiO_2 and nano/bulk ZnO treatment, respectively in comparision to control. Similarly, when curcumin (100 μM) was present along with nano/bulk TiO_2 and nano/bulk ZnO treatment, the ROS generation was high by 4.5/2.2-fold, 4.7/2.4-fold, respectively in comparision to control (Figure 2.4B).

In the chronic exposure (72 h) increase in ROS generation were 2.3-/1.6-fold for nano/bulk TiO_2 and 4.04-/2.16-fold for nano/bulk ZnO. ROS production was attenuated when antioxidant (ascorbic acid/curcumin) at 60 μM concentration was included during 72 h treatment or added after 48 h or 24 h of nano/bulk particle exposure (determined after 72 h). However, in the case of rotenone (0.025 μM), the addition of antioxidant (ascorbic acid/curcumin) even though reduced ROS production, but, it was not complete (Figure 2.4C; Control=1).

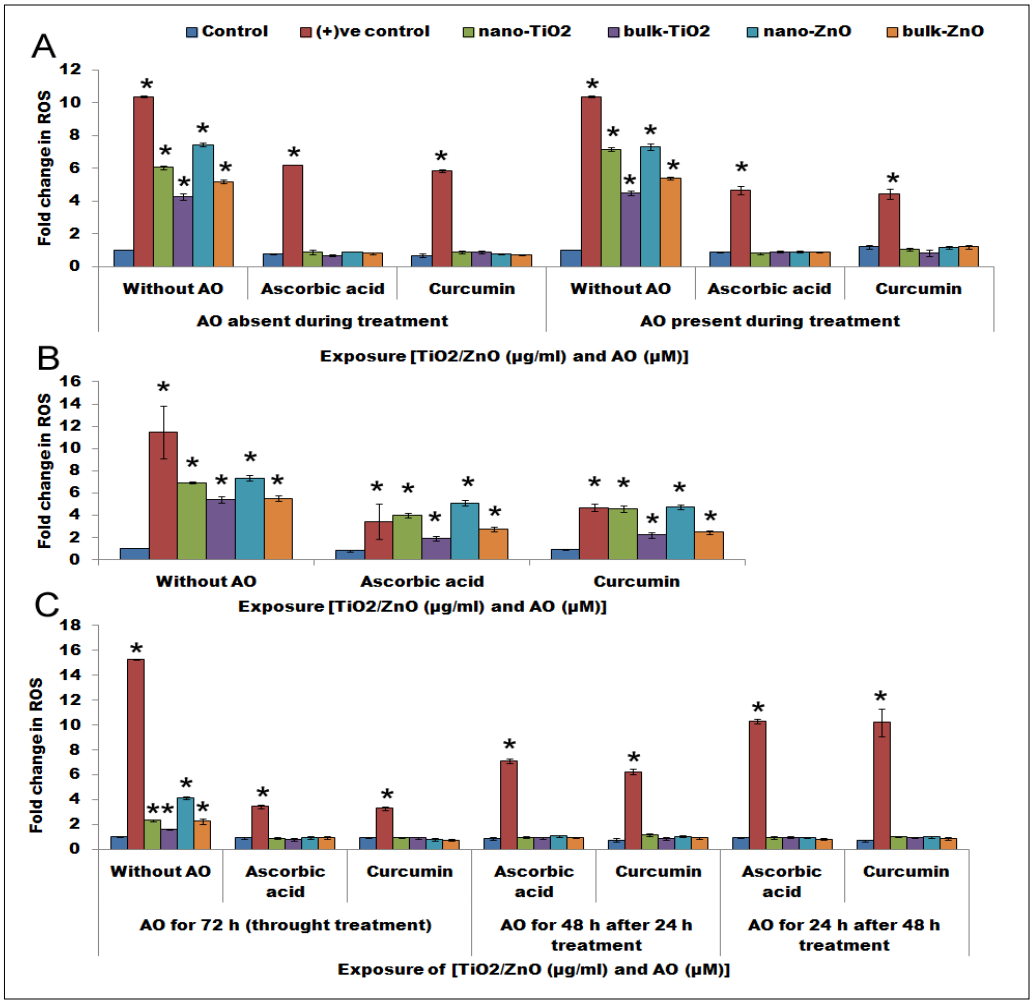

Figure 2.4 ROS generation in worms on exposed to nano/bulk TiO_2 or ZnO particles in presence/absence of antioxidants (AO) A. Worms pre-exposed to AO for 48 h followed by 24 h treatment in absence/presence of AO; B. Worms were exposed to nano/bulk TiO_2/ZnO for 24 h in presence of AO; C. AO added to the worms at different time duration of nano/bulk TiO_2/ZnO exposure. The data is normalized relative to the mean control value (Control=1). Bars represent mean±SE; Bonferroni corrected *p<0.05

2.4 Discussion

Unique physiochemical properties of nano-TiO_2 and nano-ZnO have enhanced their applicability in industrial as well as household utilities (Bui et al., 2017; Truppi et al., 2017;

Colombo et al., 2017). More applicability of NPs than respective bulk enhances their manufacture on a large scale and consequently their discharge into the environment (Dumont et al., 2015; Wu et al., 2014; Praetorius et al., 2012). Thus, raising the global concern related to their negative impact on human as well as other organism. Enormous nanotoxicity studies, including both *in-vivo* (Iannarelli et al., 2016; Shi et al., 2013; Hong et al., 2013; Iavicoli et al., 2012,) and *in-vitro* (Vinardell et al., 2017; Jimeno-Romero et al., 2016; Saliani et al., 2016; Sahu et al., 2016; Pandurangan and Kim, 2015; Hong et al., 2013) concluded that free radical generation is the major mechanism behind the NPs induced toxicity. Free radical generated subsequently enhances the chances of oxidative stress, DNA damage, cytotoxicity, apoptosis, and tumor (Iannarelli et al., 2016; Khanna et al., 2015; Manke et al., 2013). Since the use of antioxidant is an important counter measure against oxidative stress, few studies carried out in this direction has shown that dietary supplement of antioxidants minimize the free radical generation and provide prevention against ROS induced cytotoxicity (Fukui et al., 2015; Pallauf et al., 2013; Siddiqui et al., 2012). However, the ameliorating efficacy of antioxidant against NPs induced lethality at chronic and acute toxicity is unexplored. Thus, the present study was undertaken to examine the ameliorating effects of antioxidants (ascorbic acid and curcumin) against nano-TiO_2 and nano-ZnO induced toxicity employing *C. elegans* as a model organism. *C. elegans* harbor highly conserved biochemical pathways with humans, hence they are used for screening the compounds for their effect on longevity and neuronal degeneration (Maglioni et al., 2016; Chen et al., 2013). On similar lines, in the present study, we have employed *C. elegans* for determining the amelioration effects of antioxidants against NPs toxicity.

The particle characteristics such as size, agglomeration, specific surface area, charge and pH of nanoparticles suspension are important aspects for toxicological assessment. These features affect the dissolution, size distribution, bioavailability, cellular uptake, intracellular localization and cytotoxicity caused by the particles. Earlier studies have indicated that small size particles (high surface area) with more positive charge are more toxic (Shin et al., 2015). In correlation with earlier reports, in the present study nano form were found to be more toxic than the bulk form (Khare et al., 2015; Ratnasekhar et al., 2015), and nano-ZnO with positive charge was more toxic than nano-TiO_2 (Manke et al., 2013; Shin et al., 2015). An adverse effect of particle(s) exposure to organism depends on concentration, duration of exposure as well as the developmental stage of the organism. In the present study, LC_{50} of nano/bulk particles was lower for chronic exposure compared to acute, indicating higher toxicity values for increased duration of exposure for the same particle. Further, NPs exhibited dose dependent toxicity as well as nano-ZnO was more toxic compared to nano-TiO_2, similar to that reported in our previous study (Khare et al., 2011). In both acute as well as chronic exposures to NPs, the pre-, post- as well along with antioxidants supplement was found to be protective against the NPs induced mortality. These findings are in corroboration with recent report where in curcumin exposure along with cadmium quantum dots ameliorate lethality in *C. elegans* (Srivastava et al., 2016). Thus, during NPs exposure the presence of antioxidant curcumin/ascorbic acid gives protection to worms. Our study further shows that pre-anti-oxidants supplement is efficient in ameliorating nano/bulk particle induced negative effects on survival of organism.

Intracellular ROS generation is required for the regulation of cellular signaling and survival, where cell maintain the redox homeoststis by controlling the balance between ROS generation

and elimination. However, high ROS generation disturb this control and induces cytotoxicity depending upon the magnitude, duration and the site of generation (Panieri and Santoro, 2016). Healthy cells have low basal ROS output and normal metabolic regulation thus able to tolerate exogenous ROS stress. Similarly in present study, bulk-TiO_2 at nonlethal dose and duration of exposure is appears to be tolerable by the organism. On the other hand, the lethal concentration of nano-TiO_2 and nano/bulk ZnO might cause prolonged accumulation of ROS which in turn leads to cellular damage resulting in the cell death, diseases condition and death of the organism. However, reduction in ROS accumulation by employing proper antioxidants may be a useful strategy to prevent or delay these pathologic processes. High ROS generation was evident for both acute as well as the chronic exposure of worms to nano/bulk TiO_2 and ZnO. To determine the role of ROS in NPs induced lethality we employed antioxidants. Curcumin and ascorbic acid are known natural antioxidants which are traditionally used as a food ingredient in India and other countries and are also employed against various diseases/toxicity (reviewed in He et al., 2015; Harrison et al., 2014). In the present study, antioxidant (both curcumin as well as ascorbic acid) was found to have a significant ameliorating effect on the NPs induced ROS in exposed worms. Thus, during NPs exposure the presence of antioxidant curcumin/ascorbic acid gives protection to worms by reducing ROS generation. However, quenching of ROS when antioxidant supplement was given pre- or post NPs treatment was complete, however that was not so when it was given along with NPs. Thus, overall, low quantity pre-anti-oxidants supplement is an excellent means to acquire protection against NPs induced adverse effect.

2.5 Summary

- Nano-TiO_2, nano-ZnO and bulk-ZnO were found to be toxic to *C. elegans*.
- LC_{50} of 24 h exposure (acute) to nano-TiO_2 is 172 μg/ml, nano-ZnO is 1.125 μg/ml and bulk-ZnO is 4.64 μg/ml.
- LC_{50} of 72 h exposure (chronic) to nano-TiO_2 is 54.2 μg/ml, nano-ZnO is 0.18 μg/ml and bulk-ZnO is 0.93 μg/ml.
- Nano-ZnO was more toxic than nano-TiO_2 in *C. elegans*.
- In both acute as well as chronic exposures to NPs, the pre-(20 μM and above) and post-(60 μM and above) as well as along with (100 μM and above) antioxidants supplement was found to be protective against the NPs induced mortality.
- In both acute as well as chronic exposures to NPs, the pre-(20 μM) and post-(60 μM) as well as along with (100 μM) antioxidants supplement was found to be protective against the NPs induced ROS generation.
- Quenching of ROS when antioxidant supplement was given pre- or post NPs treatment was complete, however that was not so when it was given along with NPs.
- So far, our study indicates the protective role of curcumin and ascorbic acid against nano/bulk particle toxicity and a possibility of evading the nanotoxicity by incorporating these antioxidants in the everyday diet.

Objective 2

Evaluate the molecular mechanism underlying antioxidant mediated protection against nanoparticles

Sub objective

I. **To determine the effect of TiO_2 and ZnO nanoparticles on oxidative stress response pathways and its regulation by antioxidants**

II. **To evaluate oxidative damage and antioxidant enzyme regulation in *C. elegans* exposed to TiO_2 and ZnO nanoparticles in presence/absence of antioxidants**

III. **Functional validation of the protective role of antioxidants against the adverse effects of TiO_2 and ZnO nanoparticles**

Objective 2

Chapter 3

I. To determine the effect of TiO_2 and ZnO nanoparticles on oxidative stress response pathways and its regulation by antioxidants

3.1 Introduction

Oxidative stress induced signaling pathway alters the normal biological expression of gene and protein in *in-vivo*. The change in transcription and translation level of responsive gene is an important biological marker to determine the underlying toxicity mechanism of any compound. So far, worms exposed to nano TiO_2/ZnO showed elevated ROS production and the presence of antioxidants quenched ROS production (Chapter 2). Moving ahead in this chapter our aim is to find the effect on oxidative stress response signaling pathway on exposure to TiO_2/ZnO particles in presence/absence of antioxidants.

NPs (including nano-TiO_2 and nano-ZnO) and heavy metal-induced oxidative stress are known to adversely affect insulin/insulin-like growth factor-1 signaling (IIS) pathways as well as inflammatory responses in exposed organism (Nemmar et al., 2017; Song et al., 2016; Khare et al., 2015; Vigneshkumar et al., 2011; Tvermoes et al., 2010). *C. elegans* has an evolutionarily conserved IIS-pathway. The IIS-pathway in *C. elegans* has a single insulin-like growth factor receptor (IGFs), DAF-2, (Baugh and Sternberg, 2006). On binding of insulin-like peptides to a DAF-2 receptor, a signaling cascade leads to, phosphorylation of FOXO transcriptional factor DAF-16. The phosphorylation of DAF-16 is mediated by PI3K/AKT-1 kinases. Phosphorylated DAF-16 is recognized by 14-3-3 proteins PAR-5 and FTT-2 which promotes its cytoplasmic retention. Contradictory to this, in stressed condition reduced IIS-signaling induces nuclear translocation of DAF-16. In the nucleus, DAF-16 along with other transcriptional factors (SKN-1 and HSF-1) controls a subset of genes involved in stress resistance, activation of antioxidant enzyme genes, innate immunity and lifespan (Altintas et al., 2016). Generally, the moderate reduction in IIS-signaling pathway alters the expression of genes which provides stress resistance and increased longevity in *C. elegans*, where stress

response promotes lifespan extension. Therefore the reduced IIS-signaling by genetic alteration such as mutation of *daf-2* or *akt-1* also promotes stress resistance and extend lifespan. However, inactivation of *daf-16* causes sensitivity to stress and reduced lifespan. DAF-16 activation also requires the stress-activated MAPK pathway (Kondo et al., 2005) such as p38 and JNK pathway. Three isoforms of p38 PMK-1, PMK-2 and PMK-3 are present in *C.elegans*. p38 MAP kinase signaling pathway activates SKN-1 and facilitate its nuclear translocation where it interacts with other transcriptional factors such as DAF-16, HSF-1 and induces the expression of stress protective genes. Whereas, JNK-1 directly interact with phosphorylate DAF-16 and promote the nuclear translocation of DAF-16 (Oh et al., 2005). Interestingly, the JNK pathway act parallels with the DAF-2 pathway to regulate stress resistance and lifespan. Even in mammals phosphorylation of FOXO4 (a member of the mammalian FOXO family) is mediated through JNK, in normal conditions. Thus, this interaction between the MAPK and insulin/IGF- pathways is functionally conserved among species and suggests that signaling pathways for stress response and immune response are closely regulated by each other.

Adverse effects of TiO_2/ZnO particles on oxidative stress induced signaling pathways have been studied in *in-vitro* (cell lines; Ghosh et al., 2016; Pati et al., 2016; Saptarshi et al., 2015) and *in-vivo* (bacteria; Soni et al., 2017), (*C. elegans*; Khare et al., 2015), (*Drosophila melanogaster*; Ng et al., 2017), (rat/mice; Ghosh et al., 2016; Hong et al., 2016; Saptarshi et al., 2015) (plants; Tiwari et al., 2017; Hossain et al., 2015) models. High oxidative stress and alteration in the level of expression of antioxidant enzyme genes was observed in WAG cell line (Dubey et al., 2015), human lymphocyte cells (Ghosh et al., 2016), mouse macrophages (Pati et al., 2016). The oxidative stress mediated alteration in expression and function of

antioxidants on exposure to TiO_2/ZnO particles are also reported in *in-vivo* studies such as, in bacteria (Soni et al., 2017; Maurer-Jones et al., 2013), *C. elegans* (Khare et al., 2015), *Drosophila melanogaster*, (Ng et al., 2017), rat/mice (Ghosh et al., 2016; Yang et al., 2015; Shim et al., 2014 ,) and plants (Tiwari et al., 2017; Hossain et al., 2015). Further, alteration in immune responses was observed in cells (Saptarshi et al., 2015) and rat/mouse (Hong et al., 2016; Saptarshi et al., 2015; Kononenk et al., 2015; Fu et al., 2014).

Apart from this curcumin and ascorbic acid both can interct with transcriptional factors such as nuclear factor erythroid-2 related factor 2 (Nrf2), nuclear factor kappa-B (NF-κB), tumor necrosis factor alpha (TNF-α) etc. which play an important role in the regulation of genes expression (He et al., 2015). Curcumin and ascorbic acid protect metal (Cd, Hg), particulate matter and NPs (TiO_2, ZnO, cadmium quantum dots, Al_2O_3) induced alteration in the expression of stress responsive genes/protein in human bronchial epithelial cells, *C. elegans*, mice, rat and *Oreochromis niloticus* (Abdelazim et al., 2018; Liu et al., 2017; Srivastava et al., 2016; Jin et al., 2015; Fukui et al., 2015; Sangartit et al., 2014; Li et al., 2012; Agarwal et al., 2010). Further, ascorbic acid suuplement affects the expression of several genes relevant to the inhibition of cancer (Mikirova and Scimeca, 2016). Therefore, we analyzed the effect of TiO_2/ZnO particle on IIS-signaling pathway in exposed worms in presence/absence of antioxidants. The main aim of this study is to address (i) Whether the oxidative stress noticed due to exposure to TiO_2 and ZnO leads to disruption in oxidative stress response pathway or immune stress response in exposed *C. elegans*, if so (ii) Whether the presence of curcumin/ascorbic acid during treatment ameliorates the same.

3.2 Material & Methods

3.2.1 Strains employed: The standard N2 strain of *C. elegans* was employed along with the transgenic green fluorescent protein tagged to the promoter of gene of intrest such as CF1553 (*sod-3p*::GFP), CL2050 (*hsp-16.2p*::GFP), BC20329 (*skn-1p*::GFP), BC20337 (*hsf-1p*::GFP), BC20336 (*ctl-2p*::GFP) for the detection of gene expression.

3.2.2 Treatment: Age synchronized L4 worms were treated with LC_{10} concentrations of nano-TiO_2 (same concentration was applied for bulk-TiO_2) and nano/bulk ZnO in presence/absence of antioxidants, along with control (water/solvent). The exposure was for 24 h duration.

3.2.3 Real-time polymerase chain reaction:

(i) RNA extraction:

The treated worms were harvested, washed thoroughly with sterile water and collected in 1.5 ml eppendorf for RNA extraction. The worms were homogenized in 1ml of RNAzole RT (Molecular Research Centre, U.S.A) and centrifuged at 5000 rpm for 2 min at 4°C. To the homogenate, 0.3 ml of DEPC water was added and incubated at RT for 15 min. Again the sample was centrifuged at 12000 rpm for 15 min at 4°C. The supernatant was carefully transferred to a fresh eppendrof, without disturbing the pellet. RNA was precipitated by gently mixing the supernatant with 400 µl of 75% ethanol. The sample was stored at RT for 10 min and centrifuged at 12000 rpm for 10 min at 4°C. RNA forms a white pellet at the bottom of the eppendrof. The RNA pellet was washed twice with 75% ethanol and the pellet was dissolved in 25 µl of DEPC water. Finally, the RNA was quantified spectrophotometrically by Nanodrop (Thermo scientific, U.S.A).

(ii) cDNA synthesis:

Single strand cDNA was synthesized from total RNA using the high capacity cDNA reverse transcription kit (SuperScript® III Reverse Transcriptase, Invitrogen, U.S.A), following manufacturers protocol. In a 0.2 ml thin-walled PCR tube 1 µg RNA, 1 µl oligo dT and 1 µl dNTPs were added and the volume was made up to 10 µl.

The mixture was incubated in a thermal cycler at 65°C for 5 min, and then immediately placed on ice for at least 1 min. The contents of the tube were collected by brief centrifugation. The cDNA synthesis mix containing 2 µl of 10X buffer, 2 µl of 0.1 M DTT, 4 µl of 25 mM $MgCl_2$, 1 µl of RNase Out and 1 µl of superscript III was prepared by adding the component in the given order. This cDNA synthesis mix was added to the RNA mixture and gently mixed. It was subjected to 50 min incubation at 50°C, 5 min at 85°C and briefly cooled by placing the tube on ice for 1 min. Finally, 1 µl of RNAase H was added and incubated at 37°C for 20 min. The cDNA was quantified by Nanodrop (Thermo scientific, U.S.A) and was stored at -20°C.

(iii) Quantitative Real-Time Polymerase Chain Reaction (qPCR):

Genes involved in oxidative stress and immune response pathways were screened for their expression pattern employing the expression of actin gene for normalization. To design primers, the gene sequences of *C. elegans* were retrieved from database and primers were designed employing NCBI pick primers program. The primer were designed spanning exon. The primer sequences along with gene name are listed in table 3.1. The PCR conditions were initially standardized and cDNA was screened to check genomic DNA contamination, for each primer pair, it was followed by qPCR. The 5 µl reaction mixture contained 2.5 µl (1X) TaqMan Universal PCR Master Mix (SYBR

Green, Thermofisher), 0.25 µl of 100 µM of each gene primers, 100 µg cDNA and nuclease-free H_2O. qPCR assay for each gene target was performed in triplicate in 384-well plates, in QuantStudio™ 6 Flex Real-Time PCR System (Thermo scientific, US). q-PCR conditions for all the genes were 95°C initial denaturation (10 min); 40 cycles with 95°C (15 s), 60°C (1 min). Relative transcript levels were calculated using life technology Software (Version v1.3) through ΔΔCt method. The gene expression of individual sample was subtracted from its actin transcript levels, normalized against the control (C=1) and is represented in fold change.

3.2.4 GFP reporter assay (semi-quantitative):

For detection of gene expression, the transgenic lines with specific gene promoters tagged to green fluorescence protein were employed. The transgenic worms were exposed to control/treated solutions in the presence/absence of antioxidants for 24 (±0.5) h at 20°C. After exposure, worms were washed thrice with milliQ water. To detect the transgenic expression, ~1000 worms were taken in each well of black 96 well plate in 200 µl (PBST), in triplicates. PBST without worms was employed as blank. The GFP fluorescence was measured at the 485 nm excitation and 525 nm emission wavelengths in spectrophotometer. The GFP intensity of control/treated group subtracted from blank, normalized against the control (C=1) and is represented in fold change (Khare et al., 2015). All the experiments were repeated thrice.

3.2.5 Western blotting:

(i) Sample Preparation:

Worms cultured on 90 mm plates were exposed to nano/bulk particles in the presence/absence of antioxidants for 24 h. Worms were harvested, washed thrice with

sterile water and twice with sample buffer to remove bacterial and other contamination. After washing worms were centrifuged at 3000 rpm for 5 min, extra buffer was removed and worm pellet was weighed. Worms pellet was resuspended in sample buffer (4% sodium dodecyl sulfate, 100 mM Tris-Cl (pH 6.8), 20% glycerol) to make 10 % of the protein sample and boiled for 15 min. This was followed by centrifugation at 1000 g for 5 min to remove insoluble material from protein samples. The supernatant was collected in a tube (Youngman et al., 2011). The sample (20 µl) was loaded into the wells of polyacrylamide gel and resolved through electrophoresis.

(ii) SDS-PAGE casting and sample loading:

Reagents employed:

a. 40% acrylamide: A stock solution of 40% acrylamide was prepared by dissolving 39 g of acrylamide, and 1 g of bis-acrylamide in 60 ml of DW. After the complete dissolution of the chemicals, the solution was filtered, and the final volume was made up to 100 ml. The solution was kept covered from light, during its storage.

b. 1.5 M Tris: 90.86 g of Trizma base was dissolved in 450 ml of DW, the pH of the solution was adjusted to 8.8 (by conc. HCl), and the final volume was made up to 500 ml by DW.

c. 1 M Tris: 60.57 g of Trizma base was dissolved in 450 ml of DW, the pH was adjusted to 6.8 (by conc. HCl), and the final volume was made up to 500 ml by DW.

d. 20% sodium dodecyl sulfate (SDS): 20 g of SDS was added to 100 ml of DW, and the solution was kept overnight at 37ºC for dissolution.

e. 10% Ammonium persulphate: 100 mg of ammonium persulphate was dissolved in 1 ml of DW.

f. Staining solution (0.25%): Commasie brilliant blue, CBB (0.25 g) was dissolved in 40 ml of DW. To this 50 ml of methanol and 10 ml of glacial acetic acid were added, to make a final volume of 100 ml.

g. Destaining solution: For slow destaining of the gel, 25 ml methanol and 7.5 ml of glacial acetic acid were made up to 100 ml by DW. For faster destaining the proportion of methanol: acetic acid: water was kept at 4:1:5 in the solution.

h. 5X Running buffer: Tris base (15.15 g), glycine (72 g) and SDS (5 g) were dissolved in 500 ml water, and the final volume of the solution was made up to 1000 ml. The 1X working solution was prepared by five-fold dilution of the 5X running buffer.

Procedure:

For the preparation/casting of 13% polyacrylamide gel, the lower resolving gel was prepared by adding 1.5 M Tris, (pH 8.8; 5.172 ml) to water (8.654 ml). To this solution acrylamide (6.864 ml; 40% stock), SDS (156 µl; 20% stock), TEMED (13.8 µl) and APS (138 µl; 10% stock) were added. The contents were thoroughly mixed (avoiding frothing), and poured in between the glass plates, held tightly in a caster. A layer of butanol was dispersed over the casted gel, and the entire assembly was allowed to polymerize/solidfy (Ravi Ram et al., 2005).

Once the resolving gel had been polymerized, the layer of butanol was washed off, and 4% stacking gel was cast over the resolving gel. The contents of stacking gel included: 1

M Tris, (pH 6.8; 1.484 ml), Acrylamide (0.84 ml; 40% stock), water (3.522 ml), SDS (89.82 µl; 20% stock), TEMED (6.8 µl), and APS (75 µl; 10% stock). The solution was poured over the previously cast and polymerized resolving gel, and combs were placed in position for making the wells, for loading the sample. The set up was left for the stacking gel to get polymerized.

The units for the running of the gel were assembled, and the lower/upper tanks were filled with 1X running buffer. Comb was removed, the wells were flushed/cleaned, and the samples were loaded. To determine the molecular weights of the resolved protein, 10 µl of a prestained marker (range of 4 kDa to 250 kDa) was also loaded in one of the wells. Few of the empty wells, on either side of those carrying samples, were filled with the sample buffer. The electrodes were set at 100 V. The bromophenol blue, added in the sample buffer, served as a tracker and marked the extent of running the sample in the form of a blue streak. The proteins in the sample were resolved depending upon their molecular weight.

After the completion of the run, the gel was stained in staining solution overnight, and later the stain was replaced by destaining solution, which cleared off all the stain from the gel, leaving behind the stained protein bands, resolved all along the gel in a descending order of their molecular weight from top (protein with highest molecular weight to bottom (protein with lowest molecular weight).

(iii) **Semi-dry blotting:**

Reagents employed:

a. Semi-dry transfer buffer: Glycine (1.45 g) and Tris base (2.9 g) were dissolved in 200 ml DW. To this solution, methanol (100 ml), and SDS (0.925 ml of 20% SDS) were added. The solution was gently mixed to avoid frothing and the final volume was made up to 500 ml with water. The buffer was kept at 4ºC, prior to use.

b. 5X TBS: NaCl (21.91 g), and Tris base (6.06 g) were dissolved in 400 ml of water, pH was set at 7.5 (with conc. HCl), and the final volume of the solution was made up to 500 ml, with water. 1X TBS was prepared by five-fold dilution of the 5X TBS.

c. 50 mM Glycine: Dissolved glycine (0.375 g) in 100 ml of DW.

d. 0.5% Glutaraldehyde: Mixed 500 µl of glutaraldehyde in 100 ml of DW.

e. 1X TBST: Addition of 0.1% Tween-20 to 1X TBS.

f. Blocking solution: 5% milk in 1XTBST (5 g skimmed milk powder, standard grade, in 100 ml of 1X TBST).

g. Ponceau Stain: 10 mg Ponceau S; dissolved in 90 ml of water, followed by addition of 5 ml acetic acid. The final solution was made up to 100 ml.

h. Developing solution: Supersignal West Femto maximum sensitivity substrate (Thermoscientific) was employed for developing the blot. 150 µl of solution A (Luminol/Enhancer) and 150 µl of solution B (stable peroxide solution) were mixed and

poured over the blot, for the detection of chemiluminescent signals (from the protein bands tagged with HRP) following exposure to light within the Gel Doc.

Procedure:

Polyvinyl di fluoride (PVDF, Millipore, Merck Life Science, Bangalore, India) membrane (of the dimensions marginally larger than that of the gel) was dipped in methanol, washed with water, and immersed in semi-dry transfer buffer for 10 min. The gel with the resolved protein after the completion of the run was also transferred and kept immersed in semi-dry transfer buffer for the same duration as the membrane (10 min). After this incubation, the membrane and the gel were stacked between semi-dry transfer buffer soaked whatman filter wicks (4 wicks on either side of the membrane- gel doublet). This stack was placed in the semidry transfer unit, and current equivalent to 0.8 mA/cm^2 of the area of the membrane was applied for 1 h (Ravi Ram et al., 2005).

The transfer of the prestained marker was indicative of the transfer of the protein profile as well, from the gel to the PVDF membrane. In addition, the membrane was incubated in 0.1% solution of Ponceau S in 5% acetic acid for 5 min for visualization of the transferred protein bands (appeared as pink bands, on the PVDF membrane). The membrane was destained by washing it with water for 3-4 times. The membrane with the transferred protein profile was soaked in 1X TBS before transferring it to 0.5% glutaraldehyde, four times, twice for 5 min and twice again for 10 min.

This step facilitates inter-crosslinking of the protein with the matrix of the membrane. The membrane was then transferred to 50 mM glycine for 10 min, which neutralized the glutaraldehyde and impaired further crosslinking. This was followed by a 10 min wash

of the membrane with 1X TBS. After blocking for 2 h at 37°C, the membrane was incubated overnight at 4°C with primary antibody for specific protein such as JNK (1: 1000, Santa Cruz Biotechnology, INC.), p-JNK (1: 1000, Santa Cruz Biotechnology, INC.),) and β-Actin (1:2000, Sigma, USA) in 1 x Tris-buffer saline with Tween 20 (TBST).

The membrane was then washed four times, twice in 1X TBST for 5 min, and again twice in 1X TBST for 10 min. The washing was followed by incubating the membrane for 2 h at RT with secondary antibody conjugated with horseradish peroxidase (1:4000) (Jackson immune research USA) in 1X TBST. The membrane was again washed four times as previously mentioned with 1X TBST, prior to developing the signals on to the blot. The blot was developed with Supersignal West Femto maximum sensitivity HRP substrate (Thermoscientific) and the signals were documented using Versa doc Imaging system (Bio-Rad, USA). The blots were developed using β-actin was used as an internal control to normalize the data.

3.2.6 Statistical analysis:

Fold change of the transcript levels of genes and protein increased or decreased by ±0.5-fold were considered as significantly up- or down-regulated. Results were expressed as mean±S.E.M. and data were analyzed using one-way analysis of variance (ANOVA). Probability levels of $p<0.05$ and $p<0.01$ were considered statistically significant on employing Bonferroni correction.

3.3 Results

3.3.1a Effect of TiO_2/ZnO NPs on an expression of oxidative stress response genes in particle exposed worms:

The expression of oxidative stress response phosphokinases (*sek-1, nsy-1*, *pmk-1*), transcription factors (TF- *daf-2*, *daf-16, skn-1, hsf-1*) and downstream genes (such as, *sek-1, nsy-1, pmk-1, daf-2, daf-16, skn-1, hsf-1, sod-3, ctl-2, gcs-1, gst-4*, *hsp-16.2*) were determined in response to TiO_2/ZnO particle exposure.

The phosphokinases such as *sek-1, nsy-1* and *pmk-1* were significant (p<0.01) up-regulated by 9-, 2.6- and 8.7- fold, respectively in the worms exposed to nano-TiO_2. Exposure to bulk-TiO_2 lead to significant (p<0.01) up-regulation of *pmk-1* by 4.6- fold in exposed worms, while others were non-significant in comparison to control (1- fold, Figure 3.1A). The stress response genes and their TF, in the worms exposed to nano/bulk TiO_2, were significantly up-regulated, except for *daf-2* which was found to be down-regulated by 3.69-/2.06- folds respectively. TF such as *daf-16, skn-1,* and *hsf-1* were significantly (p<0.001) up-regulated by 4.44-/2.48-, 2.69-/2.19- fold and 1.95-/1.47-fold, respectively (Figure. 3.1B, C), in nano/bulk TiO_2 exposed worms in comparison to control. The expression of antioxidant enzyme genes which are downstream of DAF-16, SKN-1, and HSF-1, were found to be significantly up-regulated (p<0.01). Expression of genes such as *sod-3, ctl-2, gcs-1, gst-4* and *hsp-16.2* was up-regulated by 2.85/1.66, 2.16-/1.39-, 1.68-/1.46-, 2.75/1.69- and 1.59/1.23- fold, respectively in nano/bulk TiO_2 exposed worms in comparison to control (Figure 3.1B, C).

Similarly, in worms exposed to nano-ZnO, *sek-1, nsy-1,* and *pmk-1* were significant (p<0.01) up-regulated by 2.7-, 16.2- and 12.7- fold, respectively in comparison to control. Exposure to bulk-ZnO lead to significant (p<0.01) up-regulation of *nsy-1* and *pmk-1* by 1.6- and 6.8-fold respectively in exposed worms, while others were non-significant in comparison to control (Figure. 3.1A). IIS-signaling pathway, in worms exposed to nano/bulk ZnO, revealed significant (p<0.01) down-regulation of *daf-2* by 3.57/3.2- fold, while all other genes were significantly (p<0.01) up-regulated. TFs *daf-16, skn-1* and *hsf-1* were significantly up-regulated by 3.59/2.84-, 3.58/2.56- and 2.3/1.72- folds respectively, in comparison to control (Figure 3.1B, C). Expression of *sod-3, ctl-2, gcs-1, gst-4* and *hsp-16.2* was up-regulated by 3.32/2.63, 2.59-/2.0-, 2.99-/2.15-, 3.69/2.5- and 1.9/1.56- fold respectively, in nano/bulk ZnO exposed worms in comparison to control (Figure 3.1B, C).

Effect on expression of genes screened in worms exposed to nano-TiO_2/ZnO was significant (p<0.01) in comparison to the same in worms exposed to their respective bulk components (Figure. 3.1).

Further, through protein analysis, the phosphorylated (active) and nonphosphorylated form of JNK-1 (MAPK) was measured. A significant increase (p<0.01) in the level of p-JNK was observed on exposure to nano-TiO_2, nano-ZnO, and bulk-ZnO with an increase of 1.7-, 2.1- and 2- fold, respectively, in comparison to control (Figure 3.2). The effects noticed were non-significant between nano and bulk comparisons.

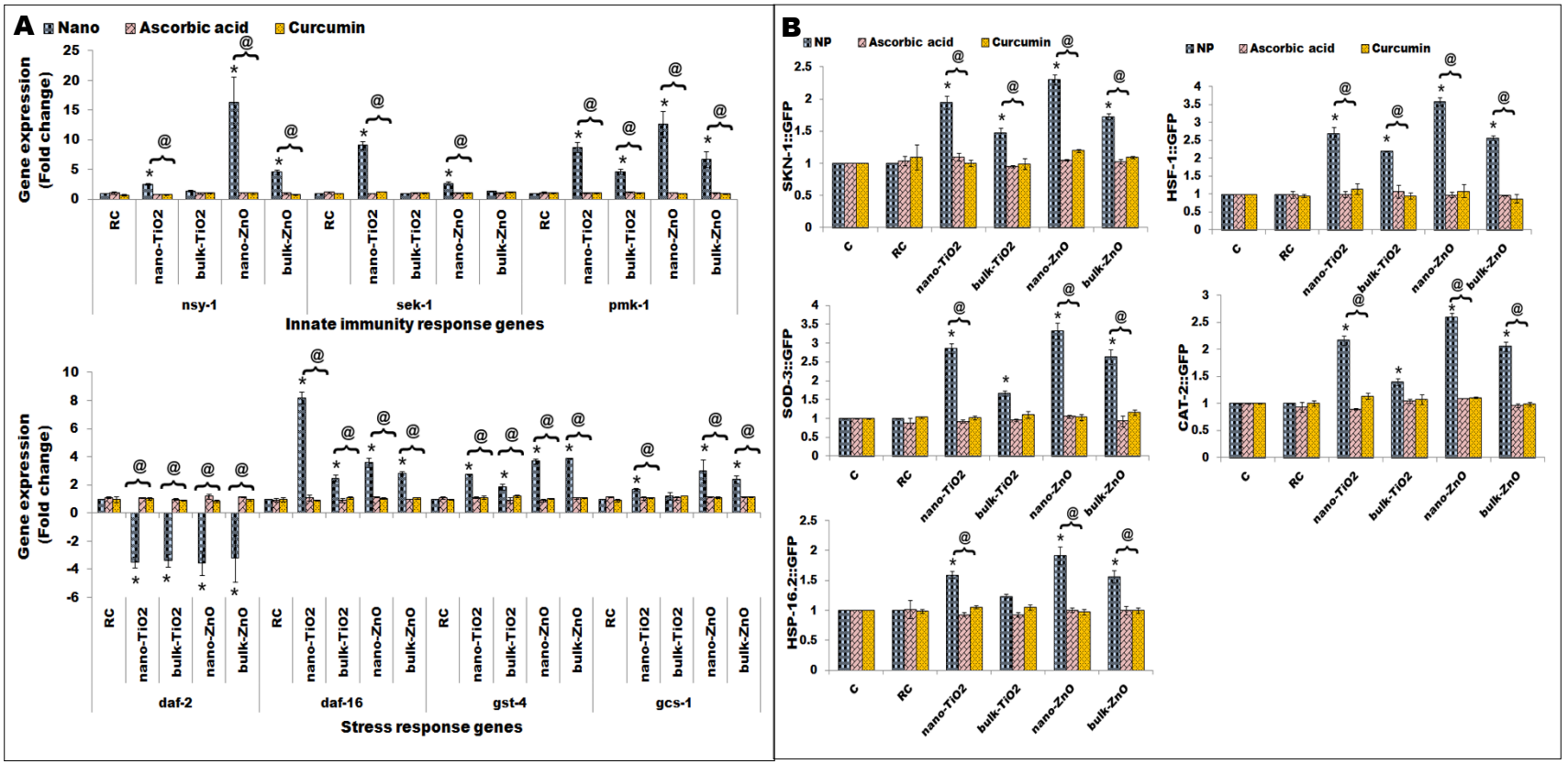

Figure 3.1 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced expression of genes in *C. elegans*. A. Determined through qPCR; B. Determined in transgenic (specific gene promoter tagged-GFP) strains of *C. elegans*. C = control; RC = reference control; n=3, bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001 = Significant against control; @p<0.001 = Significant recovery (in presence of antioxidant) against particle exposure

3.3.1b Amelioration effects of antioxidant on the gene expression of organism related to stress resistance and immune response:

In the presence of ascorbic acid and curcumin the expression of all the genes screened for oxidative stress response was non-significant compared to control (Figure. 3.1). Thus, the presence of antioxidant during nano/bulk treatment led to significant recovery in expression of all the genes screened (Figure. 3.1).

Similarly, even at protein level, phosporylated JNK-1 levels in worms treated with nano/bulk in presence of antioxidants were non-significant (Figure 3.2) in comparison to controls.

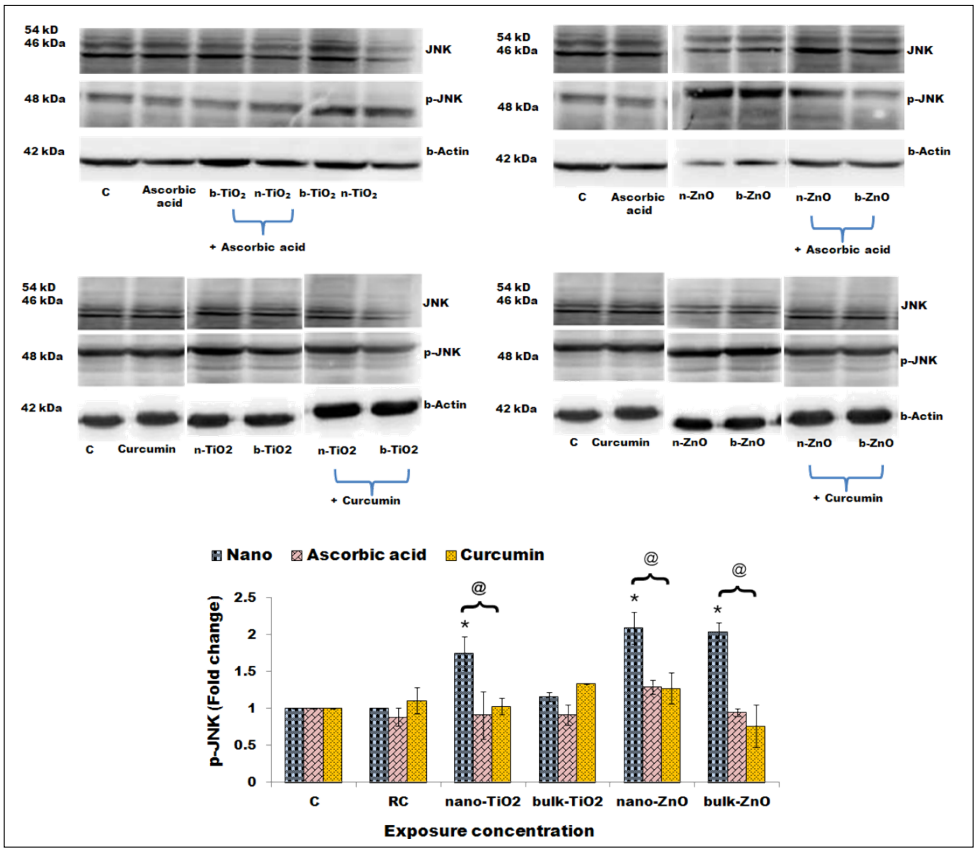

Figure 3.2 Amelioration effect of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced activation of p-JNK in *C. elegans*. C = control; RC = reference control; n=3, bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.01 = Significant against control; @p<0.01 = Significant recovery (in presence of antioxidant) against particle exposure

3.4 Discussion

Gene expression is modulated by oxidative stress induced by both physiological signals (hormones, cytokines, etc.) and environmental stimuli (physical parameters, xenobiotics, etc.). Extensive studies on the induction of stress-response genes by oxidative stress have been reported (Morel and Barouki, 1999). So far, we witnessed excessive ROS formation (Chapter

2) in worms exposed to NPs. Thus, we looked at the expression of genes involved in oxidative stress response. Previous literature signifies that nano-TiO_2 and nano-ZnO were identified to induce oxidative stress which primarily targets IIS-pathways in *C. elegans* and leads to up-regulation of stress response gene expression (Khare et al., 2015; Ratnasekhar et al., 2015). Under normal condition, DAF-2 keeps other key modulators DAF-16, HSF-1 and SKN-1 transcription factors inactivated through phosphorylation and act as a major regulatory protein in IIS-pathway. We observed significant down-regulation in the expression of *daf-2* gene, indicating inhibition of IIS-pathway. DAF-16 is a member of FOXO-transcription factor and is known to play a crucial role in stress response. Oxidative stress response genes such as *sod, ctl, gcs, gst* and *hsps* lie down-stream of DAF-16 and hence is regulated by DAF-16 (Landis and Murphy, 2010). In the present study, the up-regulation in the expression of *sod-3, ctl-2, gcs-1* and *gst-4* gene is an indication of nuclear translocation of DAF-16 and SKN-1.

DAF-16 requires SKN-1 and HSF-1 to regulate the transcription of genes in the nucleus. We observed significant up-regulation of SKN-1 and HSF-1 in transgenic GFP worms. SKN-1 in *C. elegans* is ortholog to human Nrf2 and is an important factor in oxidative stress and regulates expression of Phase-II detoxification genes (such as *gcs, gst* etc.) in the worm intestine (Landis and Murphy, 2010). Even the nuclear translocation of SKN-1 is mediated by IIS through akt-1 phosphorylation. Similarly, HSF-1 is also an important transcriptional factor which induces transcription of chaperones and proteases in heat and other stress conditions. HSF-1 and DAF-16 act together to promote longevity and small heat-shock protein genes (Brunquell et al., 2016). Therefore, exposure to TiO_2/ZnO induces stress response through IIS-pathway, and this study collaborates with gene expression studies under oxidative stress (Gonzalez-Moragas et al., 2017; Tian et al., 2016; Khare et al., 2016; Wang et al., 2014).

Further, JNK-1 is also known to activate DAF-16 under oxidative stress conditions, thus we analyzed the expression and activation of JNK-1 and our results suggest that apart from IIS-signaling pathway even the MAPK pathway is activated.

DAF-16 is also activated through immune response during oxidative stress. This requires the innate immune response NSY-1-SEK-1-PMK-1 (p38MAPK) pathway (Landis and Murphy, 2010). Thus, we tested the role of innate immune response in particle exposed worm against particle generated oxidative stress. The up-regulation in the expression of *nsy-1, sek-1*, and *pmk-1* genes indicates activation of innate immune response in worms exposed to TiO_2/ZnO. This p38MAPK also activates SKN-1 by phosphorylation, preventing its degraded by the ubiquitin-proteasome system. SKN-1 phosphorylation is mediated by PMK-1, which leads to nuclear translocation and induction of stress protective genes (Landis and Murphy, 2010). This indicates that both DAF-16 and SNK-1 are activated in worms exposed to TiO_2/ZnO particle and is regulated by both IIS-pathway as well as the innate immune signaling pathway.

However, in the presence of ascorbic acid or curcumin during particle treatment, the expression of *daf-2* and *daf-16* genes in exposed worms were comparable to that of the control. Thus, the presence of antioxidants curcumin and ascorbic acid reduces oxidative load and activates IIS-signaling and controls activation of immune response signaling. This data is in line with the reports that presence of antioxidants reduced oxidative stress and immune response in cell lines, *C.elegans*, mice and rat against NPs such as- NiO_2, ZnO, Ag, TiO_2 and Cd-quantum dots (Srivastava et al., 2016; Yang et al., 2016; Khorsandi et al., 2016; Turkez et al., 2016; Fukui et al., 2015; Jin et al., 2015; Nemenqani, 2015; Nemenqani et al., 2015; Somayeh and Mohammad 2014; Li et al., 2012; Siddiqui et al., 2012).

3.5 Summary

- Nano/bulk TiO_2 and ZnO exposure were found to induce the oxidative stress-mediated alteration in IIS and immune signaling in exposed worms.
- Exposure of nano/bulk particles alters the expression of a xenobiotic gene (*gsc-1*) and antioxidative enzyme gene (*sod-3, ctl-2, gst-4*) in *C. elegans*.
- Immune response (*nsy-1, sek-1, pmk-1,* and JNK-1) act parallel to IIS-signaling in an exposed worm.
- Co-exposure of antioxidants (curcumin and ascorbic acid) was efficient to reduce oxidative stress thus, maintain the IIS and immune signaling in worms against nano/bulk exposure.

Table 3.1 Sequence of genes primers employed in the present study

Gene name	Function	Primer sequence 5'-----3'
daf-2 (5'->3')	*daf-2* encodes a tyrosine kinase receptor, the *C. elegans* insulin/IGF receptor ortholog; DAF-2 activity is required for embryonic and larval development, longevity, reproduction, fat storage, chemotaxis learning, and stress resistance, including response to high temperature, oxidative stress, and bacterial infection	F' AACTCTCGGTGGAAAGAAGC R' GTCGGTTTCCTTGTTAAGGC
daf-16 (5'->3')	*daf-16* encodes the sole *C. elegans* forkhead (FOXO) homologue; DAF-16 functions as a transcription factor that acts in the insulin/IGF-1-mediated signaling (IIS) pathway that regulates dauer formation, longevity, fat metabolism, stress response, and innate immunity	F' GTGGCCAATGCAACAATACA R' TGATGAGGATGCATTGGATGA
gst-4 (5'->3')	*gst-4* encodes a putative glutathione-requiring prostaglandin D synthase	F' TGCAGAGGAAGAAGCTTACG R' ATGATCAGCGTCACTTCCAT
gcs-1 (5'->3')	*gcs-1* encodes the *C. elegans* ortholog of gamma-glutamine cysteine synthetase heavy chain; GCS-1 is function, in a conserved xenobiotics stress response pathway	F' AAGTAGCTATCAACGTCCCG R' AGGTTTCGCATCACGAGTAT
sek-1 (5'->3')	*sek-1* gene encodes for MAPKK and also required for PMK-1 activation and play an important function in a conserved p38 MAPK immune signaling pathway	F' GCAAACACATTCCAGAGCCG R' TGTTCGACGGTTTCACGTCT
nsy-1 (5'->3')	*nsy-1* gene encodes a *C.elegans* MAPKKK that is an activator of JNK and p38 MAPKs. NSY-1 required for PMK-1 activation and play an important function in a conserved p38 MAPK immune signaling pathway	F' TCTGTTCCCGACAAAGGCTC R' TGCAGCGTACACAGTTCCAT
pmk-1 (p38) (5'->3')	*pmk-1* gene encodes a P38 mitogen-activated *protein* kinase which is identical to mammalian p38 and selectively phosphorylated and activated by only one of the three MEK family members that recognize mammalian p38s	F' GGAACTGTTTGTGCTGCTG R' TCACGATATGTACGACGGGC
actin-1 (5'->3')	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells	F' AGAAGAGCACCCAGTCCTCC R' GAAGCGTAGAGGGAGAGGAC

Objective 2

Chapter 4

II. To evaluate oxidative damage and antioxidant enzyme regulation in C. elegans exposed to TiO_2 and ZnO nanoparticles in presence/absence of antioxidants

4.1 Introduction

In our present study, so far we have noticed curcumin and ascorbic acid protects worms against nano-TiO_2 and nano-ZnO induced lethality (Chapter 2), diminishes reactive oxygen species (ROS) production. Further, we also witnessed over-expression of oxidative stress response genes on exposure to a particle was regulated in presence of antioxidants (Chapter 3). To functionally validate the gene expression data, we analyzed antioxidant enzyme activity in *C. elegans* exposed to nano-TiO_2 and nano-ZnO in presence/absence of antioxidants. Further, we also measured the oxidative damage of macromolecules in nanoparticles exposed worms in presence/absence of curcumin and ascorbic acid.

Reactive oxygen species (ROS) such as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($^1O_2$), are byproducts of aerobic metabolism in mitochondria. Mitochondria constantly utilize oxygen in order to support energy needs of the tissue thus; it is a major site for a free radical generation. In the normal course, ROS serve as signaling molecules to regulate physiological processes including cell proliferation, host defense, signal transduction, and gene expression (Schieber and Chandel, 2014; Droge, 2002). A cellular balance between ROS generation and clearance by antioxidative defense mechanisms is crucial for normal functioning of the cell. On the other hand, high amount of ROS production leads to oxidative stress. Since ROS molecules harbor one or more unpaired electrons, they are highly reactive and readily damage the macromolecules such as lipids, proteins, polysaccharides, and DNA. However, organisms do have endogenous antioxidant defense system which includes antioxidant enzymes and molecules to scavenge the free radical. The major antioxidant enzymes are superoxide dismutase (SOD), catalase (CTL),

glutathione peroxidase (GPx), glutathione reductase and antioxidant molecules are reduced glutathione (GSH), alpha-lipoic acid, coenzyme Q etc.

Therefore, the extent of oxidative stress is measured by estimating the level of cellular enzymatic and non-enzymatic antioxidants such as SOD, CTL, reduced glutathione (GSH), and GPx. Superoxide dismutase catalyzes the breakdown of superoxide anions into oxygen and hydrogen peroxides. Catalases or glutathione peroxidases further catalyze hydrogen peroxides into water and oxygen. Similarly, reduced glutathione (GSH) is an important water-soluble antioxidant molecule that donates reducing equivalent such as proton or electron to neutralize the free radicals. Glutathione molecule itself becomes reactive after donating an electron and react with another reactive glutathione molecule to form glutathione disulfide (GSSG) which is further reduced to form reduced glutathione (GSH) and this complete cycle is accelerated by two important enzyme glutathione reductase and glutathione peroxidases.

TiO_2 and ZnO nanoparticles are reported to induce oxidative stress by the generation of ROS and altered antioxidant enzyme level in different *in-vitro* and *in-vivo* system such as cell lines (Saliani et al., 2016; Pandurangan and Kim, 2015; Srivastava et al., 2015; Dubey et al., 2015), rats/mice (Mansouri et al., 2015; Bheeman et al., 2014; Shrivastava et al., 2014), plants (Ebrahimi et al., 2016; Chichiriccò and Poma, 2015; Dolatabadi et al., 2015; Song et al., 2012), terrestrial arthropod (*Porcellio scaber, Isopoda, Crustacea*) (Drobne et al., 2009), *Daphnia magna* (Liu et al., 2014), worms (*Eisenia fetida, C. elegans*) (Khare et al., 2015; Lebedev et al., 2015; Hu et al., 2010), fish (*Oreochromis mossambicus, Tilapia zillii, Cyprinus carpio, Danio Rerio*) (Saddick et al., 2015; Liu et al., 2014; Karthigarani and Navaraj, 2012; Linhua et al., 2009).

Some naturally extracted chemicals, drugs, and antioxidants such as idebenone, carnosine, vitamin E, and glycyrrhizic acid are reported to mitigate nano-TiO_2 induced alteration in antioxidant enzyme level in mice/rat (Ali et al., 2015; Azim et al., 2015; Khorsandi et al., 2015). Similarly, protective effects of vitamin E, B, C and α-lipoic acid are determined against nano-ZnO induced oxidative stress in rat and Nile tilapia (*Oreochromis niloticus*) (Yousef and Mohamed, 2015; Fukui et al., 2015; Nemenqani, 2015; Nemenqani et al., 2015; Somayeh and Mohammad, 2014; Alkaladi et al., 2014; Rasheed et al., 2012). Thus, the main aim of this study is to evaluate (i) the disruption of antioxidant enzyme regulation and oxidative damage of macromolecules in *C. elegans* exposed to TiO_2 and ZnO particles, (ii) the presence of curcumin/ascorbic acid during treatment ameliorates the same by normalizing antioxidant enzyme activity and monitoring oxidative damage of macromolecules.

4.2 Material and Methods

All the assays in this chapter were performed at LC_1, LC_{10} and LC_{50} concentration for lethal particles, whereas for non-lethal bulk particle (bulk-TiO_2) same concentration as used for nano-TiO_2 was applied in presence/absence of antioxidants (curcumin and ascorbic acid), along with control. For determining DNA damage and apoptosis the assay was performed at LC_{10} concentration.

4.2.1 ROS assay: Followed the protocol as mentioned in chapter 2, section 2.2.5.

4.2.2 MTT Assay:

Tetrazolium MTT dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] is used to assess cellular metabolic activity. MTT is a cell-membrane permeable dye, which

is reduced by NAD(P)H-dependent cellular oxidoreductase enzymes to an insoluble purple colored formazan product. This formazan product is soluble in organic solvent and thus, measured calorimetrically. Synchronized L4 worms (~1000 worms) in triplicate were transferred to each well of transparent 96-well microtitre plate along with 50 µl of MTT (10 mg/ml) dye and incubated at 20°C for 3 h. It was centrifuged at 2000 rpm for 10 min, and the supernatant was aspirated. Formazan formed in worms was solubilized in 100 µl DMSO and measured at 595 nm in Spectrophotometer (Spectramax, Molecular Devices, UK) (James et al., 2007). MTT gives a measure of viable cells and is shown in results section as fold change in comparison to control.

4.2.3 Sample preparation for enzymatic assays:

Samples of control and treated worms were prepared as 10% by weight/volume ratio in phosphate buffer (0.1 M, pH 7.4), homogenized and centrifuged at 1,500 × g for 10 min at 4ºC. The supernatant was employed as 10% homogenate and used for measuring LPO and GSH activity. Further, the 10% homogenate was again centrifuged at 12,500 × g for 15 min at 4ºC, the supernatant was collected as post-mitochondrial fraction (PMF- for CTL, GPx, AChE estimation) and the pellet was dissolved in phosphate buffer (0.1 M, pH 7.4), and this fraction called mitochondrial fraction (MF) was used for the SOD estimation.

4.2.4 Protein estimation:

Protein concentration in tissue homogenates was measured using bovine serum albumin (BSA) as the reference standard (Lowry et al., 1951). Lowry's method employs two colour forming reactions (i) Biuret reactions: cupric ions (Cu^{2+}) in presence of a base

reacts with a peptide bond of protein under alkaline conditions reducing into cuprous ions (Cu^+) (ii) Lowry's reaction: The blue-purple color is formed due to the reduction of Folin Ciocalteu reagent (phosphomolybdo-tungstate to hetero-polymolybdenum blue) by the copper catalysed oxidation of aromatic amino acids tryptophan and tyrosine. Finally, the blue color is measured at 650-700 nm on the spectrophotometer. The reaction consists of 2 ml copper sulphate reagent [98 ml of solution A (2% sodium carbonate in 50 ml of 0.1 N NaOH solution), 2 ml solution B (1.56% copper sulphate solution in 10 ml of 2.37% sodium potassium tartrate solution)] and 0.2 ml of Folin - Ciocalteau reagent solution (1 N) was added to 0.2 ml of sample, followed by 30 min incubated in dark condition. The standard of 1 mg/ml BSA was run in parallel at different concentrations (25-200 µg/ml). Optical density was measured at 660 nm. The protein concentrations in the *C. elegans* samples were determined through BSA standard curve as mg/ml total protein.

4.2.5 Superoxide dismutase (SOD) activity *EC 1.15.1.1*:

The activity of superoxide dismutase was measured in the control and treated worms following the method of Kakkar et al., 1984. This assay consists of 1.3 ml sodium pyrophosphate buffer (0.082 M, pH 8.3), 100 µl phenazine methosulphate (186 µM), 300 µl nitro-blue-tetrazolium (300 µM), and 100 µl of MF in phosphate buffer (0.1 M, pH 7.4). The reaction was initiated by addition of 200 µl NADH (780 µM) followed by incubation at 37^{o}C for the 90 s. Glacial acetic acid (1 ml) was added to stop the reaction. The reaction mixture was mixed vigorously with 4 ml of n-butanol and the mixture was allowed to stand for 10 min followed by centrifugation for 10 min at 3,000 × g to

separate the butanol layer. The color intensity of the chromogen (purple) in butanol layer was measured at 560 nm on the spectrophotometer. A mixture without enzyme preparations was run in parallel to serve as the reagent blank. The superoxide dismutase activity is expressed in units/min/mg protein.

4.2.6 Catalase (CTL) activity *EC1.11.1.6*:

The activity of catalase in control and treated worms was assayed following the method of Aebi, 1984, using H_2O_2 as substrate. To the reaction mixture, 896 µl phosphate buffer (0.1 mM, pH 7.4), 100 µl PMF of sample, and 4 µl H_2O_2 (30 mM) was added rapidly, making its final volume to 1 ml. The decrease in optical density was measured for 150 s at 240 nm using the spectrophotometer. The activity of the enzyme was calculated using the molar extinction coefficient 43.6 M cm^{-1} and expressed as catalase activity/min/mg protein.

4.2.7 Reduced glutathione (GSH) assay:

Levels of reduced glutathione (GSH) in *C. elegans* were measured following the method of Hasan and Haider, 1989. 500 µl of 10% homogenate was deproteinized with an equal volume (500 µl) of 10% TCA and allowed to stand at 4°C for 1 h. The contents were centrifuged at 3000 × g for 15 min. The supernatant (0.5 ml) was added to 2 ml of Tris-HCl buffer (0.4 M, pH 8.9) containing EDTA (0.02 M, pH 8.9) followed by the addition of 100 µl 5,5'dithionitrobenzoic acid (DTNB, 0.01 M). The volume was made up to 3 ml by addition of 0.5 ml of DW and absorbance of yellow color was read on a spectrophotometer at 412 nm. The standard of GSH was run in parallel at different concentrations (5-100 µg/ml) and the results are expressed as µg GSH/g tissue.

4.2.8 Glutathione peroxidase (GPx) activity *EC 1.11.1.7*:

The activity of glutathione peroxidase in *C. elegans* was measured by the procedure of Flohe and Gunzler, 1984. The PMF of exposed worms was used for the estimation of glutathione peroxidase activity. Reaction mixture in a final volume of 1 ml containing 300 µl phosphate buffer (0.1 M, pH 7.4), 200 µl reduced glutathione (2 mM), 100 µl sodium azide (10 mM), 200 µl hydrogen peroxide (H_2O_2, 1 mM) and 300 µl *C. elegans* homogenate (PMF) was incubated at 37ºC for 15 min. The reaction was stopped by addition of 0.5 ml TCA (10%). The tubes were centrifuged at 2000 × g for 5 min to settle the protein. Following this, the 100 µl of supernatant was added into another tube containing 0.2 ml of phosphate buffer (0.1 M, pH 7.4) and 0.7 ml of the DTNB (0.4 mg/ml). The reaction mixture was vortexed and absorbance was recorded at 420 nm. The values are expressed as nmol GSH oxidized/min/mg protein.

4.2.9. Protein carbonyl assay:

Protein carbonyl levels in *C. elegans* was measured following the method of Levine et al., 1990 using 2,4-dinitrophenylhydrazine (DNPH) as a substrate. To 200 µl of PMF, 800 µl of 10 mM DNPH dissolved in 2 M HCl was added. Following this, samples were incubated for 1 h at RT in the dark with continuous stirring and then precipitated with 1 ml of 20% trichloroacetic acid (TCA). The tubes were kept for 10 min on ice and samples were centrifuged at 3,000 × g for 15 min at RT. The supernatant was discarded and protein pellet was washed in 10% TCA once and thrice in ethanol: ethyl acetate (1:1) to remove free DNPH and additional lipid contaminants. The protein precipitate was then dissolved in 1 ml 6 M guanidine hydrochloride solution. Finally, the absorbance

was determined using spectrophotometer at 375 nm and the amount of carbonyl content was calculated using a molar extinction coefficient (ε) of 22.0 $mM^{-1}cm^{-1}$ for aliphatic hydrazones.

4.2.10 Lipid peroxidation (LPO) assay:

As a measure of lipid peroxidation, thiobarbituric acid reactive substance (TBARS) was measured in *C. elegans* following the method of Ohkawa et al., 1979. The control and treated worms were homogenized individually in phosphate buffer (0.1 M, pH 7.4). To 50 µl 10% *C. elegans* homogenate, 1650 µl DW was added. The homogenate was incubated with 100 µl of 10% sodium dodecyl sulfate for 10 min followed by the addition of 20% acetic acid (600 µl). The reaction mixture was incubated with 600 µl 0.8% thiobarbituric acid for 2 h in boiling water bath, then cooled and centrifuged at 500 rpm for 5 min. The supernatant was transferred to new tubes. The intensity of pink chromogen formed was read at 532 nm. The amount of TBARS during the reaction was calculated using a molar extinction coefficient of 1.56 x 105 $M^{-1}cm^{-1}$.

4.2.11 Estimation of 8-OHDG:

8-hydroxy-2'-deoxyguanosine (8-OHdG) biomarker was used to determine DNA damage (Yang et al., 2013). L4 staged worms were subjected to LC_{10} of nano/bulk TiO_2 and ZnO for 24 h. After treatment, worms were washed with M9 buffer harvested and homogenized in TE buffer followed by centrifugation at 12000 rpm for 15 min at 4°C. DNA was extracted by phenol-chloroform extraction method and subjected to liquid chromatography to determine 8-OHdG and dG levels.

DNA extraction:

Control and treated worms were harvested, washed thrice with sterile water and twice with TEN buffer (40 mM Tris-HCl, pH 7.5 - 8.0, 1 mM EDTA and 150 mM NaCl) to remove bacterial and other contamination. Worms were resuspended in TEN (0.75 ml) and transferred in eppendorfs. The washed worms were freezed for 2 h at 20 ºC followed by thawing at 37 ºC, twice. 40 µl of 10% SDS and 8 µl of 10 mg/ml proteinase K were added by gentle mixing followed by the incubation at 55ºC for 1 h. To this, 8 µl of 10 mg/ml proteinase K mix gently and incubate at 55 ºC for 1 h with occasional flicking. An equal volume of phenol was added, and samples were mixed for 5 min by flipping followed by the centrifuged at 14000 rpm for 10 min at 4ºC. The aqueous phase (upper layer) was taken in fresh eppendorf tubes and an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added. Mixed for 5 min and centrifuged at 14000 rpm for 5 min at 4ºC, The aqueous phase was transferred in a fresh tube and an equal volume of chloroform:isoamyl alcohol (24:1) was added. Mixed by shaking for 3 min and was centrifuged at 14000 rpm for 5 min at 4ºC. The aqueous phase was transferred in to a fresh tube and 1/10 volume of 3 M sodium acetate (pH 5.2) and 3 volume of absolute ethanol was added at RT and was mixed by inverting the tube few times. DNA precipitated instantly and a filament was formed, centrifuged at 14000 rpm for 5 min at 4ºC. The DNA pellet was washed with chilled 70% ethanol twice and the ethanol was decanted, dried briefly. DNA pellet was resuspended in TE buffer and quantified by Nanodrop (Thermo scientific, U.S.A).

(i) Sample preparation:

200 µg of DNA was digested to the nucleotide by adding 20 units of nuclease P1 and incubating at 37°C for 2 h. Subsequently, phosphate groups were removed by addition of 6 units of alkaline phosphatase and incubation at 37°C for 2 h. Just prior to UPLC analysis, the samples were filtered with Microcon YM-10 (EMD Millipore, Billerica, MA, USA) in order to remove remaining proteins or other contamination.

(ii) Ultra-High Performance Liquid Chromatography (UHPLC):

The samples were subjected to UPLC system (Shimadzu Corporation, Kyoto, Japan.) using enable amino column (4.6X250 mm) with the elution rate 1 ml/min and acetonitrile with 10 mM ammonium acetate as mobile phase. The amount of 8-OHdG and dG were detected with PDA (Photodiode array) detector at 254 nm wavelength in exposed worm samples along with standards for 8-OHdG (Sigma Aldrich) and dG (Sigma Aldrich).

(iii) Determination of 8-OHdG:

To determine the concentration of 2-dG and 8-OHdG standard graphs were plotted and the level of 8-OHdG was represented as nmol of 8-OHdG per nmol of 2dG.

4.2.12 Apoptosis:

Germ line apoptosis was determined by employing the transgenic strain ZH814. ZH814 has CED-1::GFP and 2_FYVE::mRFP; the fluorescent markers that label the surface of phagocytic cups and maturing phagosomes, respectively (Lu et al., 2009). CED-1, a transmembrane protein is expressed as a marker on the surface of engulfing (phagocytic) cell. Thus, CED-1::GFP specifically label cell corpses that are in the process of being

engulfed. Further, 2xFYVE::mRFP act as a marker for cell corpses that remains on the phagosomal surface until the complete degradation occurs. ZH814 worms were subjected to LC_{10} of nano/bulk TiO_2 and ZnO for 24 h. After treatment, worms were washed and mounted on 2% agar pads on a clean slide. Fluorescence imaging was carried to determine GFP (FITC filter excitation 480/20 nm, emission 535/20 nm) and/or RFP (Rhodamine filter excitation 560/20 nm emission 630/30 nm) expressed in the germ line cells undergoing apoptosis. Number of CED-1::GFP and 2xFYVE:: mRFP marked cell corpses were scored in ~30 worms for each treatment under the fluoresces microscope. The results represent the number of phagosomes (GFP) and engulfed cell corpses (RFP).

5.2.13 Statistical analysis:

Results are expressed as mean±SEM, graphically. Significance analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni correction.

4.3 Results

4.3.1a Effect of TiO_2/ZnO NPs on ROS generation of an organism in comparison to bulk:

Compared to control, concentration dependent and significant (p<0.001) ROS production with 2.8-/4.4-/6.8-, 2.1-/3.4-/4.9- fold increase, was observed in worms exposed to $LC_1/LC_{10}/LC_{50}$ of nano-TiO_2, bulk-TiO_2, respectively. Further, level of ROS in worms exposed to nano was significant (p<0.05) in comparison to that of bulk (Figure 4.1).

Similarly, 3.2-/4.8-/7.3- and 2.3-/3.8-/5.4- fold increase in ROS was noticed in worms exposed to $LC_1/LC_{10}/LC_{50}$ of nano-ZnO and bulk-ZnO, respectively. Nano exposed worms were found to have significant increment ($p<0.01$) in the ROS generation compare to bulk (Figure 4.1).

4.3.1b Amelioration from nano/bulk induced ROS generation:

In presence of the antioxidants (ascorbic acid or curcumin), ROS production was found to be equivalent to that of control at LC_1 and LC_{10} concentrations of nano/bulk particles (Figure 4.1), while the recovery was not comparable to control at LC_{50} concentration.

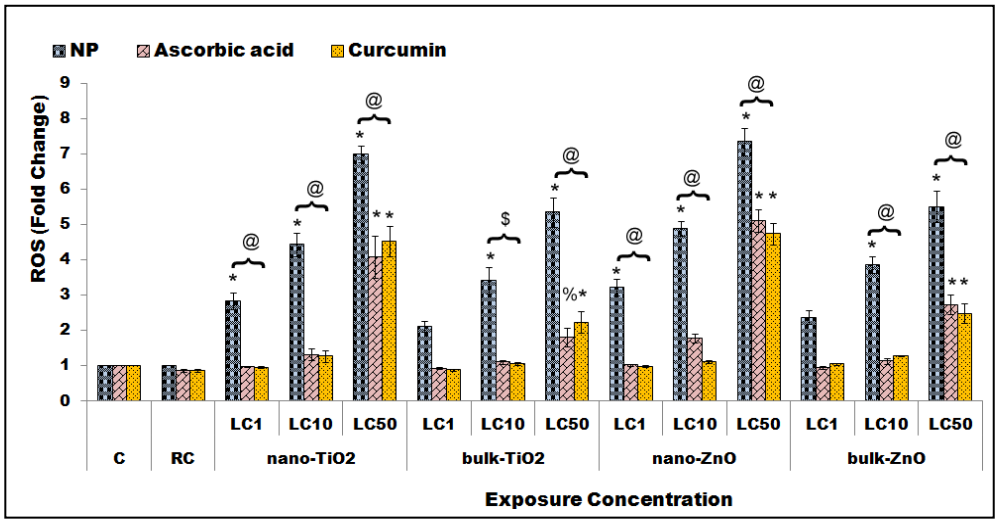

Figure 4.1 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced change in the level of reactive oxygen species in exposed *C. elegans*. C = control; RC = reference control; bar = mean±SEM of three independent experiments; Bonferroni corrected *$p<0.001$, $^{\%}p<0.01$ = Significant against control; $^{@}p<0.001$, $^{\$}p<0.05$ = Significant recovery (in presence of antioxidant) against particle exposure

4.3.2a Effect of TiO_2/ZnO NPs on mitochondrial activity of organism in comparison to bulk:

Mitochondrial activity reduces significantly ($p>0.001$) in concentration dependent manner in particle exposed organisms compared to controls. We observed 13%, 31%, 41% reduction in mitochondrial activity of the organism exposed to for nano-TiO_2 and 7%, 17%, 33% reduction in organism exposed to bulk-TiO_2 at LC_1, LC_{10}, LC_{50} concentrations, respectively. A significant decrease ($p<0.01$) in the mitochondrial activity was found in nano-TiO_2 exposed worms at LC_{10} and LC_{50} concentrations, compared to bulk particles (Figure 4.2).

Similar results were observed in nano ZnO and bulk ZnO exposed organisms where 27%, 44%, 63% and 15%, 36%, 57% reduction in mitochondrial activity was noticed at LC_1, LC_{10} and LC_{50} concentrations, respectively, in comparison to control. The decrease in mitochondrial activity of worms exposed to nano was significant ($p<0.01$) in comparison to that of bulk (Figure 4.2).

4.3.2b Amelioration from nano/bulk induced mitochondrial damage:

In the presence of antioxidants during particle treatment, the mitochondrial activity did not differ significantly from control in worms exposed to LC_1 and LC_{10} concentrations of nano/bulk particles as well as LC_{50} of the bulk-TiO_2 particle. In worms exposed to LC_{50} of nano-TiO_2 and nano-ZnO in presence of antioxidants, a significant ($p<0.001$) reduction in mitochondrial activity with 10% and 32% respectively was noticed, while a reduction of mitochondrial activity by 13% was noticed even in case of bulk-ZnO, in

presence of curcumin (Figure 4.2). Thus, overall 30% mitochondrial activity was recovered in presence of antioxidants at highest treatment concentration.

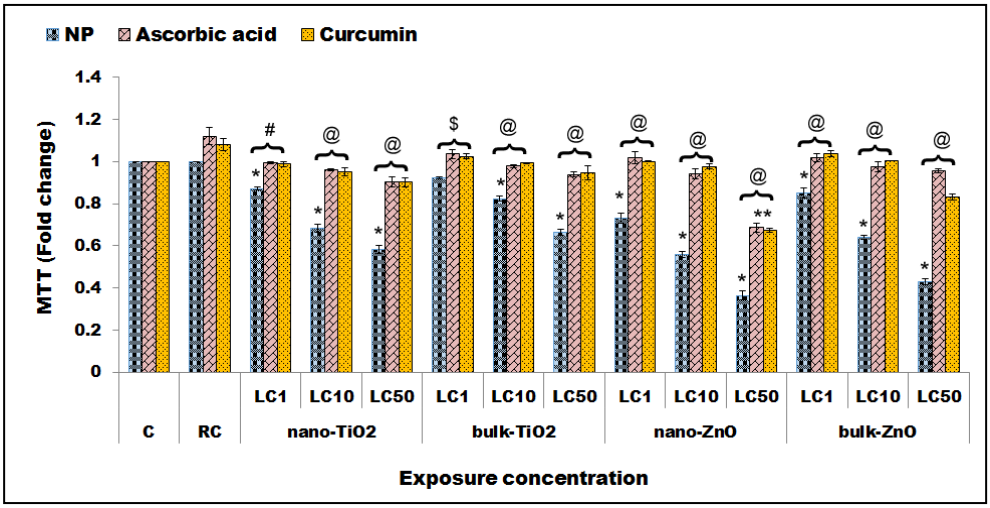

Figure 4.2 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced mitochondrial dysfunction in *C. elegans*. C = control; RC = reference control; bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001 = Significant against control; $^{@}p$<0.001, $^{\#}p$<0.01, $^{\$}p$<0.05 = Significant recovery (in presence of antioxidant) against particle exposure

4.3.3a Effect of TiO_2/ZnO NPs on superoxide dismutase (SOD) activity of organism in comparison to bulk:

The SOD activity was found to increase in concentration dependent manner on exposure of worms to nano/bulk particles. The increase in SOD activity in worms on exposure to LC_1, LC_{10} and LC_{50} concentrations was 1.15-, 1.4-, 1.7- fold for nano-TiO_2 exposure, 1.1-, 1.2-, 1.35- fold for bulk-TiO_2 exposure, 1.2-, 1.6-, 1.95- fold for nano-ZnO exposure and 1.1-, 1.2-, 1.6- fold for bulk-ZnO exposure, respectively, in comparison to

control worms (1-fold). Significant (p<0.001) increase in SOD activity was noticed in worms exposed to LC_{10} and LC_{50} concentrations of nano TiO_2/ZnO and bulk-ZnO particles, and only in worms exposed to LC_{50} concentrations of bulk-TiO_2 particles (Figure 4.3).

4.3.3b Amelioration effect of antioxidant on superoxide dismutase (SOD) activity:

In the presence of antioxidants during particle treatment, SOD activity in nano/bulk particles exposed worms was equivalent to that of control, except in worms exposed to LC_{50} of nano particles. Even at the LC_{50} concentration, 50% recovery was noticed in presence of antioxidants with the SOD activity of 1.35- fold for nano-TiO_2 and 1.5- fold for nano-ZnO particle (Figure 4.3). Thus, antioxidants attenuated SOD activity imposed by lower concentrations of nano/bulk particles and up to 50% (of SOD activity) even at highest treatment concentration (LC_{50}) of nanoparticles tested.

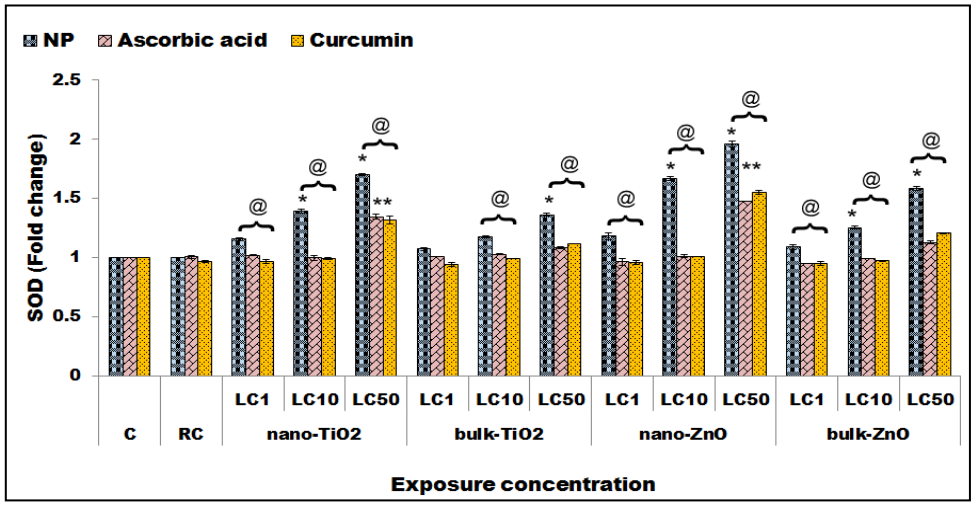

Figure 4.3 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced change in the level of super oxide dismutase enzyme in exposed *C. elegans*. C = control; RC = reference control; bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001 = Significant against control; @p<0.001 = Significant recovery (in presence of antioxidant) against particle exposure

4.3.4a Effect of TiO_2/ZnO NPs on catalase (CTL) activity of organism in comparison to bulk:

A significant (p<0.01) and concentration dependent increase in CTL activity was observed in worms exposed to LC_{10} and LC_{50} of nano-TiO_2 (1.39-, 1.75- fold); LC_{50} of bulk-TiO_2 (1.46- fold); LC_1, LC_{10} and LC_{50} of nano-ZnO (1.6-, 1.75-, 2.1- fold) and for LC_{10} and LC_{50} of bulk-ZnO (1.4-, 1.6- fold) in comparison to control (1- fold) worms (Figure 4.4).

4.3.4b Amelioration effect of antioxidant on catalase (CTL) activity:

Significant reduction in CTL activity was noticed in the presence of antioxidants during nano/bulk treatment in comparison to the absence of antioxidants during treatment. In

the presence of antioxidants, CTL activity was restored to that of control, except in worms exposed to LC_{50} of nano-TiO_2 and nano-ZnO, where it was found to be significant (p<0.001) with 1.26- fold and 1.7- fold increase, respectively (Figure 4.4). Thus, antioxidants attenuated CTL activity imposed by lower concentrations of nano/bulk particles and at LC_{50} of nano-TiO_2 and nano-ZnO, attenuation was 60% and 35% (of CTL activity), respectively.

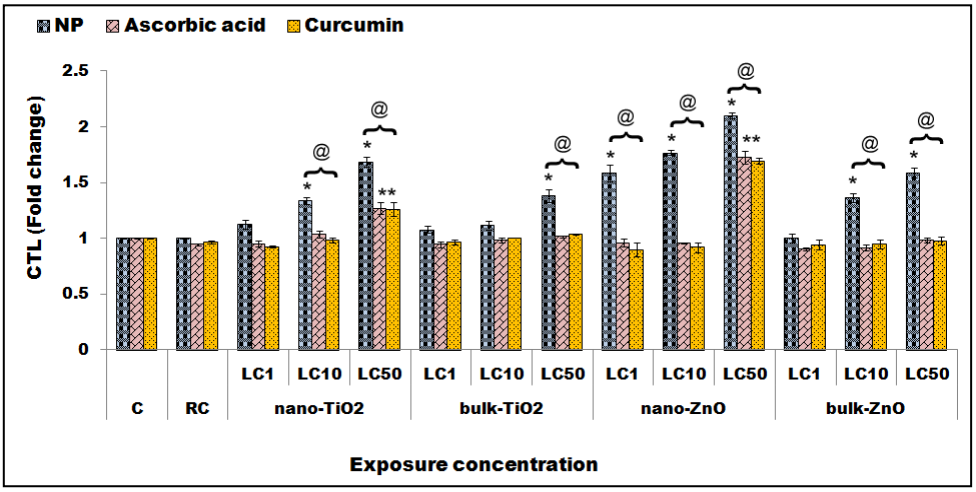

Figure 4.4 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced change in the level of catalase enzyme in exposed *C. elegans*. C = control; RC = reference control; bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001 = Significant against control; @p<0.001 = Significant recovery (in presence of antioxidant) against particle exposure

4.3.5a Effect of TiO_2/ZnO NPs on reduced glutathione (GSH) activity of organism in comparison to bulk:

Glutathione in reduced form (GSH) is a measure of cells free-radical scavenging capacity. GSH level was found to reduce significantly ($p<0.01$) in concentration dependent manner with 15%, 24%, 42%; 12%, 22.5%, 36.6%; 19.3%, 43%, 55.3% and 13%, 30%, 43% reduction in organism exposed to LC_1, LC_{10}, LC_{50} concentrations of nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO respectively, in comparison to control. The decrease in GSH levels of worms exposed to nano was non-significant in comparison to that of bulk (Figure 4.5).

4.3.5b Amelioration effect of antioxidant on reduced glutathione (GSH) activity:

In the presence of antioxidants during particle treatment, the GSH levels did not differ significantly from control, in the worms exposed to LC_1 and LC_{10} of nano/bulk particle. In worms exposed to LC_{50} of nano/bulk particles in presence of antioxidants, a significant ($p<0.001$) reduction in GSH level was noticed (Figure 4.5). The recovery in GSH levels in presence of ascorbic acid/curcumin antioxidants, against nano-TiO_2 is 24%/32%; for bulk-TiO_2 it is 45%/62%; for nano-ZnO it is 50%/42%, while for bulk-ZnO it is 78%/69%, respectively.

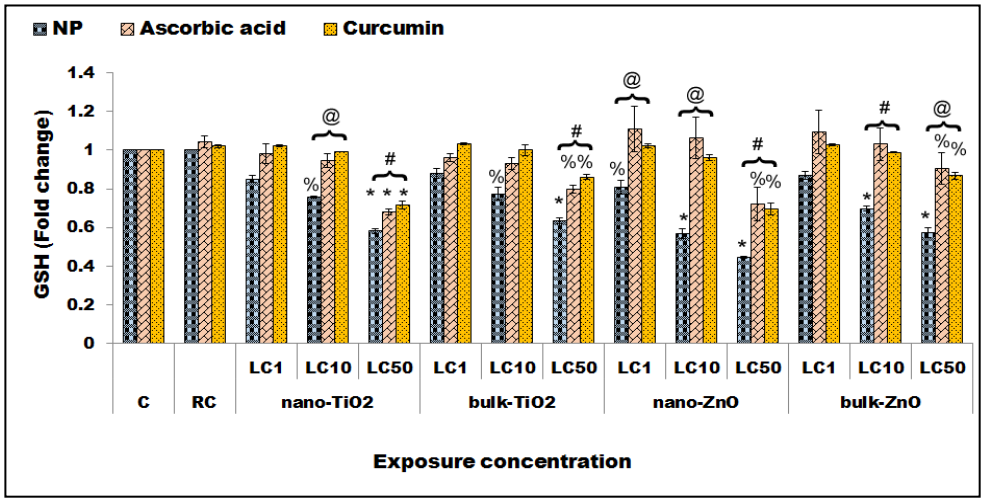

Figure 4.5 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced change in reduced glutathione level in exposed *C. elegans*. C = control; RC = reference control; bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001, $^{\%}p$<0.01 = Significant against control; $^{@}p$<0.001, $^{\#}p$<0.01 = Significant recovery (in presence of antioxidant) against particle exposure

4.3.6a Effect of TiO_2/ZnO NPs on glutathione peroxidase (GPx) activity of organism in comparison to bulk:

Glutathione peroxidase activity was found to reduce in concentration dependent manner with 10%, 15.6%, 21.4%; 3%, 8.5%, 16.8%; 7.75%, 18.7%, 33% and 5.4%, 10%, 20% reduction in organism exposed to LC_1, LC_{10}, LC_{50} concentrations of nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO respectively, in comparison to control (Figure 4.6).

Significant (p<0.01) decrease in GPx activity was noticed in worms exposed to LC_{10} and LC_{50} concentrations of NPs and only at the LC_{50} concentration of bulk particles (Figure 4.6).

4.3.6b Amelioration effect of antioxidant on glutathione peroxidase (GPx) activity:

In the presence of antioxidants during particle treatment, the GPx activity did not differ significantly from control, except for LC_{50} of nano-ZnO where a significant (p<0.001) reduction of 23% and 18% GPx activity was noticed in presence of ascorbic acid and curcumin respectively, during treatment (Figure 4.6). The recovery in GPx activity by 30% and 46% was witnessed in presence of ascorbic acid and curcumin, respectively, against LC_{50} of nano-ZnO exposure.

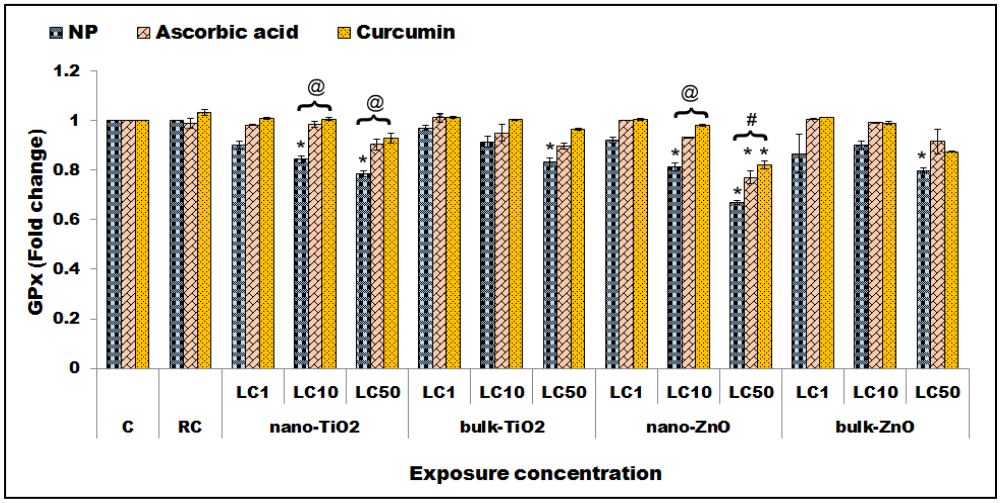

Figure 4.6 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced change in the level of glutathione peroxidase in exposed *C. elegans*. C = control; RC = reference control; bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001 = Significant against control; $^{@}p$<0.001, $^{\#}p$<0.01 = Significant recovery (in presence of antioxidant) against particle exposure

4.3.7a Effect of TiO_2/ZnO NPs on lipid peroxidation (LPO) of the organism in comparison to bulk:

A significant (p<0.001) and concentrations dependent increase in MDA content with 2.25-, 4.2- and 7.2- fold for nano-TiO_2, 1.5-, 2.07- and 2.5- fold for bulk-TiO_2, 2.95-, 4.9- and 9.0- fold nano-ZnO and 2.4-, 2.6- and 3.5- fold for bulk-ZnO exposed worms at LC_1, LC_{10} and LC_{50} respectively, in comparison to control (1- fold) was obtained (Figure 4.7).

4.3.7b Amelioration effect of antioxidant on lipid peroxidation (LPO):

Significant (p<0.001) reduction in MDA content was noticed in the presence of antioxidants during nano/bulk treatment in comparison to the absence of antioxidants during treatment. In the presence of antioxidant (ascorbic acid or curcumin) during treatment, the MDA content was equivalent to that in control among the exposed worms at the low exposure concentrations (LC_1 and LC_{10}) of nano/bulk particles as well as at LC_{50} of bulk-TiO_2. At LC_{50} concentration of nano TiO_2/ZnO 84% recovery in presence of antioxidants was noticed, whereas at LC_{50} of bulk-ZnO the recovery was 70% and 75% in presence of ascorbic acid and curcumin respectively (Figure 4.7).

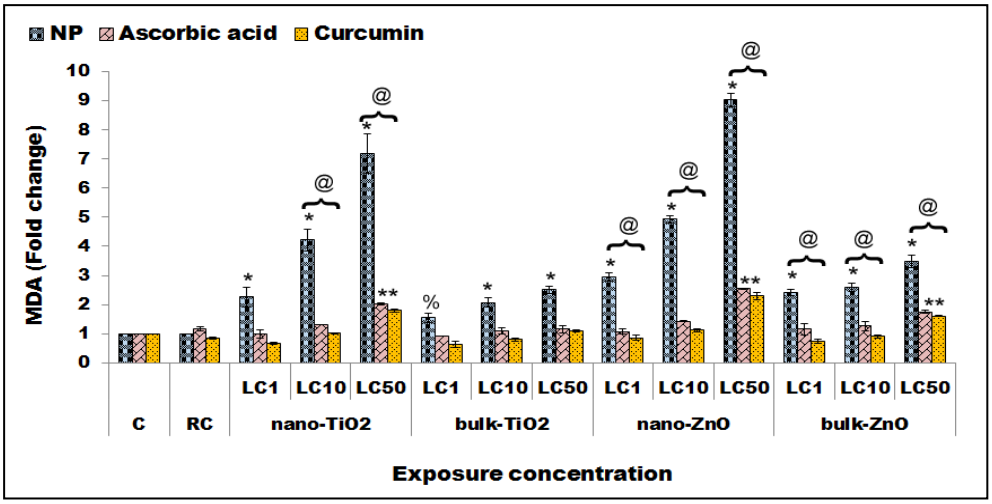

Figure 4.7 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced change in the level of malondialdehyde in exposed *C. elegans*. C = control; RC = reference control; bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001, %p<0.01 = Significant against control; @p<0.001 = Significant recovery (in presence of antioxidant) against particle exposure

4.3.8a Effect of TiO_2/ZnO NPs on protein carbonylation of the organism in comparison to bulk:

Protein carbonylation was found to increase in concentration dependent manner on exposure of worms to nano/bulk particles. The increase in protein carbonylation in worms on exposure to LC_1, LC_{10}, LC_{50} concentrations was 1.1-, 1.66-, 2.87- fold for nano-TiO_2 exposure, 1.06-, 1.1-, 1.5- fold for bulk-TiO_2 exposure, 1.2-, 2.02-, 4.1- fold for nano-ZnO exposure and 1.2-, 1.43-, 1.9- fold for bulk-ZnO exposure, respectively, in comparison to (1- fold) control worms (Figure 4.8).

Significant (p<0.01) increase in protein carbonylation was noticed in worms exposed to LC_{10} and LC_{50} concentrations of nanoparticles as well as LC_{50} of bulk particles (Figure 4.8).

4.3.8b Amelioration effect of antioxidant on protein carbonylation:

In presence of antioxidants, protein carbonylation was restored to that of control, except in worms exposed to LC_{50} of nano-TiO_2 and nano-ZnO, where it was found to be significant (p<0.001) with 1.9- and 2.28- fold in presence of ascorbic acid and 1.74 and 1.8- fold increase in presence of curcumin, respectively (Figure 4.8). Thus, antioxidants attenuated protein carbonylation imposed by lower concentrations of nano/bulk particles and even at highest treatment concentration (LC_{50}), 51-60% of nano-TiO_2 and 58-74% of nano-ZnO induced protein damage (Figure 4.8).

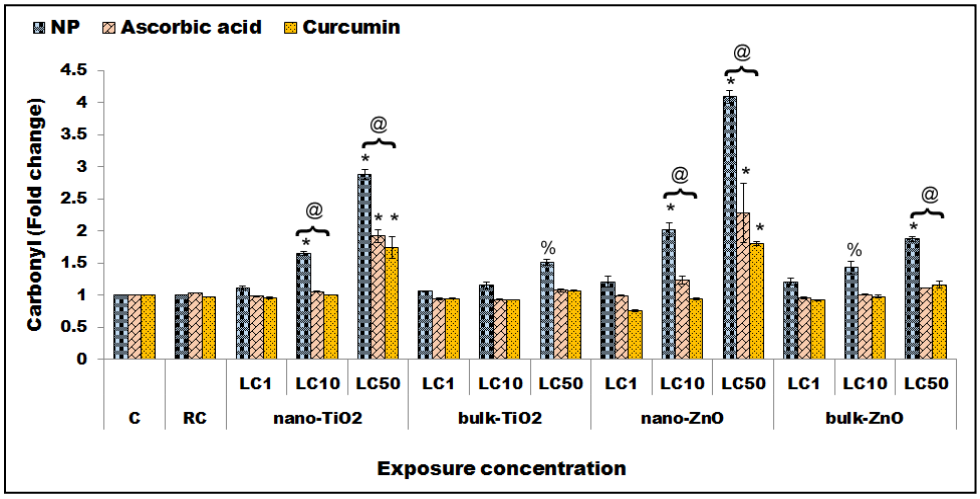

Figure 4.8 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced change in the level of carbonyl in exposed *C. elegans*. C = control; RC = reference control; bar = mean±SEM of three independent

experiments; Bonferroni corrected *p<0.001, $^{\%}p$<0.01 = Significant against control; $^{@}p$<0.001 = Significant recovery (in presence of antioxidant) against particle exposure

4.3.9a Effect of TiO_2/ZnO NPs on DNA of the organism in comparison to bulk:

The effect of NPs on DNA damage was assessed by measuring the level of oxidized DNA in *C. elegans*. Compared to control, the level of oxidized guanine base was significantly high in nano/bulk exposed worm at LC_{10} concentration. Further, DNA oxidation was found significantly high (p<0.05) in nano-TiO_2 and nano-ZnO exposed worm in comparison to the worms exposed to their respective bulk particles (Figure 4.9).

4.3.9b Amelioration from nano/bulk induced DNA damage:

The presence of curcumin or ascorbic acid protects worms from nano/bulk particles induced DNA oxidation. Further, no significant alteration was observed in the treated worms in the presence of antioxidants compared to control (Figure 4.9).

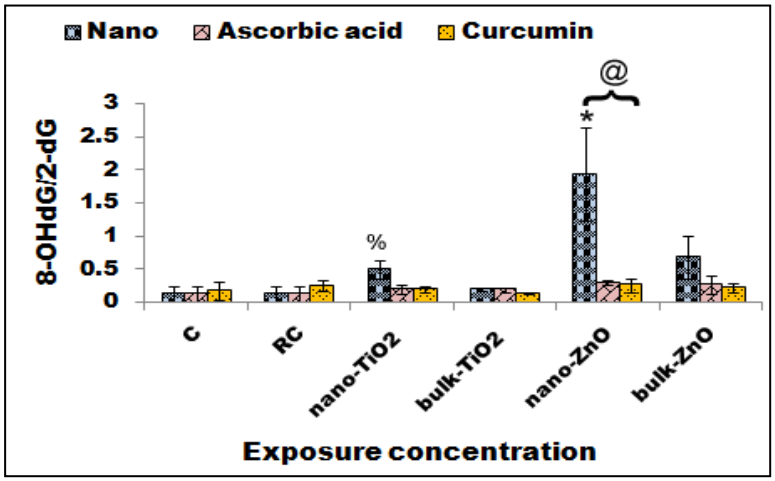

Figure 4.9 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced change in DNA damage in exposed *C. elegans*. C = control; RC = reference control; bar = mean±SEM of three independent experiments; Bonferroni corrected $^{\%}p<0.05$, $^{*}p<0.01$, = Significant against control; $^{@}p<0.001$ = Significant recovery (in presence of antioxidant) against particle exposure

4.3.10a Effect of TiO_2/ZnO NPs on germ line apoptosis of organism in comparison to bulk:

In ZH814 an apoptotic marker strain, the expression of CED-1::GFP; 2_FYVE::mRFP in the germ-line of exposed worms were significantly higher compared to controls (Figure 4.10). Further, the significant change was observed in nano-exposed ($p<0.05$) worms in comparison to worms exposed to bulk particles.

4.3.10b Amelioration from nano/bulk induced germ line apoptosis:

In the presence of antioxidants (ascorbic acid or curcumin) during the treatment, non-significant apoptosis (number of cell corpses and phagosomes) was observed in the germ-line of ZH814 in comparison to control worms (Figure 4.10).

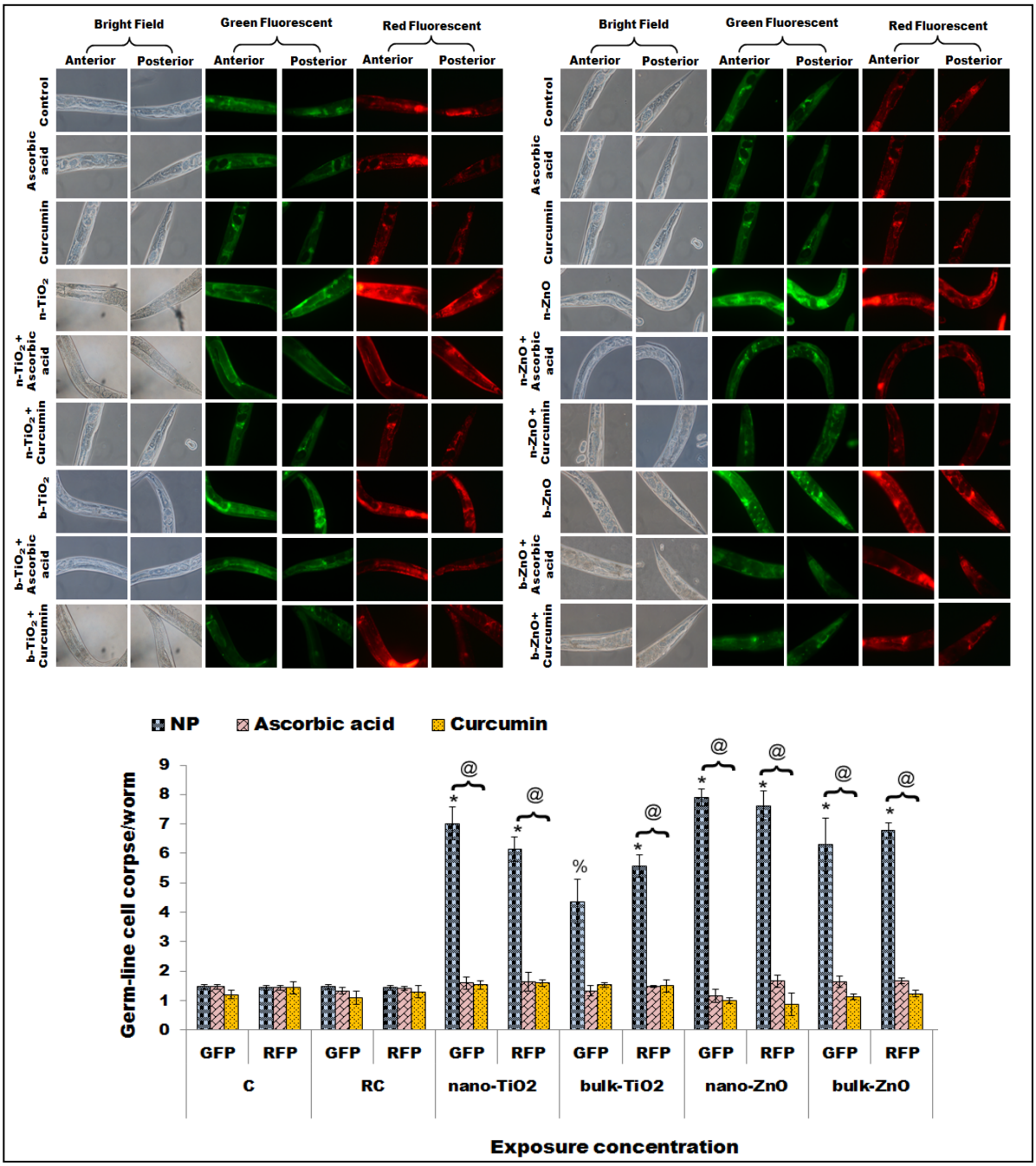

Figure 4.10 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced apoptosis in germ line of *C. elegans*. C = control; RC = reference control; n=30, bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001, $^{\%}p$<0.01 = Significant against control; $^{@}p$<0.001 = Significant recovery (in presence of antioxidant) against particle exposure

4.4 Discussion

Free radicals are highly reactive molecules with unpaired electrons in the outer shell. Free radicals convert other stable molecules into reactive species by gaining electrons from them, thus, triggering a chain reaction. Generation of ROS is omnipresent in living organism and cell has their own antioxidant networks to quench ROS therefore, there is a balance between the production and scavenging of ROS. Increased level of ROS is regulated by antioxidant defense system (antioxidative enzyme and molecules) of the organism. Antioxidants are the molecules which donate its electron and neutralize free radical. Classic antioxidant enzyme (SOD, CTL, GPx) and molecules (reduced GSH) level are considered as an important marker to study default in the antioxidant defense system of an organism. The significant increase in SOD and CTL activity was observed in nano/bulk exposed organism (supported by our GFP-transgenic results in Chapter 3). Exposure to nano/bulk increases the free radical load which in turn leads to activation of signal transduction pathways for transcription of redox-sensitive genes such as SOD, CTL, and GPx. This is in correlation with earlier studies where increased SOD and CTL activity has been reported on exposure to nano-TiO_2 or nano-ZnO in WAG-cell lines, aquatic model carp (*Cyprinus carpio*), earthworm *Eisenia fetida* and Wister rats, (Dubey et al., 2015; Hao et al., 2012; Li et al., 2012; Hu et al., 2010). Glutathione is one of the most abundant and important cellular tri-peptide antioxidants and GPx enzyme catalyzes a reaction between reduced monomeric glutathione and peroxides as a protective mechanism against oxidative damage (Birben et al., 2012; Lushchak, 2012). A dose-dependent decrement was observed in GSH/GPx level in an exposed worm. This is in correlation with *in-vivo* studies in rats on exposure to indium titanium oxide nanoparticles (InTiONPs), (Bheeman et

al., 2014) as well as nano-ZnO (Mansouri et al., 2015) significant decrease in GSH/GPx enzymes activity was reported. Similarly, in *Daphnia magna* and zebra fish embryos the reduced level of GSH in 21 day chronic exposure to TiO_2, ZnO, CuO and Au nanoparticles have been reported (Liu et al., 2014). Thus, the present study revealed that both the nano and bulk form of TiO_2 or ZnO even at sub-lethal concentrations (LC_1 and LC_{10}) induced high oxidative stress, as identified by the elevated level of ROS, and significant change in antioxidant enzyme levels.

However, under stress circumstances, the balance is shifted towards the formation of free radicals and high level of ROS can oxidize biomolecules such as lipids, protein, and DNA which, ultimately participate in the regulation of cell death. Reactions between free radicals and lipid or protein end up with the generation of reactive aldehydes [malondialdehyde (MDA) and 4-hydroxynonenal (HNE)] and/or ketones respectively. Similarly, high level of ROS leads to oxidation of DNA bases and may break DNA strand. Guanine is the most susceptible DNA base due to its low oxidation potential during the oxidation reaction. It has multiple oxidation products and the two most common modifications are 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 2, 6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua). 8-OHdG is one of the most predominant, stable, and well studied forms of free radical-induced oxidative lesions. 8-OHDG along with MDA and carbonyl content are used as an important index of the stressed condition. Many toxicity studies claimed that nano from of particle including nano- TiO_2/ZnO stimulates higher ROS production and oxidative stress than their bulk counterparts (Khare et al., 2015; Ratnasekhar et al., 2015; Xiong et al., 2011). In the present study, MDA, protein carbonyl content, as well as 8-OHdG, were found to be

significantly high in nano/bulk TiO_2/ZnO exposed worms. Therefore, results indicate that exposure to nano/bulk induces high ROS generation and oxidative damage of lipid, protein, and DNA. Similar results were reported wherein exposure of nano/bulk TiO_2/ZnO to WAG cells, eukaryotic cells and mice induce macromolecules damage which causes oxidative stress and initiates inflammatory responses (Bucchianico et al., 2017; Hong and Zhang, 2016; Saliani et al., 2016; Dubey et al., 2015). NPs induce oxidative damage of macromolecular can initiate programmed cell death in the organism (Saliani et al., 2016; Khanna et al., 2015). In the present study, we witnessed significant effect on cell viability (MTT assay) and germ cell apoptosis in worms exposed to nano/bulk TiO_2/ZnO. This data is consistent with the results obtained from *in-vitro* and *in-vivo* acute toxicity of nano-TiO_2 and nano-ZnO on WAG cell line (Dubey et al., 2015), human fetal lung fibroblasts (Zhang et al., 2011), primary mouse embryo fibroblast cells (Yang et al., 2009), *C. elegans* (Khare et al., 2015), mice (Mohamed and Hussien, 2016), rat (Meena et al., 2015). These findings indicate that exposure to nano and bulk form of TiO_2 or ZnO even at sub-lethal concentrations (LC_{10}) induced high oxidative stress, as identified by the elevated level of ROS, macromolecule damage, apoptosis and significant change in antioxidant enzyme levels.

Co-exposure with curcumin has been reported to protect against mercury, iron and arsenic trioxide-induced changes in SOD, CTL, reduced GSH-level and GPx activity in rat and mice (Badria et al., 2015; Mathews et al., 2012; Agarwal et al., 2010). Curcumin was also found to be protective against nano-nickel oxides particles induced lipid peroxidation and reduced GSH level in human airway epithelial (HEp-2) and human breast cancer (MCF-7) cells (Siddiqui et al., 2012). Co-exposure with ascorbic acid has been reported to protect against cadmium-

induced changes in biochemical parameters such as alanine aminotransferase, creatine phosphokinase and catalase activity in *Cyprinus carpio* (Banaee et al., 2015). Ascorbic acid has also been reported to help in maintaining the normal level of SOD, CTL and GSH in nano-ZnO (300 mg/kg and 1 g/kg body weight) or $CdCl_2$ (5 mg/kg body weight) exposed rats (Nemenqani et al., 2015; El-Sokkary et al., 2011). The presence of antioxidants ascorbic acid and curcumin provide protection against particle (TiO_2/ZnO) induced oxidative stress. This is in correlation with the protection provided by antioxidants against heavy metal toxicity.

In the present study, the presence of ascorbic acid and curcumin during the treatment not only reduces free radical generation (Chapter 2), it also provides significant protection to macromolecules from oxidative damage followed by apoptosis, induced by the exposure of nano/bulk particles in *C. elegans*. Our studies correlates with the finding that ascorbic acid and curcumin protects rats and miceagainst cadmium, mercury, iron, arsenic trioxide and nano-ZnO induced oxidative damage to lipid, protein and DNA in rat and mice (Badria et al., 2015; Nemenqani et al., 2015; Parveen et al., 2014; Mathews et al., 2012; El-Sokkary et al., 2011; Agarwal et al., 2010). Further, curcumin is also shown to be protective against nano-nickel oxide particles induced cell death in human airway epithelial (HEp-2) and human breast cancer (MCF-7) cells (Siddiqui et al., 2012). Ascorbic acid has shown protective effect against $PM_{2.5}$ induced human bronchial epithelial cells (16HBE) cell damage (Jin et al., 2015). Similarly, in the present study, we have shown antioxidants provide protection against TiO_2/ZnO (nano/bulk) induced macromolecular damage as well as oxidative enzyme activity in *C. elegans*.

Overall, nano/bulk TiO_2/ZnO exposure induces oxidative damage of macromolecules such as protein, lipids, and DNA, even though cell tries to counteract the same by increasing its antioxidant enzyme capacity. However, in presence of ascorbic acid and curcumin, protection is noticed against TiO_2/ZnO particle induced ROS and oxidative damage, which is reflected in the normal activity of antioxidant enzymes in *C. elegans*.

4.5 Summary

- Exposure to nano and bulk particles activates anti-oxidative stress response enzymes such as superoxide dismutase and catalases in exposed worms.
- Exposure to nano/bulk TiO_2/ZnO leads to a reduction in the glutathione and glutathione peroxide enzyme activity in *C. elegans*.
- Exposure to nano/bulk TiO_2/ZnO leads to lipid peroxidation, protein carbonylation, DNA damage and apoptosis in the exposed worm.
- Exposure to particle at sub-lethal concentrations (LC_1 and LC_{10}) in the presence of antioxidants efficiently protects the organisms from nano/bulk particles induced oxidative damage.
- At LC_{50} concentrations of nano/bulk particles presence of antioxidants provide 50-80% recovery against the particles induced oxidative stress and other consequences in worms.
- Thus, the presence of antioxidants during particle treatment normalizes enzymatic activity in exposed worms and also mitigates the macromolecular damage.

Objective 2

Chapter 5

III. Functional validation of the protective role of antioxidants against the adverse effects of TiO_2 and ZnO nanoparticles

5.1 Introduction

Organism's response to environmental variation is crucial to understand the impact of environment on the well-being of living entities. Organism's condition is quantified by assessing the physiological responses such as its performance, the efficiency with which it functions under conditions of environmental stress and pollution. For that, *Caenorhabditis elegans* can provide a compelling platform because it has a stereotypic pattern for development along with a large repertoire of scorable phenotypes (Hunt, 2017; O'Reilly et al., 2014). In the previous chapters, we observed high ROS generation (Chapter 2) and oxidative stress (Chapter 4) in worms as a consequence of exposure to nano/bulk TiO_2 and ZnO. ROS generation can modulate the redox environment inside the organism which can affect the feeding behaviors and neuronal activity of the organism, also interacts with major sperm protein thus cumulatively may have an effect on the reproduction, growth, and behavior of the organism. Along with this, ROS also works as signaling molecules and regulate the major signaling pathways (immune pathway, insulin signaling pathway, aging, apoptosis, etc) when it interacts with transcriptional factors. In chapter 3, we have reported that exposure of nano/bulk TiO_2 and ZnO affected the signaling of IIS and immune response pathway. In *C. elegans* both the pathways are involved in the longevity, reproduction, behavior, immune response, stress response etc (Miranda-Vizuetea and Veal, 2017; McCallum and Garsin, 2016). The eco-toxicological impacts of nano-TiO_2 and nano-ZnO on the physiology have been investigated in a number of model organisms. Adverse effect of nano-TiO_2 and nano-ZnO has been reported on growth of bacteria (Ko et al., 2015; Lin et al., 2014; Maurer-Jones et al., 2013; Ge et al., 2011; Heinlaan et al., 2008), *Saccharomyces cerevisiae* (Usatii et al., 2016), diatoms (Peng et al., 2011), marine and freshwater green algae (Hazeem et al., 2016;

Cardinale et al., 2012; Kulacki et al., 2012), *Daphnia* (Liu et al., 2014; Zhu et al., 2010), zebra fish (Vicario-Pares et al., 2014; Liu et al., 2014; Zhu et al., 2009), *C. elegans* (Khare et al., 2015; Wu et al., 2013; Li et al., 2012), earth worm (Alahdadi and Behboudi, 2015), *Drosophila melanogaster* (Sabat et al., 2016), mice (Wang et al., 2016) and plants (Zafar et al., 2016; Burke et al., 2015; Masarovicova and Kralova, 2013; Yoon et al., 2013; Du et al., 2011). Similarly negative effect of nano-TiO_2 and nano-ZnO on fertility has been reported in *Daphnia* (Liu et al., 2014; Lopes et al., 2013; Zhu et al., 2010; Heinlaan et al., 2008), earthworm (Alahdadi and Behboudi, 2015; Schlich et al., 2012), zebra fish (Vicario-Pares et al., 2014; Liu et al., 2014; Zhu et al., 2008), *C. elegans* (Khare et al., 2015; Wu et al., 2013; Li et al., 2012), mouse/rats (Brohi et al., 2017; Amara et al., 2015). Further impaired behavior has been reported in *Daphnia* (Fekete-Kertesz et al., 2016; Lopes et al., 2013; Zhu et al., 2009), rainbow trout (Boyle et al., 2013), *C. elegans* (Khare et al., 2015; Wu et al., 2013; Li et al., 2012), *Drosophila melanogaster* (Sabat et al., 2016) and mice (Xie et al., 2012) on exposure to nano-TiO_2 and nano-ZnO. Even the lifespan in *Caenorhabditis elegans* was found to be adversely affected when worms were treated with nano-TiO_2 and nano-ZnO (Kumar et al., 2016). The harmful effects of different NPs on the physiology of aquatic and terrestrial organisms have been reported (reviewed in Peng et al., 2017; Da, 2016; Exbrayat et al., 2015; Ivask et al., 2014). Even we have shown adverse impairment in worm physiology in response to nano/bulk TiO_2 and ZnO exposure (Ratnasekhar et al., 2015; Khare et al., 2015). In this chapter, our aim is to investigate whether antioxidants that quench ROS (Chapter 2) and subsequently protect organisms against over-expression of oxidative stress response gene and protein (Chapter 3 and 4), are capable of attenuating the nano-induced adverse effects on the physiology of worms.

Therefore, the main aim of the study is to determine physiological variations in worms on exposure to nano-TiO_2 and nano-ZnO followed by the ability of ascorbic acid and curcumin to overcome the same. To achieve the same we tested physiological parameters such as growth, reproduction, locomotion behavior and lifespan in exposed worms in presence or absence of antioxidants.

5.2 Material and methods

Nano/bulk of TiO_2 and ZnO along with antioxidant (curcumin/ascorbic acid) were employed in the present study for determining their effects on the physiological response of worm. Synchronized L4 worms were exposed to concentrations of LC_1, LC_{10} and LC_{50} of nano/bulk particles. Treatment of particles (in the presence and absence of antioxidants) was given along with control (water or solvent control) for 24 h (±0.5) at 20°C. Physiological endpoints like growth, reproduction, locomotion behavior (head thrash & body bend) and lifespan were assayed in the exposed worms in comparison to control worms.

5.2.1 Growth Assay:

After treatment, the worms were washed and treated with 125 mM sodium azide (NaN_3) to inhibit the movement of worms. Subsequently, images were captured using an inverted microscope (Nikon Eclipse TE2000-S with DXM1200f camera) at 10X magnification. The growth of worm was measured as the mean length of worm and the analysis was carried out using Image-J software using 3252 pixels = 1mm as scale. The experiment was repeated thrice with 30 replicates for each group.

5.2.2 Fertility Assay:

Age synchronized L4 worms were individually placed in each well of a 12-well plate. Treatment was given at the lethal concentrations of nano/bulk TiO_2 and ZnO along with food (2X) in the presence or absence of antioxidant for 72 h (±0.5) at 20°C. After three days, the number of offspring at all stages except the eggs were counted (Middendorf and Dusenbery, 1993). Experiments were repeated thrice with twelve replicates in each group.

5.2.3 Behavior Assay:

After treatment duration, the nematode behavior was monitored under the microscope by tracking body bend and head trashes frequency (Tsalik and Hobert, 2003). Worms were harvested and individually (from each treatment group) were transferred on to an unseeded NGM plate. After 1 min recovery period individual worm was manually scored under the microscope, 1 min for head trashes and 20 sec for body bends. A head thrash is defined as the change in the direction of bending at the midbody. A body bend is counted as a change in the direction of the part of the nematodes corresponding to the posterior bulb of the pharynx along the Y-axis, assuming that the nematode was traveling along the X-axis. The experiment is repeated trice with 30 replicates for each group.

5.2.4 Lifespan assay:

Synchronized L4 worm was treated for 24 h and then 30 worms from each treatment group were transferred to seeded NGM plates containing 50 µM 5-fluoro-2'-deoxyuridine (FUdR). FUdR is known to block cell division hence inhibit progeny production in worms. Every day, nematodes were observed under the microscope for their survival and periodically moved to fresh plates to keep growth condition mold free.

Animals were scored as dead if they are failed to respond to the gentle touch of Pt-wire (Kwon et al., 2010).

5.2.5 Statistical analysis:

Physiological assays were expressed as mean±SEM. Significance was analyzed using one-way analysis of variance (ANOVA). $p<0.05$, 0.01 and 0.001 were considered statistically significant on Bonferroni correction. Percent effect was determined by taking control as 100% and evaluating effects in treatment groups. Recovery was calculated assuming 0% recovery in the treatment group (without supplement).

5.3 Results

5.3.1a Effect of TiO_2/ZnO NPs on the growth of the organism in comparison to bulk:

Growth was observed in terms of average length of the nematodes. The control worms measured 0.97±0.04 mm. There was a significant reduction ($p<0.01$) in the body length by 7.08% was observed when the worms were exposed to the LC_{50} concentration of the nano-TiO_2 but not that of bulk. However, among all the other comparisons the reduction in growth was found to be non-significant with respect to control. The decrease in length of worms exposed to nano was non-significant in comparison to that of bulk (Figure 5.1). When worms were exposed to nano-ZnO significant reduction ($p<0.001$) of 6.9%, 9.5% and 10.9% in the length of the worm was observed at LC_1, LC_{10}, and LC_{50} concentrations, respectively. In worms exposed to the bulk-ZnO the reduction in the length of worm observed was 2.7%, 2.7% and 4.5% at LC_1, LC_{10}, and LC_{50}

concentrations, respectively. The decrease in length of worms exposed to nano was significant to that of bulk at all the concentrations (Figure 5.1).

5.3.1b Amelioration effects of antioxidant on the growth of an organism:

Significant reduction in the growth of worms on exposure to particles was attenuated when exposure was carried out in presence of either curcumin or ascorbic acid (Figure 5.1).

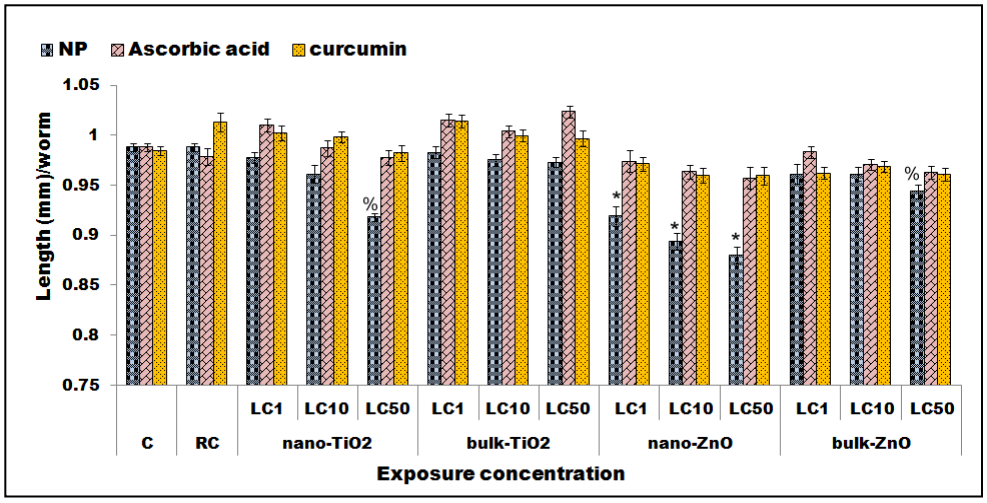

Figure 5.1 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO on the growth of *C. elegans*. C = control; RC = reference control; n=90, bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001, $^{\%}p$<0.01 = Significant against control

5.3.2a Effect of TiO_2/ZnO NPs on the reproduction of organism in comparison to bulk:

The average progeny count in control worms was 138.9±0.69. Significant (p<0.05) reduction in progeny count was noticed in worms exposed to both nano/bulk forms of TiO_2/ZnO particles at all the three concentrations tested (Figure 5.2). In worms exposed

to LC_1, LC_{10} and LC_{50} of nano-TiO_2, reduction in progeny count was observed by 15%, 30%, and 50%, respectively, in comparison to that of control. Similarly, an organism exposed to bulk-TiO_2 at LC_1, LC_{10} and LC_{50} concentrations 14.6%, 22.6% and 35.5% reduction in the progeny count was observed, respectively. A significant ($p<0.05$) decline in progeny count was noticed in nano-TiO_2 exposed worms at the LC_{50} concentration in comparison to bulk (Figure 5.2).

The reduction in the progeny of nano-ZnO/bulk-ZnO exposed worms at LC_1 concentration was 33%/9%, at LC_{10} concentration, the reduction was 49.6%/22.7% and at LC_{50} concentration the reduction was, 67.3%/ 45.6% respectively. Further, nano-ZnO at all the exposed concentrations (LC_1, LC_{10}, and LC_{50}) was found significant ($p<0.01$) in comparison to that of bulk (Figure 5.2).

5.3.2b Amelioration effects of antioxidant on the reproduction of organism:

The progeny count did not differ significantly between control, solvent control and among the worms exposed to only antioxidants. In presence of antioxidant (curcumin or ascorbic acid) during treatment, the progeny count was equivalent to that in control among the exposed worms indicating complete protection at low exposure concentrations (LC_1 and LC_{10}) of nano/bulk particles. Even at the LC_{50} concentration of nano/bulk particles in presence of antioxidants, significant ($p<0.001$) recovery of about 77-80% in the exposed worms was noticed (Figure 5.2) in comparison to control.

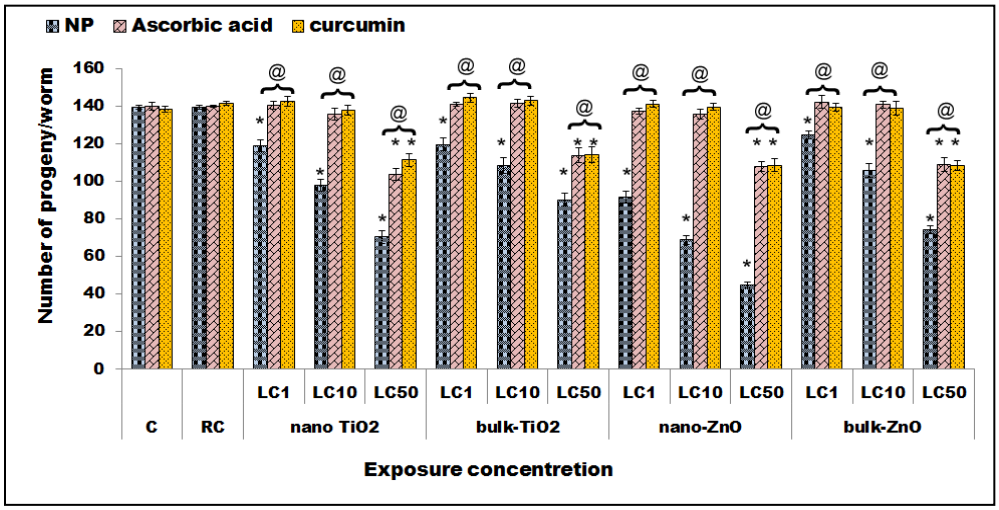

Figure 5.2 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced reproductive toxicity in *C. elegans*. C = control; RC = reference control; n=36, bar = mean±SEM of three independent experiments; Bonferroni corrected *p<0.001 = Significant against control; @p<0.001 = Significant recovery (in presence of antioxidant) against particle exposure

5.3.3a Effect of TiO_2/ZnO NPs on behavior of the organism in comparison to bulk:

The average head thrash count per minute in control worm was 136.7±0.43. Significant (p<0.01) reduction in head thrash count was noticed in worms exposed to both nano/bulk forms of TiO_2/ZnO particles at all the three concentrations tested (Figure 5.3A). In comparison to the control, a significant reduction (p<0.001) in head thrash count in worms exposed to LC_1, LC_{10}, LC_{50} concentrations was observed by 31.6%, 49.5%, 57.8% in nano-TiO_2 as well as 16.4%, 34.6%, 43.8% in bulk-TiO_2, respectively. The significant reduction (p<0.01) in the head thrashes was observed at LC_{10} and LC_{50} of nano-TiO_2 compared to the same concentrations of bulk particles.

Significant reduction (p<0.001) was noticed in the head thrashes count by 48.5%, 60.4%, 69.7% in worms exposed to nano-ZnO and 17.1%, 35.3%, 53.1% in worms

exposed to bulk-ZnO, at LC_1, LC_{10}, LC_{50} concentrations, respectively, in comparison to that of controls. Further, at all the exposure concentrations reduction in head thrash count of worms exposed to nano-ZnO was found significant ($p<0.01$) in comparison to that of bulk-ZnO (Figure 5.3A).

The average body bends per 20 seconds in control worm was 17.3±0.08. Body bends count of worms exposed to the nano/bulk were observed to be significantly reduced ($p<0.001$) at all the tested concentrations, in comparison to control. We found 38%, 54%, 62% reduction in response to nano-TiO_2 and 24%, 32%, 45% reduction in bulk-TiO_2 was observed at LC_1, LC_{10} and LC_{50} concentrations, respectively. A significant decline ($p<0.05$) in body bends count of worms was observed at the LC_{10} concentration of nano- TiO_2 compared to respective bulk (Figure 5.3B).

The reduction in the body bend by 39%, 58%, 68% was found in worms exposed to nano-ZnO and 29%, 38%, 53% reduction in worms exposed to bulk-ZnO at LC_1, LC_{10} and LC_{50} concentrations respectively, compared to control. On the similar line, a significant decline in body bends count of worms exposed at LC_{10} ($p<0.05$) and LC_{50} ($p<0.01$) of nano-ZnO was observed compared to bulk-ZnO (Figure 5.3B).

5.3.3b Amelioration effects of antioxidant on the behavior of organism:

In presence of the antioxidant, no significant alteration in the behavior of worms was noticed between control, solvent control and among the worms exposed to only antioxidants. Nano/bulk exposed worms (at LC_1, LC_{10} and LC_{50} concentrations) showed a significant recovery ($p<0.001$) in both the head thrashes as well as body bend behavior. At LC_1 and LC_{10} concentrations, the head thrash, as well as body bend-count of worms exposed to nano/bulk particles in the presence of antioxidants, was non-significant to

that in control. However at the LC_{50} concentration of nano/bulk particles, in presence of antioxidants 86%-95% recovery was found in case of head thrashes (Figure 5.3A). Similarly in case of body bend behavior in worms exposed to LC_{50} concentrations of nano/bulk TiO_2, in presence of antioxidants 50-60% recovery was observed; and 60-70% recovery in case of worms exposed to LC_{50} concentrations of nano/bulk ZnO in presence of antioxidants (Figure 5.3B).

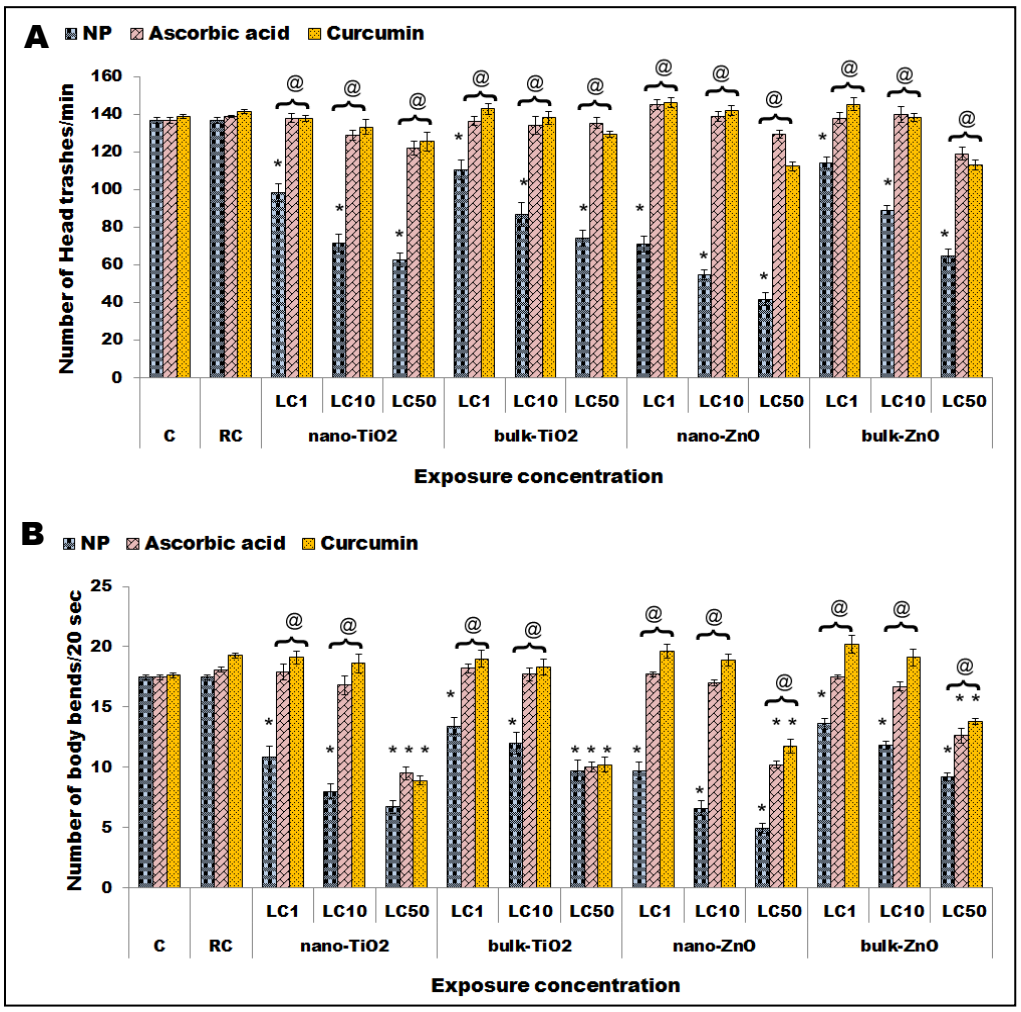

Figure 5.3 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO induced behavioral toxicity in *C. elegans*. (A) Head trashes (B) Body bends. C = control; RC = reference control; n=30, bar =

mean±SEM of three independent experiments; Bonferroni corrected *p<0.001 = Significant against control; @p<0.001 = Significant recovery (in presence of antioxidant) against particle exposure

5.3.4a Effect of TiO_2/ZnO NPs on the lifespan of the organism in comparison to bulk:

Compared to control (mean lifespan; 16.5 days), the mean lifespan was significantly reduced (p<0.01) among nano-TiO_2 exposed worms at LC_{10} and LC_{50} concentration (mean lifespan; 14.6 and 14 days) while bulk-TiO_2 was not observed to have any effect on the lifespan of the organism. The significant reduction (p<0.05) in the lifespan of worms exposed to LC_{10} and LC_{50} of nano-TiO_2 was observed, compared to bulk particles (Figure 5.4).

However, nano-ZnO exposed worms have significant reduction (p<0.001) in mean lifespan even at LC_1 concentration (mean lifespan; 12.2 days) while in bulk-ZnO exposed worms significant reduction (p<0.01) in mean lifespan was noticed at LC_{10} and LC_{50} concentration (mean lifespan; 14.6 and 14 days) (Figure 5.4). Further, the reduction in the life span of nano-ZnO exposed worms was significant (p<0.05) with respect to bulk at all the tested concentrations (Figure 5.4).

5.3.4b Amelioration effects of antioxidant on the lifespan of an organism:

Presence of ascorbic acid and curcumin (mean lifespan; 18.4 and 18.8 days) significantly (p<0.01) increases the lifespan of the organism, in comparison to control (mean lifespan; 16.5 days). Similarly in presence of antioxidants during particle treatment the exposed worms showed mean lifespan of 17.5-18, equivalent to only antioxidant treatment,

indicating significant (p<0.05) recovery and amelioration of particle effect on lifespan (Figure 5.4).

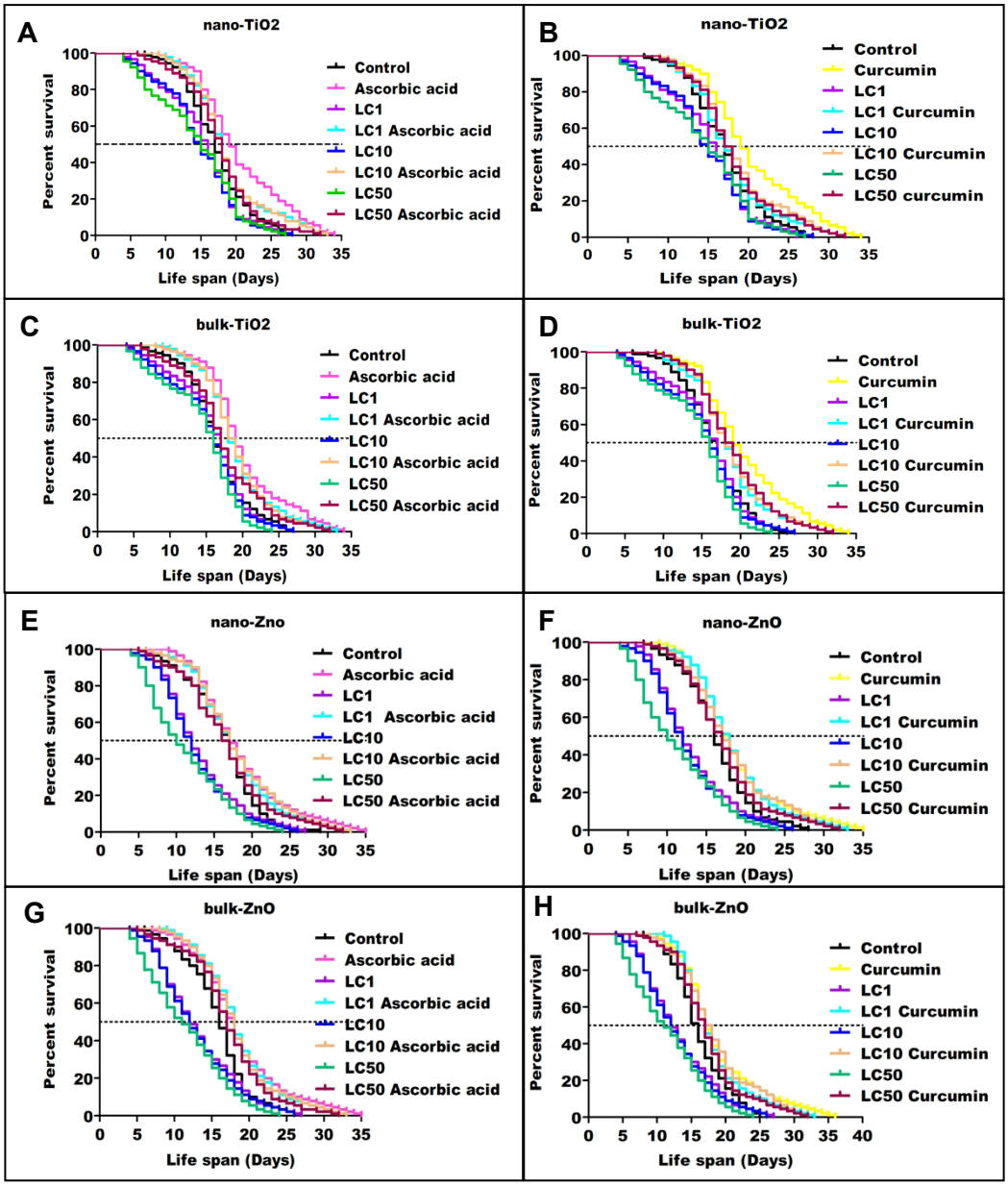

Figure 5.4 Amelioration effects of ascorbic acid and curcumin on nano-TiO_2 (A, B), bulk-TiO_2 (C, D), nano-ZnO (E, F) and bulk-ZnO (G, H) induced change in mean lifespan of worms; Kaplan-Meier survival curve. *p<0.05

5.4 Discussion

Alteration in physiological response is certainly an indicator of cellular stress and *C. elegans* offers several endpoints that can serve as markers of cellular response in adverse conditions, such as- lethality, growth, reproduction, lifespan and behavioral changes. All the assays reflect the larval, embryonic, neuronal and cellular developmental of the organism. Growth, reproduction, and lifespan are an indicator of nutrient availability and proper development of the organism at a cellular and organizational level. While, behavior assays (body bends, head trashes, pharyngeal pumping, distance traveled etc.) indicates neuronal heath of the organism. In stress conditions, reduction in the growth (in terms of length) and reproduction reflect a delay in developmental progression and this may contribute to the prolonged lifespan of an organism. However, damage to neurons or network between the neurons can have the negative impact on the behavior of the organism. Thus, with the aim to determined physiological variations in worms on exposure to nano/bulk TiO_2 and nano/bulk ZnO we measured the effect on growth, reproduction, behavior, and lifespan of an organism.

In the present study, the growth of *C. elegans* (reflected by the length of the organism) was significantly affected by the nano/bulk TiO_2/ZnO exposure. Similar results on growth have been reported by others on exposure to nano-TiO_2 in *C. elegans* (Roh et al., 2010; Wang et al., 2009) they have shown the involvement of the *cyp35a2* (xenobiotic metabolism gene) gene as well as particle dissolution as the cause for reduction in growth. Further, it has been reported that the nano-CeO_2 induced oxidative stress and also has a strong affinity to bind with *E. coli* leading to the restricted dietary intake of food, resulting in the decrease in the growth of exposed worm (Rogers et al., 2015). However, environmentally relevant concentrations both

nano-TiO_2 as well as nano-CeO_2 did not have the significant effect on the growth of organism (Roh et al., 2008).

Stress alters the reproductive speed, locomotion and life cycle in *C. elegans*. In the present study on exposure to TiO_2/ZnO particles, we noticed an adverse effect on the reproductive potential, locomotion behavior as well as the lifespan of the *C. elegans*, in a dose-dependent manner. These studies are in collaboration with the published reports wherein, they have shown that nano-TiO_2 and nano-ZnO affect the reproduction of organisms such as bacteria (Ge et al., 2010), *C. elegans* (Khare et al., 2015; Ratnasekhar et al., 2015; Wang et al., 2009), earthworm (Alahdadi and Behboudi, 2015) and *Daphnia* (Lopes et al., 2013). In Zebra fish (Liu et al., 2014) NPs are known to penetrate the blood-testis barrier and damage the spermatogenesis (Brohi et al., 2017; Lan and Yang, 2012). Even in case of mammalian system (rats) exposure to nano-TiO_2 was found to be associated with the suppressed spermatogenesis as a consequence of which significant reduction in progeny count was noticed (Hong et al., 2015). Reproductive organs and neurons are the secondary target organ for NPs in nematodes (Brohi et al., 2017; Jiang et al., 2016; Nouara et al., 2013; Zhao et al., 2013). Similarly, defect in locomotion behavior of *C. elegans* has been reported on exposure to a broad spectrum of chemical and environmental stress including heavy metals and metal oxide NPs (Scharf et al., 2016; Soria et al., 2015; Gupta et al., 2015; Suganthi et al., 2015; Khare et al., 2015; Rui et al., 2013; Chen et al., 2013; Xie et al., 2012). On exposure to metal ($CuSO_4$, Cd, Mn, Zn) and metal NPs (Ag NPs), negative effect on the lifespan of *C. elegans* has also been reported (Piechulek et al., 2017; Koch et al., 2014; Chen et al., 2013). Thus, it appears that there is a close correlation between ROS production and negative impact on organism's morphological as well as behavioral traits such as growth, reproduction, locomotion behavior and lifespan in

nematodes. To prove this concept, we analyzed the impact of particles on morphological and behavioral traits in worms exposed to particles in presence of antioxidants. Indeed, the amelioration of negative impact of the particle on morphological as well as behavioral traits in presence of antioxidants in exposed worms indicates that antioxidants provide protection not only against ROS but also against ROS induced adverse effects. The quenching of ROS production in presence of antioxidant(s) during treatment (Chapter 2) followed by recovery in oxidative stress gene and protein expression (Chapter 3 and 4) would consequently reduce the impact of particles on morphological and behavioral traits of exposed worms. Thus, in this chapter, we provide functional validation for the protective role of antioxidants against the adverse effect of particles. We also demonstrate quenching of ROS is co-related with the amelioration of particle-induced effects on growth, locomotion behavior, reproduction and lifespan of the exposed worm.

5.5 Summary

- Exposure to nano-TiO_2 at LC_{50} concentration and nano-ZnO at all tested concentrations caused the significant reduction in the growth of exposed worms.
- Worms exposed to either nano/bulk TiO_2/ZnO exhibited the significant decline in progeny count as well as locomotion and foraging behavior.
- Nano-TiO_2 and bulk-ZnO at LC_{10} and LC_{50} concentration, as well as nano-ZnO at all tested concentrations, had the significant negative impact on the lifespan of the exposed worms.
- The negative impact on growth due to particle treatment on worms was ameliorated in presence of antioxidants during treatment.

- Adverse effects on reproduction and behavior in worms exposed to particles were not noticed when antioxidants were given along with treatment, at low treatment concentrations.
- In worms exposed to the LC_{50} concentration of particles in presence of antioxidants, 75-80% recovery was noticed in case of progeny count, but recovery was not complete.
- In worms exposed to the LC_{50} concentration of particles in presence of antioxidants, 80-90%, and 50-60% recovery was noticed in case of locomotion behavior and foraging behavior, respectively.
- The presence of antioxidants during particle treatment led to significant ($p<0.05$) recovery and amelioration of particle effect on lifespan.
- Significant recovery in morphological and behavioral traits of particle exposed worms in presence of antioxidants is functional validation for the protective role of antioxidants against the adverse effect of particles.

Conclusion

6. Conclusion

The present research study entitled "**Evaluation of antioxidants in amelioration of toxic effects of engineered nanoparticles using *Caenorhabditis elegans* as a basic *in-vivo* alternative model"** was carried out to determine the adverse effects of TiO_2 and ZnO nanoparticles (NPs) and the ameliorating properties of ascorbic acid and curcumin. First, we determined the physiochemical characteristics of NPs using TEM, SEM and DLS techniques and determined its stability in exposure medium. The average particle size (determined through TEM) of the nano and bulk TiO_2 was observed to be 11 nm and 124 nm, while for ZnO nano and bulk particles were 21 nm and 242 nm. The hydrodynamic size (determined through Zeta Sizer; DLS) for nano-TiO_2, bulk-TiO_2, nano-ZnO and bulk-ZnO were 240±10 nm, 346±1.7 nm, 153±0.6 nm and 1589±213 nm and zeta-potential (determined through Zeta Sizer; Zeta potential) were found to be -14±0.7 mV, -21±1.6 mV, 22±0.6 mV and -19±0.9 mV, respectively. The NPs were in nano range, uniformly dispersed and observed to be stable for 72 h in the absence/presence of antioxidants. Then, we determined the LC_{50} concentrations (lethal concentration at which 50% worms were dead) of the nano/bulk particles both at chronic (72 h, L1 to L4) and acute (24 h) exposure. The LC_{50} values for chronic exposure of nano-TiO_2, nano-ZnO, bulk-ZnO were found to be 54.2 µg/ml, 0.18 µg/ml and 0.93 µg/ml respectively, while bulk-TiO_2 was found non-lethal up to 200 µg/ml. Similarly, the LC_{50} values for acute exposure in young adult were found to be 172 µg/ml, 1.125 µg/ml and 4.64 µg/ml for nano-TiO_2, nano-ZnO and bulk-ZnO, respectively. However, bulk-TiO_2 was found non-lethal even at the 500 µg/ml. The results indicate that nano forms of the particles were more toxic than the respective bulk and nano-ZnO was more toxic than nano-TiO_2 in *C.*

elegans. Further, the toxicity of the particles was dose and exposure duration dependent. To determine the protective potential of antioxidants (ascorbic acid and curcumin) against the NPs induces lethality antioxidant exposure was given prior, post and along with particles treatment. In both acute as well as chronic exposures to NPs, the pre-(20 μM and above) and post-(60 μM and above) as well along with (100 μM and above) antioxidants supplement was found to be protective against the NPs induced mortality. Thus, the antioxidants efficiently protect the worm against NPs induced lethality in acute as well as chronic exposure. Pre-antioxidant supplementation was most effective approach against the NPs induced lethality in worms.

Reactive oxygen species (ROS) generation in worms exposed to NPs was significant in comparison to control as well as bulk particles. On acute exposure (24 h) of worms to nano/bulk TiO_2 and ZnO, 6.3-/4.4- fold and 7.4-/5.4- fold increase in ROS generation was observed at LC_{50} concentration. Similarly, ROS generation in worms on chronic exposure (72 h) to nano/bulk TiO_2/ZnO was 2.3-/1.6-fold and 4.04-/2.16-fold increase, respectively. In both acute as well as chronic exposures to NPs, the pre-(20 μM) and post-(60 μM) as well along with (100 μM) antioxidants supplement was found to be protective against the NPs induced ROS generation. Quenching of ROS when antioxidant supplement was given pre- or post NPs treatment was complete, however that was not so when it was given along with NPs. Thus, we further studies the co-exposure conditions for investigating the molecular mechanism underlying the adverse effects of nano/bulk TiO_2/ZnO in exposed worms and its attenuation in presence of antioxidants.

To determine the molecular mechanism for particle induced toxicity, we examined the role of the major oxidative stress response pathway, the insulin/insulin-like growth factor signaling

pathway (IIS-pathway; Figure 6.1A). IIS-pathway is an important, evolutionarily conserved pathway involved in the growth, development, metabolic homoeostasis, fecundity, stress resistance and the lifespan of an organism. In *C. elegans* DAF-2, DAF-16, SKN-1 and HSF-1 are the major transcriptional factor (TF) of IIS-pathway and immune response pathway therefore, we determined the gene expression of these TF as well as their downstream genes. We found significant down-regulation in the expression of *daf-2* while up-regulation in the expression of other TF such as, *daf-16*, *skn-1* and *hsf-1* and their downstream genes *gst-4, gcs-1*, *sod-3*, *ctl-2*, *hsf-16.2*. We also noticed activation of superoxide dismutase and catalases in exposed worms and reduction in the glutathione and glutathione reductase enzyme activity in exposed worms, through enzymatic assays. Oxidative stress was also found to activate immune response in nano/bulk TiO_2/ZnO exposed worms, since expression of the genes involved in the immune response (p-38-pathway; *skn-1, nsy-1, pmk-1*) were significantly up-regulated. We have also witnessed significant increase in phosphorylated JNK levels in NPs exposed worms (Figure 6.1B). Therefore both nano/bulk TiO_2/ZnO exposures were found to induce oxidative stress mediated alteration in IIS and immune signaling in exposed worms. Further we also noticed macro-molecule damage in particle exposed worms. Exposure to nano/bulk TiO_2/ZnO leads to lipid peroxidation, protein carbonylation and DNA damage and apoptosis. All these effects ultimately culminated into significant reduction in growth, progeny count, locomotion and foraging behavior as well as lifespan in worms exposed to nano/bulk TiO_2/ZnO particles. Therefore, exposure to nano/bulk particles causes oxidative stress and leads to cytotoxicity, genotoxicity, macromolecular damage and adverse affect on the physiology of worms. The adverse effects are more prominent in NPs exposed worms

compared to control or bulk exposed worms. ZnO particles are more toxic compared to TiO_2 particles in *C. elegans*.

Finally, the co-exposure of antioxidants based amelioration of toxic effects of NPs was determined. Exposure of particle at sub-lethal concentrations (LC_1 and LC_{10}) in the presence of antioxidants efficiently protects the organisms from nano/bulk particles induced oxidative damage (Figure 6.1C). At LC_{50} concentrations of nano/bulk particles presence of antioxidants provide 50-80% recovery against the particles induced oxidative stress and other consequences in worms. Thus, presence of antioxidants during particle treatment normalizes enzymatic activity, mitigates the macromolecular damage as well as adverse effect on growth, reproduction, behavior and lifespan of exposed organism. Significant recovery in morphological and behavior traits of particle exposed worms in presence of antioxidants is functional validation for the protective role of antioxidants against adverse effect of particles. Therefore, our study indicates the protective role of curcumin and ascorbic acid against nano/bulk particle toxicity and a possibility of evading the nanotoxicity by incorporating these antioxidants in the everyday diet.

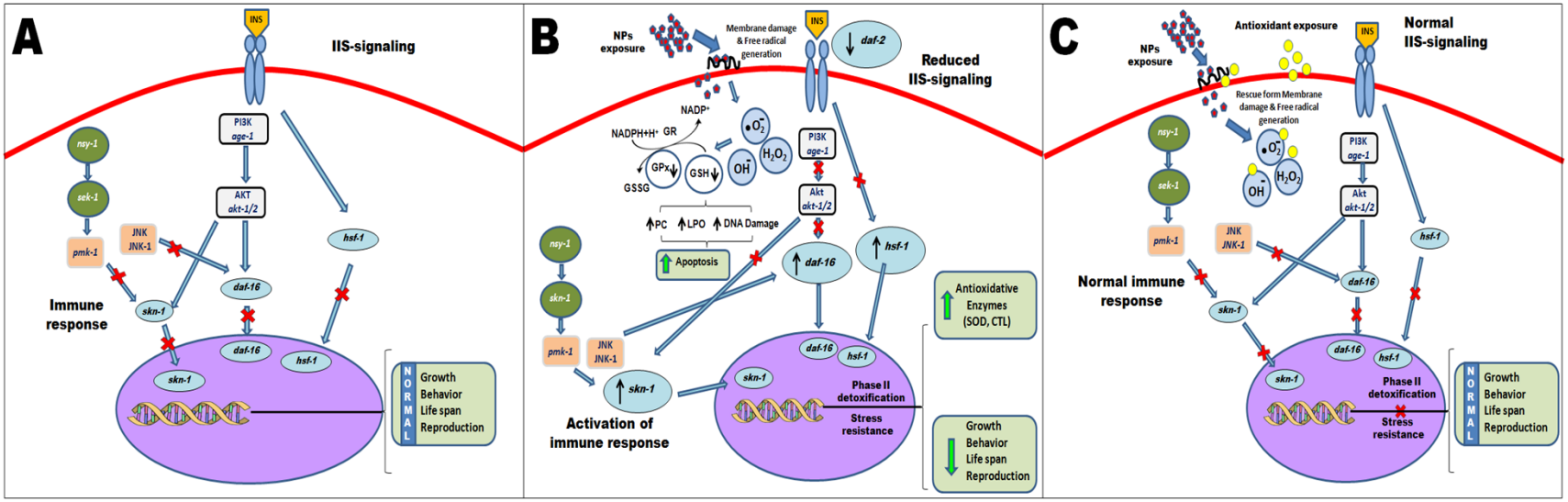

Figure 6.1 Graphical representations to describe the mode of TiO_2/ZnO particle toxicity and its amelioration in *C. elegans* A) Control worms B) Particle exposed worms C) Worms exposed to particle in presence of antioxidants

Nano-TiO_2/ZnO leads to ROS production leading to activation of oxidative stress response genes. The down-regulation of *daf-2* and up-regulation of DAF-16/SKN-1/HSF-1 transcription factors leads to stress regulation by enhancing the antioxidant enzyme production as well as activity. In addition to IIS pathway, oxidative stress also leads to activation of SKN-1 through p-38 pathway and DAF-16 through MAPK-JNK-1 pathway. High oxidative stress leads to depletion of GSH which in turn fails to reduce peroxidase formation. Accumulation of malondialdehyde (MDA) and protein carbonyl (PC) along with reduction in mitochondrial activity triggers DNA damage leading to apoptosis. Overall TiO_2/ZnO negatively affects growth, behavior, reproduction and lifespan in *C. elegans*. Presence of antioxidants during particle exposure protects worms from particle induced oxidative damage.

References

7. REFERENCES

1. Abbasalipourkabir, R., Moradi, H., Zarei, S., Asadi, S., Salehzadeh, A., Ghafourikhosroshahi, A., Mortazavi, M., Ziamajidi, N., 2015. Toxicity of zinc oxide nanoparticles on adult male Wistar rats. *Food and Chemical Toxicology*, 84,154-160.

2. Abdelazim, A.M., Saadeldin, I.M., Swelum, A.A., Afifi, M.M., Alkaladi, A., 2018. Oxidative stress in the muscles of the fish Nile tilapia caused by zinc oxide nanoparticles and its modulation by vitamins C and E. *Oxidative Medicine and Cellular Longevity,* 2018, 1-9.

3. Abdel-Daim, M.M., Ghazy, E.W., 2015. Effects of Nigella sativa oil and ascorbic acid against oxytetracycline-induced hepato-renaltoxicity in rabbits. *Iranian Journal of Basic Medical Sciences,* 18(3), 221-227.

4. Abdulqader, S., Mustafa, I., 2014. The protective role of vitamin C against formaldehyde induced-hepatotoxicity and nephrotoxicity in male rats. *IOSR journal of pharmacy and biological sciences*, 9(4), 21-6.

5. Adedara, I.A., Subair, I.T., Ego, V.C., Oyediran, O., Farombi, E.O., 2017. Chemoprotective role of quercetin in manganese-induced toxicity along the brain-pituitary-testicular axis in rats. *Chemico-Biological Interactions,* 263, 88-98.

6. Aebi, H., 1984. Catalase in vitro. *Methods in Enzymology,* 105, 121-126.

7. Afifi, M., Saddick, S., Abu Zinada, O.A., 2016. Toxicity of silver nanoparticles on the brain of *Oreochromis niloticus* and *Tilapia zillii*. *Saudi Journal of Biological Sciences,* 23(6), 754-760.

8. Afifi, O.K., Embaby, A.S., 2016. Histological study on the protective role of ascorbic acid on cadmium induced cerebral cortical neurotoxicity in adult male albino rats. *Journal of Microscopy,* 4(1), 36-45.

9. Agarwal, R., Goel, S.K., Behari, J.R., 2010. Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. *Journal of Applied Toxicology*, 30(5), 457-468.

10. Aggarwal, B.B., Harikumar, K.B., 2009. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *International Journal of Biochemistry & Cell Biology*, 41(1), 40-59.

11. Akinrinde, A.S., Omobowale, O., Oyagbemi, A., Asenuga, E., Ajibade, T., 2016. Protective effects of kolaviron and gallic acid against cobalt-chloride-induced cardiorenal dysfunction via

suppression of oxidative stress and activation of the ERK signaling pathway. *Journal of Physiology and Pharmacology*, 94(12), 1276-1284.

12. Akinyemi, A.J., Onyebueke, N., Faboya, O.A., Onikanni, S.A., Fadaka, A., Olayide, I., 2017. Curcumin inhibits adenosine deaminase and arginase activities in cadmium-induced renal toxicity in rat kidney. *Journal of Food and Drug Analysis*, 25(2), 438-446.

13. Alahdadi, I., Behboudi, F., 2015. The effects of CuO and ZnO nanoparticles on survival, reproduction, absorption, overweight, and accumulation in Eisenia fetida earthworm tissues in two substrates. *International Journal of Environmental Research,* 9, 35-42.

14. Al-Attar, A. M., 2011. Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. *Saudi Journal of Biological Sciences,* 18(1), 63-72.

15. Alavez, S., Vantipalli M.C., Zucker, D.J.S., Klang, I.M., Lithgow, G.J., 2011. Amyloid-binding compounds maintain protein homeostasis during ageing and extend lifespan. *Nature,* 472(7342), 226-229.

16. Albanese, A., Tang, P.S., Chan, W.C., 2012. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annual Review of Biomedical Engineering,* 14, 1-16.

17. AL-Harbi, M. S., Hamza, R. Z., Dwary, A. A., 2014. Ameliorative effect of selenium and curcumin on sodium fluoride induced hepatotoxicity and oxidative stress in male mice. *Journal of Chemical and Pharmaceutical Research,* 6(4), 984-998.

18. Alkaladi, A., Afifi, M., Mosleh, Y., Abu-Zinada, O., 2014. Ultra structure alteration of sublethal concentrations of zinc oxide nanoparticals on Nil Tilapia (*Oreochromis niloticus*) and the protective effects of vitamins C and E. *Life Science Journal,* 11(10), 257-262.

19. Al-Rasheed, N., Faddah L., Ibrahim, H., Mohamed, A. M., Al- Rasheed, N., Abdelbaky, N., 2015. Role of carnosine and melatonin in ameliorating cardiotoxicity of titanium dioxide nanoparticles in the rats. *Brazilian Archives of Biology and Technology,* 58 (4), 577-586.

20. Altintas, O., Park, S., Lee, S.J., 2016. The role of insulin/IGF-1 signaling in the longevity of model invertebrates, *C. elegans* and *D. melanogaster*. *BMB Reports,* 49(2), 81-92.

21. Altun, Z.F. and Hall, D.H. 2009. *Introduction.* In WormAtlas. doi:10.3908/wormatlas.1.1

22. Amara, S., Slama, I.B., Omri, K., El Ghoul, J., El Mir, L., Rhouma, K.B., Abdelmelek, H., Sakly, M., 2015. Effects of nanoparticle zinc oxide on emotional behavior and trace elements homeostasis in rat brain. *Toxicology & Industrial Health,* 31(12), 1202-1209.

23. Amer, S.A.M., AL-Harbi, M.S., AL-Zahrani, Y.A.A., 2016. Protective role of some antioxidants on arsenic toxicity in male mice: Physiological and histopathological perspectives. *Biological Medicine*, 8(1), 1-8.

24. Angelstorf, J.S., Ahlf, W., von der Kammer, F., Heise, S., 2014. Impact of particle size and light exposure on the effects of TiO_2 nanoparticles on *Caenorhabditis elegans*. *Environmental Toxicology and Chemistry,* 33(10), 2288–2296.

25. Antonini, J.M., 2003. Health effects of welding. *Critical Reviews in Toxicology,* 33(1), 61-103.

26. Aruoja, V., Dubourguier, H.C., Kasemets, K., Kahru, A., 2009. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. *Science of the Total Environment,* 407(4), 1461-1468.

27. Asouri, M., Ataee, R., Ahmadi, A, A., Amini, A., Moshaei, M, R., 2013. Antioxidant and free radical scavenging activities of curcumin. *Asian Journal of Chemistry,* 25(13), 7593-7595.

28. Assimos, D.G., 2004. Vitamin C supplementation and urinary oxalate excretion. *Reviews in Urology*, 6(3), 167.

29. American Society for Testing and Materials. 2001. *Standard guide for conducting laboratory soil toxicity tests with the nematode Caenorhabditis elegans*. E2172. In Annual Book of ASTM Standards, 11.05. West Conshohocken, PA, 1606–1616.

30. Ates, M., Daniels, J., Arslan, Z., Farah, I.O., Rivera, H.F., 2013. Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp *(Artemia salina*) larvae: Effects of particle size and solubility on toxicity. *Environmental Science: Processes & Impacts,* 15(1), 225-233.

31. Atsumi, T., Tonosaki, K., Fujisawa, S., 2007. Comparative cytotoxicity and ros generation by curcumin and tetrahydrocurcumin following visible-light irradiation or treatment with horseradish peroxidase. *Anticancer Research*, 27(1A), 363-371.

32. Avalos, A., Haza, A.I., Mateo, D., Morales, P., 2015. Effects of silver and gold nanoparticles of different sizes in human pulmonary fibroblasts. *Toxicology Mechanisms and Methods,* 25(4), 287-295.

33. Azab, A.E., Albasha, M.O., Elsayed, A.S.I., 2016. Prevention of hepatotoxicity with curcuma longa and rosmarinus officinalis in gentamicin treated guinea pigs. *Indo American Journal of Pharmaceutical Sciences,* 6(03), 4791-4802.

34. Azim, S.A.A., Darwisha, H.A., Rizkb, M.Z., Ali, S.A., Kadry, M.O., 2015. Amelioration of titanium dioxide nanoparticles-induced liver injury in mice: Possible role of some antioxidants. *Experimental and Toxicologic Pathology*, 67, 305-314.

35. Baba, N.A., Raina, R., Verma, P.K., Sultana, M., Prawez, S., Nisar, N.A., 2013. Toxic effects of fluoride and chlorpyrifos on antioxidant parameters in rats: protective effects of vitamin C and E. *Fluoride*, 46(2), 73-79.

36. Babu, P.S., 2016. Application of nanotechnology in commercial field. *Indian Journal of Biotechnology and Pharmaceutical Research*, 4(6), 252-254.

37. Badria, F.A., Ibrahim, A.S., Badria, A.F., Elmarakby, A.A., 2015. Curcumin attenuates iron accumulation and oxidative stress in the liver and spleen of chronic iron-overloaded rats. *PLoS One*, 10(7), 1-13.

38. Bahadar, H., Maqbool, F., Niaz, K., Abdollahi, M., 2016. Toxicity of nanoparticles and an overview of current experimental models. *Iranian Biomedical Journal,* 20(1), 1-11.

39. Baky, N.A.A., Faddah, L.M., Al-Rasheed, N.M., Shebali, W., 2013. Role of quercetin and L-arginine in alleviating zinc oxide nanoparticle hepatotoxicity in rats. *Chiang Mai Journal of Science,* 40(3), 577-592.

40. Banaee, M., Mehrpak, M., Nematdoost, Haghi, B., Noori, A., 2015. Amelioration of cadmium-induced changes in biochemical parameters of the muscle of Common Carp (*Cyprinus carpio*) by Vitamin C and Chitosan. *International Journal of Aquatic Biology,* 3(6), 362-371.

41. Baugh, L.R., Sternberg, P.W., 2006. DAF-16/FOXO regulates T\transcription of cki-1/Cip/Kip and repression of lin-4 during *C. elegans* L1 arrest. *Current Biology,* 16(8), 780-785.

42. Ben Abdallah, F., Fetoui, H., Zribi, N., Fakfakh, F., Ammar-Keskes, L., 2012. Antioxidant supplementations *in vitro* improve rat sperm parameters and enhance antioxidantenz yme activities against dimethoate-induced sperm damages. *Andrologia,* 44(Suppl 1), 272-279.

43. Ben-Slama, I., Amara, S., Mrad, I., Rihane, N., EL Mir, L., Sakly, M., Amara, S., 2015. Sub-acute oral toxicity of zinc oxide nanoparticles in male rats. *Journal of Nanomedicine and Nanotechnology,* 6(284), 1-6.

44. Bhattacharya, S., 2017. Medicinal plants and natural products in amelioration of arsenic toxicity: A short review. *Pharmaceutical Biology,* 55(1), 349-354.

45. Bheeman, D., Sugumaran, S., Mathan, R., Dakshanamoorthy, S., Bellan, C. S., 2014. Oxidative stress induced biochemical alterations due to novel InTiO nanoparticles exposure. *Nanoscience and Nanotechnology Letters*, 6(6), 457-463.

46. Bhuvaneswari, P., 2014. Antioxidants in oral healthcare. *Journal of Pharmaceutical Sciences and Research*, 6(4), 206-209.

47. Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative stress and antioxidant defense. *World Allergy Organization Journal.,* 5(1), 9-19.

48. Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., Gluud, C., 2012. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Sao Paulo Medical Journal,* 133(2), 164-165.

49. Blinova, I., Ivaska, A., Heinlaan, M., Mortimer, M., Kahru, A., 2010. Ecotoxicity of nanoparticles of CuO and ZnO in natural water. *Environmental Pollution,* 158(1), 41-47.

50. Bogdan Allemann, I., Baumann, L., 2008. Antioxidants used in skin care formulations. *Skin Therapy Letter,* 13(7), 5-9.

51. Boonpeng, S., Siripongvutikorn, S., Sae-wong, C., Sutthirak, P., 2014. The antioxidant and anti-cadmium toxicity properties of garlic extracts. *Food Science & Nutrition.,* 2(6), 792-801.

52. Boonyanitipong, P., Kositsup, B., Kumar, P., Baruah, S., Dutta, J., 2011. Toxicity of ZnO and TiO_2 nanoparticles on germinating rice seed *Oryza sativa* L. *International Research Journal of Biochemistry and Bioinformatics,* 1(2011), 282-285.

53. Bossa, N., Chaurand, P., Levard, C., Borschneck, D., Miche, H., Vicente, J., Geantet, C., Aguerre-Chariol, O., Michel, F.M., Rose, J., 2017. Environmental exposure to TiO_2 nanomaterials incorporated in building material. *Environmental Pollution,* 220(Pt B), 1160-1170.

54. Boyle, D., Al-Bairuty, G.A., Ramsden, C.S., Sloman, K.A., Henry, T.B., Handy, R.D., 2013. Subtle alterations in swimming speed distributions of rainbow trout exposed to titanium dioxide nanoparticles are associated with gill rather than brain injury. *Aquatic Toxicology,* 126 (2013), 116- 127.

55. Brenner, S., 1974. The genetics of *Caenorhabditis elegans*. *Genetics*, 77, 71-94.

56. Brohi, R.D., Wang, L., Talpur, H.S., Wu, D., Khan, F. A., Bhattarai, D., Rehman, Z.U., Farmanullah, F., Huo, L. J., 2017. Toxicity of nanoparticles on the reproductive system in animal models: A review. *Frontiers in pharmacology,* 8(606), 1-22.

57. Brown, M.K., Evans, J.L., Luo, Y., 2006. Beneficial effects of natural antioxidants EGCG and alpha-lipoic acid on life span and age-dependent behavioral declines in *Caenorhabditis elegans*. *Pharmacology Biochemistry & Behavior.,* 85(3), 620-628.

58. Brunquell, J., Morris, S., Lu, Y., Cheng, F., Westerheide, S.D., 2016. The genome-wide role of HSF-1 in the regulation of gene expression in *Caenorhabditis elegans*. *BMC Genomics,* 17(559), 1-18.

59. Bucchianico, S.D., Cappellini, F., Le Bihanic, F., Zhang, Y., Dreij, K., Karlsson, H.L., 2017. Genotoxicity of TiO_2 nanoparticles assessed by mini-gel comet assay and micronucleus scoring with flow cytometry. *Mutagenesis*, 32(1), 127-137.

60. Bui, V., Park, D., Lee, Y.C., 2017. Chitosan combined with ZnO, TiO_2 and Ag nanoparticles for antimicrobial wound healing applications : A mini review of the research trends. *Polymers,* 9, 1-24.

61. Bulboaca, A., Bolboaca, S., Suci, S., 2016. Protective effect of curcumin in fructose-induced metabolic syndrome and in streptozotocin-induced diabetes in rats. *Iranian Journal of Basic Medical Sciences,* 19(6), 585-593.

62. Burke, D.J., Pietrasiak, N., Situ, S.F., Abenojar, E.C., Porche, M., Kraj, P., Lakliang, Y., Samia, A.C., 2015. Iron oxide and titanium dioxide nanoparticle effects on plant performance and root associated microbes. *International Journal of Molecular Sciences,* 16(10), 23630-23650.

63. Canas, J.E., Qi, B., Li, S., Maul, J.D., Cox, S.B., Das, S., Green, M.J., 2011. Acute and reproductive toxicity of nano-sized metal oxides (ZnO and TiO_2) to earthworms (*Eisenia fetida*). *Journal of Environmental Monitoring,* 13, 3351-3357.

64. Cao, Z., Wu, Y., Curry, K., Wu, Z., Christen, Y., Luo, Y., 2007. Ginkgo biloba extract EGb 761 and Wisconsin ginseng delay sarcopenia in *Caenorhabditis elegans*. *Journals of gerontology. Series A, Biological sciences and medical sciences,* 62(12), 1337-1345.

65. Cavallo, D., Ciervo, A., Fresegna, A.M., Maiello, R., Tassone, P., Buresti, G., Casciardi, S., Iavicoli, S., Ursini, C.L., 2015. Investigation on cobalt-oxide nanoparticles cyto-genotoxicity and inflammatory response in two types of respiratory cells. *Journal of Applied Toxicology,* 35(10), 1102-1113.

66. Ceron, J., Cabello, J., Monje, J.M., Miransa-Vizuete, A., Villanueva, A., Schwartz, S., Lehner, B., 2008. *Applications of RNAi in C. elegans Research.* New York, USA, Nova Science Publishers.

67. Chakraborty, C., Sharma, A.R., Sharma, G., Lee, S.S., 2016. Zebrafish: A complete animal model to enumerate the nanoparticle toxicity. *Journal of Nanobiotechnology,* 14(65), 1-13.

68. Chang, X., Zhang, Y., Tang, M., Wang, B., 2013. Health effects of exposure to nano-TiO_2: A meta-analysis of experimental studies. *Nanoscale Research Letter,* 8(1), 1-10.

69. Chang, Z., Huo, L., Li, P., Wu, Y., Zhang, P., 2015. Ascorbic acid provides protection for human chondrocytes against oxidative stress. *Molecular Medicine Reports,* 12(5), 7086-7092.

70. Chanvorachote, P., Pongrakhananon, V., Wannachaiyasit, S., Luanpitpong, S., Rojanasakul, Y., Nimmannit, U., 2009. Curcumin sensitizes lung cancer cells to cisplatin-induced apoptosis through superoxide anion-mediated Bcl-2 degradation. *Cancer Investigation,* 27(6), 624-635.

71. Chao, Y.Y., Hong, C.Y., Kao, C.H., 2010. The decline in ascorbic acid content is associated with cadmium toxicity of rice seedlings. *Plant Physiology and Biochemistry*, 48(5), 374-381.

72. Charao, M.F., Souto, C., Brucker, N., Barth, A., Jornada, D.S., Fagundez, D., Ávila, D.S., Eifler-Lima, V.L., Guterres, S.S., Pohlmann, A.R., Garcia, S.C., 2015. *Caenorhabditis elegans* as an alternative *in vivo* model to determine oral uptake, nanotoxicity, and efficacy of melatonin-loaded lipid-core nanocapsules on paraquat damage. *International Journal of Nanomedicine,* 10, 5093-5106.

73. Chege, P.M., McColl, G., 2014. *Caenorhabditis elegans*: a model to investigate oxidative stress and metal dyshomeostasis in Parkinson's disease. *Frontiers in Aging Neuroscience,* 6(89), 1-15.

74. Chen, B.J., Niu, C.J., Yuan, L., 2015. Ascorbic acid regulation in stress responses during acute cold exposure and following recovery in juvenile Chinese soft-shelled turtle (*Pelodiscus sinensis*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* 184, 20-26.

75. Chen, J., Dong, X., Xin, Y., Zhao, M., 2011. Effects of titanium dioxide nano-particles on growth and some histological parameters of zebrafish (*Danio rerio*) after a long-term exposure. *Aquatic Toxicology,* 101(3-4), 493-499.

76. Chen, P., Martinez-Finley, E.J., Bornhorst, J., Chakraborty, S., Aschner, M., 2013. Metal-induced neurodegeneration in *C.elegans. Frontiers in Aging Neurosciences,* 5(18), 1-11.

77. Chichiricco, G., Poma, A., 2015. Penetration and toxicity of nanomaterials in higher plants. *Nanomaterials(Basel),* 5(2), 851-873.

78. Chirnomas, D., Taniguchi, T., de la Vega, M., Vaidya, A.P., Vasserman, M., Hartman, A.R., Kennedy, R., Foster, R., Mahoney, J., Seiden, M.V., D'Andrea, A.D., 2006. Chemosensitization to cisplatin by inhibitors of the Fanconi anemia/BRCA pathway. *Molecular Cancer Therapeutics,,* 5(4), 952- 961

79. Cho, C.Y., Park, S.J., 2016. Enhanced optical output and reduction of the quantum-confined Stark effect in surface plasmon-enhanced green light-emitting diodes with gold nanoparticles. *Optics Express.,* 24(7), 7488-7494

80. Choi, J.R., Shin, D.M., Song, H., Lee, D., Kim, K., 2016. Current achievements of nanoparticle applications in developing optical sensing and imaging techniques. *Nano Convergence.*, 3(1), 1-13.

81. Chuengsamarn, S., Rattanamongkolgul, S., Luechapudiporn, R., Phisalaphong, C., Jirawatnotai, S., 2012. Curcumin extract for prevention of type 2 diabetes. *Diabetes Care,* 35(11), 2121-2127.

82. Cochran, D.B., Wattamwar, P.P., Wydra, R., Hilt, J.Z., Anderson, K.W., Eitel, R. E., Dziubla, T.D., 2013. Suppressing iron oxide nanoparticles toxicity by vascular targeted antioxidant polymer nanoparticles. *Biomaterials,* 34(37), 9615-9622.

83. Colombo, E., Li, W., Bhangu, S. K., Ashokkumar, M., 2017. Chitosan microspheres as a template for TiO_2 and ZnO microparticles: studies on mechanism, functionalization and applications in photocatalysis and H_2S removal. *RSC Advances,* 7, 19373-19383.

84. Crosera, M., Prodi, A., Mauro, M., Pelin, M., Florio, C., Bellomo, F., Adami, G., Apostoli, P., De Palma, G., Bovenzi, M., Campanini, M., Filon, F.L., 2015. Titanium dioxide nanoparticle penetration into the skin and effects on HaCaT cells. *International Journal of Environmental Research and Public Health,* 12(8), 9282-9297.

85. Da, P. M., 2016. A review on toxicology of industrially useful nanooxides. *International Journal of Innovative Research and Development*., 5(11), 1-6

86. da Mota, G.S.T., Arantes, A.B., Sacchetti, G., Spagnoletti, A., Ziosi, P., Scalambra, E., Vertuani, S., Manfredini, S., 2014. Antioxidant activity of cosmetic formulations based on novel extracts from seeds of Brazilian *Araucaria angustifolia* (bertoll) kuntze. *Journal of Cosmetics, Dermatological Sciences and Applications*, 4, 190-202.

87. Dabrunz, A., Duester, L., Prasse, C., Seitz, F., Rosenfeldt, R., Schilde, C., Schaumann, G.E., Schulz, R., 2011.Biological surface coating and molting inhibition as mechanisms of TiO_2 nanoparticle toxicity in *Daphnia magna*. *PLoS One.,* 6(5), e20112.

88. Dalai, S., Pakrashi, S., Kumar, R.S.S., Chandrasekaran, N., Mukherjee, A., 2012. A comparative cytotoxicity study of TiO_2 nanoparticles under light and dark conditions at low exposure concentrations. *Toxicological Research..,* 1, 116-130.

89. Das, K., Roychoudhury, A., 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers of Environ Sciences,* 2(53), 1-13.

90. Das, K.K., Saha, S., 2010. L-ascorbic acid and α tocopherol supplementation and antioxidant status in nickel- or lead-exposed rat brain tissue. *Journal of Basic Clinical Physiology and Pharmacology,* 21(4), 325-346.

91. Daverey, A., Agrawal, S.K., 2016. Curcumin alleviates oxidative stress and mitochondrial dysfunction in astrocytes. *Neuroscience,* 333, 92-103.

92. Deng, Y., White, J.C., Xing, B., 2014. Interactions between engineered nanomaterials and agricultural crops: implications for food safety. *Zhejiang University SCIENCE A (Applied Physics & Engineering),* 15, 552-572.

93. Devi, K.R., Raju, M.M., 2012. Protective *in-vivo* effects of curcumin on chromium induced genotoxicity in germ cells of mice. *Intenational Journal of Pharmacology and Biological Sciences*, 3(1), 243-250.

94. Dissanayake, N.M., Current, K.M., Obare, S.O., 2015. Mutagenic effects of iron oxide nanoparticles on biological cells. *International Journal of Molecular Sciences,* 16(10), 23482-23516.

95. Dobias, J., Bernier-Latmani, R., 2013. Silver release from silver nanoparticles in natural waters. Environmental *Sciences and Technology,* 47(9), 4140-4146.

96. Dolatabadi, A., Sani, B., Moaveni, P., 2015. Impact of nanosized titanium dioxide on agronomical and physiological characteristics of annual medic (*Medicago scutellata* L.). *Cercetari Agronomice Moldova,* 48, 53-61.

97. Donkin, S.G., Williams, P.L., 1995. Influence of developmental stage, salts and food presence on various end-points using *Caenorhabditis elegans* for aquatic toxicity testing. *Environmental Toxicology and Chemistry*., 14, 2139-2147.

98. Dottermusch, M., Lakner, T., Peyman, T., Klein, M., Walz, G., Neumann Haefelin, E., 2016. Cell cycle controls stress response and longevity in *C. elegans*. *Aging,* 8(9), 2100-2115.

99. Drobne, D., Jemec, A., Pipan Tkalec, Z., 2009. *In vivo* screening to determine hazards of nanoparticles: Nanosized TiO_2. *Environmental Pollution,* 157 (4), 1157-1164.

100. Droge, W., 2002. Free radicals in the physiological control of cell function. *Physiology Reviews,* 82(1), 47-95.

101. Du, L., Miao, X., Jiang, Y., Jia, H., Tian, Q., Shen, J., Liu, Y., 2013. An effective strategy for the synthesis of biocompatible gold nanoparticles using danshensu antioxidant: prevention of cytotoxicity via attenuation of free radical formation. *Nanotoxicology,* 7(3), 294-300.

102. Du, W., Sun, Y., Ji, R., Zhu, J., Wu, J., Guo, H., 2011. TiO_2 and ZnO nanoparticles negatively affect wheat growth and soil enzyme activities in agricultural soil. *Journal of Environmental Monitoring,* 13(4), 822-828.

103. Dubey, A., Goswami, M., Yadav, K., Chaudhary, D., 2015. Oxidative stress and nano-toxicity induced by TiO_2 and ZnO on WAG cell line. *PLoS One,* 10(5), e0127493.

104. Dumont, E., Johnson, A.C., Keller, V.D., Williams, R.J., 2015. Nano silver and nano zinc-oxide in surface waters - Exposure estimation for Europe at high spatial and temporal resolution. *Environmental Pollution,* 196, 341-349.

105. Durga, M., Nathiya, S., Devasena, T., 2015. Protective role of fenugreek leaf extract and quercetin against petrol exhaust nanoparticle induced lipid peroxidation and oxidative stress in rat erythrocytes *in vitro*. *Asian Journal of Pharmcology and Clinical Research*, 8(1), 237-241.

106. Ebrahimi, A., Galavi, M., Ramroudi, M., Moaveni, P., 2016. Effect of TiO_2 nanoparticles on antioxidant enzymes activity and biochemical biomarkers in pinto bean (*Phaseolus vulgaris L.*). *Journal of Molecular Biology Research,* 6(1), 58-66.

107. Ekstrand-Hammarström, B., Hong, J., Davoodpour, P., Sandholm, K., Ekdahl, K.N., Bucht, A., Nilsson, B., 2015. TiO_2 nanoparticles tested in a novel screening whole human blood model of toxicity triggeradverse activation of the kallikrein system at low concentrations. *Biomaterials*, 51, 58-68.

108. El-Deeb, M.E.E., Abd-El-Hafez, A.A.A., 2015. Can vitamin C affect the $KBrO_3$ induced oxidative stress on left ventricular myocardium of adult male albino rats? A histological and immunohistochemical study. *Journal of Microscopy and Ultrastructure*, 3, 120-136.

109. EL-Desoky, G. E., Abdel-Ghaffar, A., Al-Othman, Z.A., Habila, M.A., Al-Sheikh, Y.A., Ghneim, H. K., Giesy, J. P., Aboul-Soud, M. A., 2017. Curcumin protects against tartrazine-mediated oxidative stress and hepatotoxicity in male rats. *European Review for Medical and Pharmacological Sciences,* 21(3), 635-645.

110. EL-Gendy, K.S., Aly, N.M., Mahmoud, F.H., Kenawy, A., El-Sebae, A.K.H., 2010. The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. *Food and Chemical Toxicology,* 48 (1), 215-221.

111. Elhalwagy, M.E.A., Nahas, A.A., Ziada, R.M., Farid, H.E.A., 2015. Potential effect of vitamin C and curcumin on oxidative stress and skin lesion induced by dermal intoxication with cypermethrin. *American Chemical Science Journal*, 8(1), 1-12.

112. Elsayed, A.S.I., Hegazi, M.A., Mostafa, H.A.E., Hegazi, M.M., 2015. The protective role of curcumin against oxidative stress caused by gasoline. *International Journal of Applied Biology and Pharmaceutical Technology,* 7(1), 56-66.

113. El-Sokkary, G. H., Awadalla, E. A., 2011. The protective role of vitamin C against cerebral and pulmonary damage induced by cadmium chloride in male adult albino rat. *The Open Neuroendocrinology Journal,* 4, 1-8.

114. El-Tantawy, W. H., 2015. Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *Journal of Traditional and Complementary Medicine,* 6(4), 327-331.

115. Eng, S. A., Nathan, S., 2015. Curcumin rescues Caenorhabditis elegans from a Burkholderia pseudomallei infection. *Frontiers in Microbiology.* 6(290), 1-15.

116. EPA 2010. *STREAMS final report.* Aug 2010.

117. Exbrayat, J.M., Elara, N., Moudilou, E.N., Lapied, E., 2015. Harmful effects of nanoparticles on animals. *Journal of Nanotechnology*, 2015, 1-10.

118. Faddah, L.M., Abdel Baky, N.A., Al-Rasheed, N.M., Al-Rasheed, N.M., Fatani, A.J., Atteya, M., 2012. Role of quercetin and arginine in ameliorating nano zinc oxide-induced nephrotoxicity in rats. *BMC Complementary and Alternative Medicine*, 12(60), 1-14.

119. Fadus, M.C., Lau, C., Bikhchandani, J., Lynch, H.T., 2016. Curcumin: An age-old anti-inflammatory and anti-neoplastic agent. *Journal of Traditional and Complementary Medicine,* 7(3), 339-346.

120. Faisal, M., Saquib, Q., Alatar, A.A., Al-Khedhairy, A.A., Ahmed, M., Ansari, S.M., Alwathnani, H.A., Dwivedi, S., Musarrat, J., Praveen, S., 2016. Cobalt oxide nanoparticles aggravate DNA damage and cell death in eggplant via mitochondrial swelling and NO signaling pathway. *Biological Research,* 49(20), 1-13.

121. Falcaro, P., Ricco, R., Yazdi, A., Imaz, I., Furukawa, S., Maspoch, D., Ameloot, R., Evans, J.D., Doonan, C.J., 2016. Application of metal and metal oxide nanoparticles@MOFs. *Coordination Chemistry Reviews,* 307, 237-254.

122. Fekete-Kertesz, I., Maros, G., Gruiz, K., Molnar, M., 2016. The effect of TiO_2 nanoparticles on the aquatic ecosystem: A comparative ecotoxicity study with test organisms of different trophic levels. *Periodica Polytechnica Chemical Engineering,* 60, 231-243.

123. Firdaus, F., Zafeer, M. F., Anis, E., Fatima, M., Hossain, M. M., Afzal, M., 2016. Antioxidant potential of thymoquinone against arsenic mediated neurotoxicity. *Free Radicals and Antioxidants,* 6(1), 115- 123.

124. Flohe, L., Gunzler, W.A., 1984. Assays of glutathione peroxidase. *Methods in Enzymology,* 105, 114-121.

125. Flora, S.J., Bhadauria, S., Kannan, G.M., Singh, N., 2007. Arsenic induced oxidative stress and the role of antioxidant supplementation during chelation: A review. *Journal of Environmental Biology,* 28(2 Suppl), 333-347.

126. Fragiorge, E. J., Spano, M. A., Antunes, L. M. G., 2007. Modulatory effects of the antioxidant ascorbic acid on the direct genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*. *Genetics and Molecular Biology,* 30(2007), 449-455.

127. Fu, Y., Zhang, Y., Chang, X., Zhang, Y., Ma, S., Sui, J., Yin, L., Pu, Y., Liang, G., 2014. Systemic immune effects of titanium dioxide nanoparticles after repeated intratracheal instillation in rat. *International Journal of Molecular Sciences*, 15(4), 6961-6973.

128. Fukui, H., Iwahashi, H., Endoh, S., Nishio, K., Yoshida, Y., Hagihara, Y., Horie, M., 2015. Ascorbic acid attenuates acute pulmonary oxidative stress and inflammation caused by zinc oxide nanoparticles. *Journal of Occupational Health,* 57(2), 118-125.

129. Fukumura, H., Sato, M., Kezuka, K., Sato, I., Feng, X., Okumura, S., Fujita, T., Yokoyama, U., Eguchi, H., Ishikawa, Y., Saito, T., 2012. Effect of ascorbic acid on reactive oxygen species production in chemotherapy and hyperthermia in prostate cancer cells. *Journal of Physiological* Sciences, 62(3), 251-257.

130. Gaiser, B.K., Biswas, A., Rosenkranz, P., Jepson, M.A., Lead, J.R., Stone, V., Tyler, C.R., Fernandes, T.F., 2011. Effects of silver and cerium dioxide micro- and nano-sized particles on *Daphnia magna. Journal of Environmental Monitoring*, 13(5), 1227-1235.

131. Ganapathi, A.P., Devaki, R., Thuniki, N.R., Manna, J., Tirumuru, B., Gopu, C.R., Deepthi, S.B., Trivedi, R., Rana, R.K., Hasan, A., 2014. In vitro assessment of Ag and TiO_2 nanoparticles cytotoxicity. *International Journal of Research in Medical Sciences*, 2, 1360–1367.

132. Garcia-Nino, W.R., Pedraza-Chaverri, J., 2014. Protective effect of curcumin against heavy metals-induced liver damage. *Food and Chemical Toxicology,* 69, 182–201.

133. Ge, Y., Schimel, J.P., Holden, P.A., 2011, Evidence for negative effects of TiO_2 and ZnO nanoparticles on soil bacterial communities. *Environmental Sciences and Technology,* 45(4), 1659-1664.

134. Gerloff, K., Fenoglio, I., Carella, E., Kolling, J., Albrecht, C., Boots, A.W., Forster, I., Schins, R. P., 2012. Distinctive toxicity of TiO_2 rutile/anatase mixed phase nanoparticles on Caco-2 cells. *Chemical Research in Toxicology,* 25(3), 646-655.

135. Ghosh, M., Bandyopadhyay, M., Mukherjee, A., 2010. Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: Plant and human lymphocytes. *Chemosphere,* 81 (10), 1253-1262.

136. Ghosh, M., Sinha, S., Jothiramajayam, M., Jana, A., Nag, A., Mukherjee, A., 2016. Cyto-genotoxicity and oxidative stress induced by zinc oxide nanoparticle in human lymphocyte cells *in vitro* and Swiss albino male mice *in vivo. Food and Chemical Toxicology,* 97, 286-296.

137. Gianeti, M.D., Maia Campos, P.M., 2014. Efficacy evaluation of a multifunctional cosmetic formulation: The benefits of a combination of active antioxidant substances. *Molecules,* 19(11), 18268-18282.

138. Gonzalez-Moragas, L., Maurer, L.L., Harms, V.M., Meyer, J.N., Laromaine, A., Roig, A., 2017. Materials and toxicological approaches to study metal and metal-oxide nanoparticles in the model organism *Caenorhabditis elegans*. *Materials Horizons,* 4(5), 719-746.

139. Gottschalk, F., Sonderer, T., Scholz, R.W., Nowack, B., 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, Fullerenes) for different regions. *Environmental Science and Technology,* 43(24), 9216-9222.

140. Gottschalk, F., Kost, E., Nowack, B., 2013. Engineered nanomaterials in water and soils: A risk quantification based on probabilistic exposure and effect modeling. *Environmental Toxicology and Chemistry,* 32(6), 1278-1287.

141. Guaiquil, V.H., Vera, J.C., Golde, D.W., 2001. Mechanism of vitamin C inhibition of cell death induced by oxidative stress in glutathione-depleted HL-60 cells. *Journal of Biology and Chemistry,* 276(44), 40955-40961.

142. Gueroui, M., Kechrid, Z., 2016. Evaluation of some biochemical parameters and brain oxidative stress in experimental rats exposed chronically to silver nitrate and the protective role of vitamin E and selenium. *Toxicological Research,* 32(4), 301-309.

143. Guo, H., Zhang, J., Boudreau, M., Meng, J., Yin, J. J., Liu, J., Xu, H., 2016. Intravenous administration of silver nanoparticles causes organ toxicity through intracellular ROS-related loss of inter-endothelial junction. *Particle and Fibre Toxicology,* 13(21), 1-13.

144. Gupta, S., Kushwah, T., Vishwakarma, A., Yadav, S., 2015. Optimization of ZnO-NPs to investigate their safe application by assessing their effect on soil nematode *Caenorhabditis elegans*. *Nanoscale Research Letter,* 10(1), 1-9.

145. Gupta, S.C., Kim, J.H., Prasad, S., Aggarwal, B.B., 2010. Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. *Cancer and Metastasis Reviews,* 29(3), 405-434.

146. Gupta, S.C., Patchva, S., Aggarwal, B.B., 2013. Therapeutic roles of curcumin: Lessons learned from clinical trials. *Advances in Applied & Pharmaceutical Sciences Journal,* 15(1), 195-218.

147. Guzior, N., Wieckowska, A., Panek, D., Malawska, B., 2015. Recent development of multifunctional agents as potential drug candidates for the treatment of alzheimer's disease. *Current Medicinal Chemistry,* 22(3), 373-404.

148. Habibian, M., Moosavi, S.J., Farzanegi, P., 2016. Regular exercise combined with curcumin supplementation: Protective effects against lead- induced cerebellar oxidative damage in an animal model. *Neurophysiology,* 48 (1), 17-22.

149. Hamzeh, M., Sunahara, G.I., 2013. *In vitro* cytotoxicity and genotoxicity studies of titanium dioxide (TiO_2) nanoparticles in Chinesehamster lung fibroblast cells. *Toxicology In Vitro,* 27(2), 864-873.

150. Han, Z., Yan, Q., Ge, W., Liu, Z. G., Gurunathan, S., De Felici, M., Shen, W., Zhang, X.F., 2016. Cytotoxic effects of ZnO nanoparticles on mouse testicular cells. *International Journal of Nanomedicine,* 11, 5187-5203.

151. Hao, L., Chen, L., 2012. Oxidative stress responses in different organs of carp (*Cyprinus carpio*) with exposure to ZnO nanoparticles. *Ecotoxicology and Environmental Safety,* 80, 103-110.

152. Harrison, F.E., 2012. A critical review of vitamin C for the prevention of age-related cognitive decline and Alzheimer'sdisease. *Journal of Alzheimers Disease.*, 29(4), 711-726.

153. Harrison, F. E., Bowman, G.L., Polidori, M.C., 2014. Ascorbic acid and the brain: rationale for the use against cognitive decline. *Nutrients,* 6(4), 1752-1781.

154. Hasan, M., Haider, S.S., 1989. Acetyle-homocysteine thiolactone protect against some neurotoxic effects of thallium. *Neurotoxicology,* 10, 257-262.

155. He, Y., Yue, Y., Zheng, X., Zhang, K., Chen, S., Du, Z., 2015. Curcumin, inflammation, and chronic diseases: How are they linked? *Molecules,* 20(5), 9183-9213.

156. Heaney, M.L., Gardner, J.R., Karasavvas, N., Golde, D.W., Scheinberg, D.A., Smith, E.A., O'Connor, O. A., 2008. Vitamin C antagonizes the cytotoxic effects of antineoplastic drugs. *Cancer Research,* 68(19), 8031-8038.

157. Heim, J., Felder, E., Tahir, M. N., Kaltbeitzel, A., Heinrich, R. U., Brochhausen, C., Mailänder, V., Tremel, W., Brieger, J., 2015. Genotoxic effect of ZnO nanoparticles. *Nanoscale,* 7(19), 8931-8938.

158. Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H.C., Kahru, A., 2008. Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere,* 71(7), 1308-1316.

159. Heng, B.C., Zhao, X., Xiong, S., Ng, K.W., Boey, F.Y., Loo, J.S., 2010. Toxicity of zinc oxide (ZnO) nanoparticles on human bronchial epithelial cells (BEAS-2B) is accentuated by oxidative stress. *Food and Chemical Toxicology,* 48 (6), 1762–1766.

160. Hong, F., Wang, Y., Zhou, Y., Zhang, Q., Ge, Y., Chen, M., Hong, J., Wang, L., 2016. Exposure to TiO_2 nanoparticles induces immunological dysfunction in mouse testitis. *Journal of Agricultural and Food Chemistry.* 64(1), 346-355.

161. Hong, J., Zhang, Y.Q., 2016. Murine liver damage caused by exposure to nano-titanium dioxide. *Nanotechnology,* 27(11):112001.

162. Hong, T.K., Tripathy, N., Son, H.J., Ha, K.T., Jeong, H.S., Hahn, Y.B., 2013. A comprehensive *in vitro* and *in vivo* study of ZnO nanoparticles toxicity. *Journal of Materials Chemistry.* B1(23), 2985-2992.

163. Hossain, Z., Mustafa, G., Komatsu, S., 2015. Plant responses to nanoparticle stress. *International Journal of Molecular Sciences,* 16(11), 26644-26653.

164. Hu, C.W., Li, M., Cui, Y.B., Li, D.S., Chen, J., Yang, L.Y., 2010. Toxicological effects of TiO_2 and ZnO nanoparticles in soil on earthworm *Eisenia fetida*. *Soil Biology & Biochemistry*, 42(4), 586-591.

165. Hu, H., Guo, Q., Wang, C., Ma, X., He, H., Oh, Y., Feng, Y., Wu, Q., Gu, N., 2015. Titanium dioxide nanoparticles increase plasma glucose via reactive oxygen species-induced insulin resistance in mice. *Journal of Applied Toxicology,* 35(10), 1122-1132.

166. Huang, C.C., Aronstam, R.S., Chen, D.R., Huang, Y.W., 2010. Oxidative stress, calcium homeostasis, and altered gene expression in human lung epithelial cells exposed to ZnO NPs. *Toxicolology In-Vitro.,* 24(1), 45-55.

167. Huang, Y.W., Wu, C.H., Aronstam, R.S., 2010. Toxicity of transition metal oxide nanoparticles: Recent insights from *in vitro* studies. *Materials (Basel),* 3(10), 4842-4859.

168. Huang, H.C., Lin, C.J., Liu, W.J., Jiang, R.R., Jiang, Z.F., 2011. Dual effects of curcumin on neuronal oxidative stress in the presence of Cu(II). *Food and Chemical Toxicology.* 49(7), 1578-1583.

169. Huang, C.W., Li, S.W., Hsiu-Chuan Liao, V., 2017. Chronic ZnO-NPs exposure at environmentally relevant concentrations results in metabolic and locomotive toxicities in *Caenorhabditis elegans. Environmental Pollution,* 220(Pt B), 1456-1464.

170. Huerta-García, E., Pérez-Arizti, J.A., Márquez-Ramírez, S.G., Delgado-Buenrostro, N. L., Chirino, Y.I., Iglesias, G. G., López-Marure, R., 2014. Titanium dioxide nanoparticles induce strong oxidative stress and mitochondrial damage in glial cells. *Free Radical Biology and Medicine*, 73, 84-94.

171. Hunt, P.R., 2017. The *C. elegans* model in toxicity testing. *Journal of Applied Toxicology,* 37(1), 50-59.

172. Hussein, H.K., Elnaggar, M.H., Al-Dailamy, J.M., 2012. Protective role of Vitamin C against hepatorenal toxicity of fenvalerate in male rats. *Global Advanced Research Journal of Environmental Science and Toxicology,* 1(4), 060-065.

173. Hyseni, S., 2016. *Toxicological effects of nanomaterials on aqueous and terrestrial ecosystems*. CDS publication, Getzville, New York.

174. Iannarelli, L., Giovannozzi, M., Morelli, F., Viscotti, F., Bigini, P., Maurino, V., Spoto, G., Martra, G., Ortel, E., Hodoroaba, V.D., Rossi, A. M., Diomede, L., 2016. Shape engineered TiO_2 nanoparticles in *Caenorhabditis elegans* : A Raman imaging based approach to assist tissue-specific toxicological studies. *Royal Society of Chemistry*., 6, 70501-70509

175. IARC 2010. *International Agency for Research on Cancer: Carbon Black, Titanium Dioxide, and Talc IARC Monographs on the Evaluation of Carcinogenic Risks to Human*. 93: 193-275.

176. Iavicoli, I., Leso, V., Bergamaschi, A., 2012. Toxicological effects of titanium dioxide nanoparticles : A review of *in vivo* studies. *Journal of Nanomaterial,* 15, 481-508.

177. Ibrahim, M.A., Khalaf, A.A., Galal, M.K., Ogaly, H.A., Hassan, A.H.M., 2015. Ameliorative influence of green tea extract on copper nanoparticle-induced hepatotoxicity in rats. *Nanoscale Research Letter,* 10(363), 1-9.

178. Ismail, A.F., Salem, A.A.M., 2016. Renoprotective effect of curcumin on acetaminophen-induced nephrotoxicity in rats. *Journal of Chemistry and Pharmacy Research,* 8(2016), 773-779.

179. Ivask, A., Bondarenko, O., Jepihhina, N., Kahru, A., 2010. Profiling of the reactive oxygen species-related ecotoxicity of CuO, ZnO, TiO_2, silver and fullerene nanoparticles using a set of recombinant luminescent Escherichia coli strains: differentiating the impact of particles and solubilised metals. *Analytical and Bioanalytical Chemistry,* 398(2), 701-716.

180. Ivask, A., Juganson, K., Bondarenko, O., Mortimer, M., Aruoja, V., Kasemets, K., Blinova, I., Heinlaan, M., Slaveykova, V., Kahru, A., 2014. Mechanisms of toxic action of Ag, ZnO and

CuO nanoparticles to selected ecotoxicological testorganisms and mammalian cells in vitro: a comparative review. *Nanotoxicology,* 8(Suppl 1), 57-71.

181. Jacob, A.R., Sotoudeh, G., 2002. Vitamin C function and status in chronic diseases. *Nutrition in Clinical Care*, 5 (2), 66-74.

182. Jaiswal, S.K., Sharma, A., Gupta, V.K., Singh, R.K., Sharma, B., 2016. Curcumin mediated attenuation of carbofuran induced oxidative stress in rat brain. *Biochemical Research International,* 2016, 1-7.

183. James, C.E., Davey, M.W., 2007. A rapid colorimetric assay for the quantitation of the viability of free-living larvae of nematodes *in vitro*. *Parasitology Research,* 101(4), 975-980.

184. Jiang, Y., Chen, J., Wu, Y., Wang, Q., Li, H., 2016. Sublethal toxicity endpoints of heavy metals to the nematode *Caenorhabditis elegans*. *PLoS One,* 11(1), e0148014.

185. Jimeno-Romero, A., Oron, M., Cajaraville, M.P., Soto, M., Marigómez, I., 2016. Nanoparticle size and combined toxicity of TiO_2 and DSLS (surfactant) contribute to lysosomal responses in digestive cells of mussels exposed to TiO_2 nanoparticles. *Nanotoxicology*, 10(8), 1168-1176

186. Jin, X., Song, L., Liu, X., Chen, M., Li, Z., Cheng, L., Ren, H., 2014. Protective efficacy of vitamins C and E on p,p'-DDT-induced cytotoxicity via the ROS-mediated mitochondrial pathway and NF-kB/FasL pathway. *PLoS One,* 9(12), e113257.

187. Jin, X., Su, R., Li, R., Song, L., Chen, M., Cheng, L., Li Z., 2016. Amelioration of particulate matter-induced oxidative damage by vitamin c and quercetin in human bronchial epithelial cells. *Chemosphere*, 144, 459-466.

188. Juganson, K., Ivask, A., Blinova, I., Mortimer, M., Kahru, A., 2015. NanoE-Tox: New and in-depth database concerning ecotoxicity of nanomaterials. *Beilstein Journal of Nanotechnology*, 6, 1788-1804.

189. Kadasa, N.M., Abdallah, H., Afifi, M., Gowayed, S., 2015. Hepatoprotective effects of curcumin against diethyl nitrosamine induced hepatotoxicity in albino rats. *Asian Pacific Journal of Cancer Prevention*, 16 (1), 103-108.

190. Kahru, A., Dubourguier, H.C., 2010. From ecotoxicology to nanoecotoxicology. *Toxicology*, 269 (2-3), 105–119.

191. Kakkar, P., Das, B., Viswanathan, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry & Biophysics*., 21(2), 130-132

192. Kaletta, T., Hengartner, M.O., 2006. Finding function in novel targets: C. elegans as a model organism. *Nature Reviews Drug Discovery*, 5(5), 387-399.

193. Kamarehei, M., Yazdanparast, R., Aghazadeh, S., 2014. Curcumin protects SK-N-MC cells from H_2O_2-induced cell death by modulation of notch signaling pathway. *Cell Biology,* 3, 72-86.

194. Kamat, A.M., Sethi, G., Aggarwal, B.B., 2007. Curcumin potentiates the apoptotic effects of chemotherapeutic agents and cytokines through down-regulation of nuclear factor-KB and

nuclear factor-KB–regulated gene products in IFN-A–sensitive and IFN-A–resistant human bladder cancer cells. *Molecular Cancer Therapeutics.,* 6(3), 1022-1030

195. Karaytug, S., Sevgiler, Y., Karayakar, F., 2014. Comparison of the protective effects of antioxidant compounds in the liver and kidney of Cd- and Cr-exposed common carp. *Environmental Toxicology,* 29(2), 129-137.

196. Karthigarani, M., Dr. Navaraj, P. S., 2012. Impact of nanoparticle on enzymes activity in *Oreochromis Mossambicus. International Journal of Science and Technology Research.,* 1(10), 13-17

197. Kasnaviyeh, M.H., Nasiri, E., Mohammadi, F., Vaziri, S., Mosaddegh, R., Noyani, A., Dehghani, A., Masoumi, G., 2017. Protective effects of Vitamin C on kidney performance of an adult male rat exposed to formaldehyde. *Biosciences Biotechnology Research Communication*, 10(1), 118-121.

198. Kaushik, M., Moores, A., 2016. Review: Nanocelluloses as versatile supports for metal nanoparticles and their applications in catalysis. *Green Chemistry.,* 18(3), 622-637

199. Keller, A. A., McFerran, S., Lazareva, A., Suh S., 2013. Global life cycle releases of engineered nanomaterials. *Journal of Nanopartical Research.,* 15, 1-17.

200. Khalil, F.A., Ali, N.H., 2011. Protective effect of dietary antioxidants curcumin, vitamin C and ginko biloba on oxidative stress in colonic rats induced by butylated hydroxyanisol. *Austrilian Journal of Basic and Applied Science,* 5(10), 1489-1495.

201. Khalil, R.M., Khedr, N.F., 2016. Curcumin protects against monosodium glutamate neurotoxicity and decreasing NMDA2B and mGluR5 expression in rat hippocampus. *Neurosignals*, 24(1), 81-87.

202. Khan, I., Saeed, K., Khan, I., 2017. Nanoparticles: Properties, applications and toxicities. *Arabian Jornal of Chemistry.,* doi.org/10.1016/j.arabjc.2017.05.011

203. Khanna, P., Ong, C., Bay, B.H., Baeg, G.H., 2015. Nanotoxicity: An interplay of oxidative stress, inflammation and cell death. *Nanomaterials(Basel),* 5(3), 1163-1180

204. Khare, P., Sonane, M., Nagar, Y., Moin, N., Ali, S., Gupta, K.C., Satish, A., 2015. Size dependent toxicity of zinc oxide nano-particles in soil nematode *Caenorhabditis elegans*. *Nanotoxicology,* 9(4), 423-432.

205. Khare, P., Sonane, M., Pandey, R., Ali, S., Gupta, K.C., Satish A., 2011. Adverse effects of TiO_2 and ZnO nanoparticles in soil nematode, *Caenorhabditis elegans*. *Journal of Biomedical and Nanotechnology,* 7(1), 116-117.

206. Khatri, D.K., Juvekar, A.R., 2016. Abrogation of locomotor impairment in a rotenone-induced *Drosophila melanogaster* and zebrafish model of Parkinson's disease by ellagic acid and curcumin. *International Journal of Nutrition, Pharmacology, Neurological Diseases,* 6, 90-96.

207. Khorsandi, L., Mansouri, E., Orazizadeh, M., Jozi, Z., 2016. Curcumin attenuates hepatotoxicity induced by zinc oxide nanoparticles in rats. *Balkan Medical Journal.* 33(3), 252-257.

208. Khorsandi, L., Orazizadeh, M., Mansori, E., Fakhredini, F., 2015. Glycyrrhizic acid attenuated lipid peroxidation induced by titanium dioxide nanoparticles in rat liver. *Bratislavske Lekarske Listy.,* 116 (6), 383- 388.

209. Kim, I.Y., Joachim, E., Choi, H., Kim, K., 2015. Toxicity of silica nanoparticles depends on size, dose, and cell type. *Nanomedicine,* 11(6), 1407-1416.

210. Kim, M.S., Louis, K.M., Pedersen, J.A., Hamers, R.J., Peterson, R.E., Heideman, W., 2014. Using citrate-functionalized TiO_2 nanoparticles to study the effect of particle size on zebrafish embryo toxicity. *Analyst - Royal Society of Chemistry,* 139(5), 964-972.

211. Kim, Y.H., Fazlollahi, F., Kennedy, I.M., Yacobi, N.R., Hamm-Alvarez, S.F., Borok, Z., Kim, K.J., Crandall, E.D., 2010. Alveolar epithelial cell injury due to zinc oxide nanoparticle exposure. *American Journal of Respiratory and Critical Care Medicine,* 182(11), 1398-1409.

212. Kizhakekuttu, T.J., Widlansky, M.E., 2010. Natural antioxidants and hypertension: promise and challenges. *Cardiovascular Therapy,* 28(4), 20-32.

213. Klibet, F., Boumendjel, A., Khiari, M., El Feki, A., Abdennour, C., Messarah, M., 2016. Oxidative stress-related liver dysfunction by sodium arsenite: Alleviation by *Pistacia lentiscus* oil. *Pharmaceutical Biology.* 54(2), 354-363.

214. Ko, K.S., Ha, K., Kong, I.C., 2015. Effects of monotypic and binary mixtures of metal oxide nanoparticles on microbial growth in sandy soil collected from artificial recharge sites. *International Journal of Molecular Sciences*, 16(11), 27967-27977.

215. Ko, K.S., Koh, D.C., Kong, I.C., 2018. Toxicity Evaluation of Individual and Mixtures of Nanoparticles Based on Algal Chlorophyll Content and Cell Count. *Materials (Basel)*, 11(1), pii: E121.

216. Koch, K., Havermann, S., Buchter, C., Watjen, W., 2014. *Caenorhabditis elegans* as model system in pharmacology and toxicology: Effects of flavonoids on redox-sensitive signalling pathways and ageing. *Scientific World Journal*, 2014, 1-15.

217. Kondo, M., Yanase, S., Ishii, T., Hartman, P.S., Matsumoto, K., Ishii, N., 2005. The p38 signal transduction pathway participates in the oxidative stress-mediated translocation of DAF-16 to *Caenorhabditis elegans* nuclei. *Mechanisms of Ageing and Development.* 126 (6-7), 642-647.

218. Kononenko, V., Narat, M., Drobne, D., 2015. Nanoparticle interaction with the immune system. *Archives of Industrial Hygiene and Toxicology l.,* 66(2), 97-108.

219. Kosyan, D.B., Rusakova, E.A., Sizova, E.A., Yausheva, E.V., Miroshnikov, S.A.2016. Toxic effect and mechanisms of nanoparticles on freshwater infusoria. *International Journal of Geomate*, 11(23), 2170-2176.

220. Krzyzewska, I., Kyziol-Komosinska, J., Rosik-Dulewska, C., Czupioł, J., Antoszczyszyn-Szpicka, P., 2016. Inorganic nanomaterials in the aquatic environment: behavior, toxicity, and interaction with environmental elements. *Archives of Environmental Protection.,* 42(1), 87-101

221. Kukongviriyapan, U., Apaijit, K., Kukongviriyapan, V., 2016. Oxidative stress and cardiovascular dysfunction associated with cadmium exposure: beneficial effects of curcumin and tetrahydrocurcumin. *Tohoku Journal of Experimental Medicine,* 239(1), 25-38.

222. Kulikova, O.I., Fedorova, T.N., Lopachev, A.V., Orlova, V.S., Grachev, V.A., 2016. Effects of antioxidants on the viability of the human neuroblastoma SH-SY5Y cell culture under the conditions of heavy metal toxicity. *Biology and Medicine,* 8(4), 1-5.

223. Kumar, A., Pandey, A.K., Singh, S.S., Shanker, R., Dhawan, A., 2011. Engineered ZnO and TiO(2) nanoparticles induce oxidative stress and DNA damage leading to reduced viability of *Escherichia coli. Free Radical Biology and Medicine,* 51(10), 1872-1881.

224. Kumar, Y., Yadav, D.N., Ahmad, T., Narsaiah, K., 2015. Recent trends in the use of natural antioxidants for meat and meat products. *Comprehensive Reviews in Food Science and Food Safety,* 14(6), 796-812.

225. Kumar, J., Barhydt, T., Awasthi, A., Lithgow, G.J., Killilea, D.W., Kapahi, P., 2016. Zinc levels modulate lifespan through multiple longevity pathways in *Caenorhabditis elegans*. *PLoS One.,* 11(4), e0153513.

226. Kwon, E.S., Narasimhan, S.D., Yen, K., Tissenbaum, H.A., 2010. A new DAF-16 isoform regulates longevity. *Nature,* 466(7305), 498-502.

227. Lai, E.P.C., Alsudir, S., 2017. Chemical analysis of biochemically toxic metal oxide nanoparticles in environmental water. *Journal of Research Analytica,* 3(2), 52-61.

228. Lampe, V., Milobedzka, J., 1913. Studien uber Curcumin. *Berichte der Deutschen Chemischen Gesellschaft,* 46, 2235-2237.

229. Lan, Z., Yang, W.X., 2012. Nanoparticles and spermatogenesis: how do nanoparticles affect spermatogenesis and penetrate the blood-testis barrier. *Nanomedicine (Lond),* 7(4), 579-596.

230. Landis, J.N., Murphy, C.T., 2010. Integration of diverse inputs in the regulation of *C. elegans* DAF-16/FOXO. *Devlopmental Dynamics,* 239(5), 1405-1412.

231. Lantto, T.A., Colucci, M., Závadová, V., Hiltunen, R., Raasmaja, A., 2009. Cytotoxicity of curcumin, resveratrol and plant extracts from basil, juniper, laurel and parsley in SH-SY5Y and CV1-P cells. *Food Chemistry,* 117(3), 405-411.

232. Lapied, E., Nahmani, J.Y., Moudilou, E., Chaurand, P., Labille, J., Rose, J., Exbrayat, J.M., Oughton, D.H., Joner E.J., 2011. Ecotoxicological effects of an aged TiO_2 nanocomposite measured as apoptosis in the anecic earthworm *Lumbricus terrestris* after exposure through water, food and soil. *Environment International*, 37(6), 1105-1110.

233. Larue, C., Laurette, J., Herlin-Boime, N., Khodja, H., Fayard, B., Flank, A.M., Brisset, F., Carriere, M., 2012. Accumulation, translocation and impact of TiO_2 nanoparticles in wheat

(*Triticum aestivum spp*.): influence of diameter and crystal phase. *Science of Total Environ*ment*,* 431, 197-208.

234. Lebedev, S., Yausheva, E., Galaktionova, L., Sizova, E., 2015. Impact of Zn nanoparticles on growth, survival and activity of antioxidant enzymes in *Eisenia fetida*. *Modern Applied Science*, 9, 34-44.

235. Lee, I.C., Ko, J.W., Park, S.H., Shin, N.R., Shin, I.S., Moon, C., Kim, J.H., Kim, H.C., Kim, J.C., 2016. Comparative toxicity and biodistribution assessments in rats following subchronic oral exposure to copper nanoparticles and microparticles. *Particle and Fibre Toxicology,* 13 (1), 1-16.

236. Lee, I.C., Ko, J.W., Park, S.H., Lim, J.O., Shin, I.S., Moon, C., Kim, S.H., Heo, J.D., Kim, J.C., 2016. Comparative toxicity and biodistribution of copper nanoparticles and cupric ions in rats. *International Journal of Nanomedicine,* 11, 2883-2900.

237. Lei, C., Zhang, L., Yang, K., Zhu, L., Lin, D., 2016. Toxicity of iron-based nanoparticles to green algae: Effects of particle size, crystal phase, oxidation state and environmental aging. *Environmental Pollution,* 218, 505-512.

238. Levine, R,L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S., Stadtman, E.R., 1990. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymolology,* 186, 464-478.

239. Li, J., Liu, X., Zhang, Y., Tian, F., Zhao, G., Yu, O., Jiang, F.I, Liu, Y., 2012. Toxicity of nano zinc oxide to mitochondria. *Toxicology Research,* 1, 137–144.

240. Li, L.Z., Zhou, D.M., Peijnenburg, W.J., van Gestel, C.A., Jin, S.Y., Wang, Y.J., Wang, P., 2011. Toxicity of zinc oxide nanoparticles in the earthworm, *Eisenia fetida* and subcellular fractionation of Zn. *Environment International,* 37(6), 1098-1104.

241. Li, M., Pokhrel, S., Jin, X., Madler, L., Damoiseaux, R., Hoek, E.M.V., 2011. Stability, bioavailability, and bacterial toxicity of ZnO and iron-doped ZnO nanoparticles in aquatic media. *Environmental Science and Technology,* 45(2), 755-761.

242. Li, Y., Wang, W., Wu, Q., Li, Y., Tang, M., Ye, B., Wang, D., 2012. Molecular control of TiO_2-NPs toxicity formation at predicted environmental relevant concentrations by Mn-SODs proteins. *PLoS One,* 7(9), e44688.

243. Li, Y., Yu, S., Wu, Q., Tang, M., Pu, Y., Wang, D., 2012. Chronic Al_2O_3-nanoparticle exposure causes neurotoxic effects on locomotion behaviors by inducing severe ROS production and disruption of ROS defense mechanisms in nematode *Caenorhabditis elegans*. *Journal of Hazardious Material*, 219 (220), 221-230.

244. Liao, V.H., Yu, C.W., Chu, Y.J., Li, W.H., Hsieh, Y.C., Wang, T.T., 2011. Curcumin-mediated lifespan extension in *Caenorhabditis elegans*. *Mechanism of Ageing Development,* 132(10), 480-487.

245. Lin, Y.G., Kunnumakkara, A.B., Nair, A., Merritt, W.M., Han, L.Y., Armaiz-Pena, G.N., Kamat, A.A., Spannuth, W.A., Gershenson, D.M., Lutgendorf, S.K., Aggarwal, B.B., Sood, A.K., 2007.

Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kappaB pathway. *Clinical Cancer Research,* 13(11), 3423-3430.

246. Lin, C., Choi, Y.S., Park, S.G., Gwon, L.W., Lee, J.G., Yon, J.M., Baek, I.J., Lee, B.J., Yun, Y.W., Nam, S.Y., 2016. Enhanced protective effects of combined treatment with β-carotene and curcumin against hyperthermic spermatogenic disorders in mice. *BioMed Research International,* 2016, 1-8.

247. Lin, X., Li J., Ma, S., Liu, G., Yang, K., Tong, M., Lin, D., 2014. Toxicity of TiO_2 nanoparticles to *Escherichia coli*: Effects of particle size, crystal phase and water chemistry. *PLoS One*, 9(10):e110247.

248. Lindberg, H.K., Falck, G.C., Suhonen, S., Vippola, M., Vanhala, E., Catalán, J., Savolainen, K., Norppa, H., 2009. Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanofibres in human bronchial epithelial cells *in vitro*. *Toxicology Letter,* 186(3), 166-173.

249. Linhua, 2009, Hao, L., Wang, Z., Xing, B., 2009. Effect of sub-acute exposure to TiO_2 nanoparticles on oxidative stress and histopathological changes in Juvenile Carp (*Cyprinus carpio*). *Journal of Environmental Science,* 21(10), 1459-1466.

250. Liu, J., Fan, D., Wang, L., Shi, L., Ding, J., Chen, Y., Shen, S., 2014. Effects of ZnO, CuO, Au, and TiO_2 nanoparticles on *daphnia magna* and early life stages of zebrafish *Danio rerio. Environment Protection Engineering*, 40(1), 139-149.

251. Liu, Y.K., Deng, X.X., Yang, H.L., 2016. Cytotoxicity and genotoxicity in liver cells induced by cobalt nanoparticles and ions. *Bone and Joint Research,* 5 (10), 461-469.

252. Liu, W., Xu, Z., Li, H., Guo, M., Yang, T., Feng, S., Xu, B., Deng, Y., 2017. Protective effects of curcumin against mercury-induced hepatic injuries in rats, involvement of oxidative stress antagonism, and Nrf2-ARE pathway activation. *Human and Experimental Toxicology,* 36(9), 949-966.

253. Lopes, S., Ribeiro, F., Wojnarowicz, J., Lojkowski, W., Jurkschat, K., Crossley, A., Soares, A.M., Loureiro, S., 2014. Zinc oxide nanoparticles toxicity to Daphnia magna: size-dependent effects and dissolution. *Environmental Toxicology and Chemistry,* 33(1), 190-198.

254. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chem*istry, 193(1), 265-275.

255. Lu, N., Yu, X., He, X., Zhou, Z., 2009. Detecting apoptotic cells and monitoring their clearance in the nematode *Caenorhabditis elegans. Methods in Molecular Biology,* 559, 357-370.

256. Lu, J.M., Lin, P.H., Yao, Q., Chen, C., 2010. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *Journal of Cellular and Molecular Medicine,* 14(4), 840-860.

257. Lushchak, V.I., 2012. Glutathione homeostasis and functions: Potential targets formedical interventions. *Journal of Amino Acids,* 2012, 1-26.

258. Lykkesfeldt, J., Michels, A.J., Frei, B., 2014. Vitamin C. *Advances in Nutrition,* 5(1), 16-18.

259. Ma, L., Liu, J., Li, N., Wang, J., Duan, Y., Yan, J., Liu, H., Wang, H., Hong, F., 2010. Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO_2 delivered to the abdominal cavity. *Biomaterials,* 31(1), 99-105.

260. Mackevica, A., Revilla, P., Brinch, A., Hansen, S.F., 2016. Current uses of nanomaterials in biocidal products and treated articles in the EU. *Environmental Science:Nano*, 3, 1195-1205.

261. Maglioni, S., Arsalan, N., Ventura, N., 2016. *C. elegans* screening strategies to identify pro-longevity interventions. *Mechanism of Ageing and Development,* 157, 60-69.

262. Mahmoud, A. M., Ahmed, O. M., Galaly, S. R., 2014. Thymoquinone and curcumin attenuate gentamicin induced renal oxidative stress, inflammation and apoptosis in rats. *Experimental and Clinical Sciences International Journal,* 13, 98-110.

263. Mamede, A.C., Pires, A.S., Abrantes, A.M., Tavares, S.D., Gonçalves, A.C., Casalta-Lopes, J.E., Sarmento-Ribeiro, A.B., Maia, J.M., Botelho, M.F., 2012. Cytotoxicity of ascorbic acid in a human colorectal adenocarcinoma cell line (WiDr): *In vitro* and *in vivo* studies. *Nutrition and Cancer*, 64(7), 1049-1057.

264. Mandil, R., Rahal, A., Prakash, A., Garg, S.K., Gangwar, N.K., Swain, D.K., 2016. Ameliorative potential of a-tocopherol against flubendiamide and copper-induced testicular-insult in Wistar rats. *Chemical Biology Interaction,* 260, 91-101.

265. Manju, M., Akbarsha, M.A., Oommen, O.V., 2012. *In vivo* protective effect of dietary curcumin in fish *Anabas testudineus* (Bloch). *Fish Physiology and Biochemistry*, 38(2), 309-318.

266. Manke, A., Wang, L., Rojanasakul, Y., 2013. Mechanisms of nanoparticle-induced oxidative stress and toxicity. *BioMed Research International,* 2013, 1-15.

267. Mansouri, E., Khorsandi, L., Orazizadeh, M., Jozi, Z., 2015. Dose-dependent hepatotoxicity effects of zinc oxide nanoparticles. *Nanomedicine Journal,* 2(4), 273-282.

268. Manzo, S., Buono, S., Rametta, G., Miglietta, M., Schiavo, S., Di Francia, G., 2015. The diverse toxic effect of SiO_2 and TiO_2 nanoparticles toward the marine microalgae *Dunaliella tertiolecta*. *Environmental Science and Pollution Research International,* 22(20), 15941-1551.

269. Masarovicova, E., Kralova, K., 2013. Metal nanoparticles and plants. *Ecological Chemistry and Engineerings,* 20, 9-22.

270. Mastrangelo, D., Massai, L., Fioritoni, G., Lo Coco, F., Nuti, R., 2016. The cure from nature: The extraordinary anticancer properties of ascorbate (vitamin C). *Journal of Integrative Oncology,* 5(1), 1-7.

271. Mates, J.M., 2000. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology,* 153(1-3), 83-104.

272. Mathews, V., Binu, P., Sauganth-Paul, M., Abhilash, M., Manju, A., Nair, R., 2012. Hepatoprotective efficacy of curcumin against arsenic trioxide toxicity. *Asian Pacific Journal of Tropical Biomedicine*, 2, S706-S711.

273. Maurer-Jones, M.A., Gunsolus, I.L., Murphy, C.J., Haynes, C.L., 2013. Toxicity of engineered nanoparticles in the environment. *Analytical Chemistry,* 85(6), 3036-3049.

274. Maynard, R.L., 2012. Nano-technology and nano-toxicology. *Emerging Health Threats Journal*, 5, 1-8.

275. Mazdeh, M., Rahiminejad, M.E., Nili-Ahmadabadi, A., Ranjbar, A., 2016. Neurological disorders and oxidative toxic stress: A role of metal nanoparticles. *Jundishapur Journal of Natural Pharmaceutical Product*, 11(1), e27628.

276. McCallum, K.C., Garsin, D.A., 2016. The Role of reactive oxygen species in modulating the *Caenorhabditis elegans* immuneresponse. *PLoS Pathogens,* 12(11), e1005923.

277. McWilliams, A., 2014. *Global markets for nanocomposites, nanoparticles, nanoclay and nanotubes*. https://www.bccresearch.com/market-research/nanotechnology/nanocomposites-market-nan021f.html [accessed 21 March 2016].

278. McWilliams, A., 2016. *Graphene: Technoligies, Applicaltion and Market.* https://www.bccresearch.com/ market-research/advanced-materials/graphene-technologies-applications-markets-report-avm075d.html [accessed 21 March 2016].

279. Meena, R., Kumar, S., Paulraj, R., 2015. Titanium oxide (TiO_2) nanoparticles in induction of apoptosis and inflammatory response in brain. *Journal of Nanoparticle Research*, 17, 1-14.

280. Mendes, R., Fernandes, A.R., Baptista, P.V., 2017. Gold nanoparticle approach to the selective delivery of gene silencing in cancer-The case for combined delivery? *Genes (Basel)*, 8(3), 1-16.

281. Mendez Medrano, M.G., Kowalska, E.K., Lehoux, A. Herissan, A., Ohtani, B., Bahena Uribe, D., Colbeau-Justin, C., Lopez, J., Remita, H., Briois, V., 2016. Surface modification of TiO_2 with Ag nanoparticles and CuO nanoclusters for application in photocatalysis. *Journal of Physical Chemistry C,* 120(9), 5143-5154.

282. Middendorf, P.J., Dusenbery, D.B., 1993. Fluoroacetic acid is a potent and specific inhibitor of reproduction in the nematode *Caenorhabditis elegans*. *Journal of Nematology*, 25(4), 573-577.

283. Minetto, D., Libralato, G., Volpi Ghirardini, A., 2014. Ecotoxicity of engineered TiO_2 nanoparticles to saltwater organisms: An overview. *Environment International,* 66, 18-27.

284. Miranda-Vizuete, A., Veal, E.A., 2017. *Caenorhabditis elegans* as a model for understanding ROS function in physiology and disease. *Redox Biology,* 11, 708-714.

285. Mishra, S., Palanivelu, K., 2008. The effect of curcumin (turmeric) on Alzheimer's disease: An overview. *Annals Indian Acadamy of Neurology,* 11(1), 13-19.

286. Miyasaka, T., Xie, C., Yoshimura, S., Shinzaki, Y., Yoshina, S., Kage-Nakadai, E., Mitani, S., Ihara, Y., 2016. Curcumin improves tau-induced neuronal dysfunction of nematodes. *Neurobiology Aging,* 39, 69-81.

287. Mohamed, H.R., Hussien, N.A., 2016. Genotoxicity studies of titanium dioxide nanoparticles (TiO_2 NPs) in the brain of mice. *Scientifica (Cairo),* 2016, 1-7.

288. Mohamed, W.A., Abd-Elhakim, Y.M., Farouk, S.M., 2016. Protective effects of ethanolic extract of rosemary against lead-induced hepato-renal damage in rabbits. *Experimantal and Toxicologic Pathology, 68 (8*), 451-461.

289. Morel, Y., Barouki, R., 1999. Repression of gene expression by oxidative stress. *Biochemical Journal,* 342(3), 481-496.

290. Murphy CT, Hu PJ. 2013. *Insulin/insulin-like growth factor signaling in C. elegans.* doi/10.1895/wormbook.

291. Mut-Salud, N., Alvarez, P. J., Garrido, J.M., Carrasco, E., Aranega, A., Rodriguez-Serrano, F., 2016. Antioxidant intake and antitumor therapy: Toward nutritional recommendations for optimal results. *Oxidative Medicine and Cellulae Longevity,* 2016, 1-19.

292. Naidu, K.A., 2003. Vitamin C in human health and disease is still a mystery? An overview. *Nutriton Journal,* 2(7), 1-10.

293. Naidu, K.A., Tang, J.L., Naidu, K.A., Prockop, L.D., Nicosia, S.V., Coppola, D., 2001. Antiproliferative and apoptotic effect of ascorbyl stearate in human glioblastoma multiforme cells: Modulation of insulin-like growth factor-I receptor (IGF-IR) expression. *Journal of Neurooncology*, 54 (1), 15-22.

294. National center for complementary and integrative research. 2013. *Antioxidants: In depth.* https://nccih.nih.gov/health/ antioxidants/introduction.htm#ususe [accessed 11 August 2016].

295. National Institute for Occupational Safety and Health. 2011. *Occupational Exposure to Titanium Dioxide. Current Intelligence Bulletin 63.* https://www.cdc.gov/niosh/docs/2011-160/pdfs/2011-160.pdf [accessed 12 May 2016].

296. Nelson, P.J., Pruitt, R.E., Henderson, L.L., Jenness, R., Henderson, L.M., 1981. Effect of ascorbic acid deficiency on the *in vivo* synthesis of carnitine. *Biochimica Biophysica Acta*, 672(1), 123-127.

297. Nemenqani, D., 2015. The ameliorating effect of vitamin C against cardiopulmonary toxicity of zinc oxide nanoparticals. *International Research Journal of Appllied and Basic Sciences*, 9, 1102-1109.

298. Nemenqani, D., El-gharib, O., Ahmed, A.M., Baiuomy, A.R., 2015. The protective effects of antioxidant (vitamin C) against hepatic oxidative damage induced by zinc oxide nanoparticals. *International Research Journal of Appllied and Basic Sciences,* 9, 502-509.

299. Nemmar, A., Yuvaraju, P., Beegam, S., Fahim, M.A., Ali B.H., 2017. Cerium oxide nanoparticles in lung acutely induce oxidative stress, inflammation, and DNA damage in various organs of mice. *Oxidative Medicine and Cellulae Longevity,* 2017, 1-12.

300. Ng, C.T., Yong, L.Q., Hande, M.P., Ong, C.N., Yu, L.E., Bay, B.H., Baeg, Y.H., 2017. Zinc oxide nanoparticles exhibit cytotoxicity and genotoxicity through oxidative stress responses in human lung fibroblasts and *Drosophila melanogaster. International Journal of Nanomedicine*, 12, 1621-1637.

301. Nimse, S.B., Pal, D., 2015. Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Advances,* 5, 27986-28006.

302. Nisar, N.A., Mudasir Sultana, H.A. Waiz, P.A. Para, N.A. Baba, F.A. Zargar and W.H. Raja, 2013. Experimental study on the effect of vitamin C administration on lipid peroxidation and antioxidant enzyme activity in rats exposed to chlorpyriphos and lead acetate. *Veterinary World,* 6(8), 461-466.

303. Nouara, A., Wu, Q., Li, Y., Tang, M., Wang, H., Zhao, Y., Wang, D., 2013. Carboxylic acid functionalization prevents the translocation 1 of multi-walled carbon nanotubes at predicted environmental relevant concentrations into targeted organs of nematode *Caenorhabditis elegans. Nanoscale,* 5(13), 6088-6096.

304. O'Donnell, B., Huo, L., Polli, J.R., Qiu, L., Collier, D.N., Zhang, B., Pan, X., 2017. From the cover: ZnO nanoparticles enhanced germ cell apoptosis in *Caenorhabditis elegans*, in comparison with $ZnCl_2$. *Toxicology Science,* 156(2), 336-343.

305. O'Reilly, L.P., Luke, C.J., Perlmutter, D.H., Silverman, G.A., Pak, S.C., 2014. *C. elegans* in high-throughput drug discovery. *Advance Drug Delivery Reviews*, 69, 247-253.

306. Oh, S.W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R.J., Tissenbaum, H.A., 2005. JNK regulates lifespan in *Caenorhabditis elegans* by modulating nuclear translocation of forkhead transcription factor/ DAF-16. *Proceedings of the National Academy of Sciences of the United States of America,* 102(12), 4494-4499.

307. Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry,* 95(2), 351-358.

308. Ohno, S., Ohno, Y., Suzuki, N., Soma, G., Inoue, M., 2009. High-dose vitamin C (Ascorbic Acid) therapy in the treatment of patients with advanced cancer. *Anticancer Research,* 29(3), 809-815.

309. Omar, H.E.D.M., Ibrahim, A.T., Magdy, M.A., Ahmed, E.A., 2016. The protective effects of zinc and vitamin E supplementation against kidney toxicity by lithium in rats. *European Journal of Biological Research,* 6 (1), 21-27.

310. Oukarroum, A., Barhoumi, L., Pirastru, L., Dewez, D., 2013. Silver nanoparticles toxicity effect on growth and cellular viability of the aquatic plant *Lemnagibba. Environmental Toxicology and Chemistry*, 32(4), 902-907.

311. Ozbakkaloglu, T., Chen, J.F., Smith, S.T., Dai, J.G., 2016. Applications of fiber reinforced polymer composites. *International Journal of Polymer Science,* 2016, 0-1.

312. Ozkaleli, M., Erdem, A., 2018. Biotoxicity of TiO_2 nanoparticles on *Raphidocelis subcapitata* microalgae exemplified by membrane deformation. *International Journal of Environmental Research and Public Health*, 15, 1-12.

313. Padayatty, S.J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J.H., Chen, S., Corpe, C., Dutta, A., Dutta, S.K., Levine, M., 2003. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. *Journal of American College of Nutrition,* 22 (1), 18-35.

314. Pakrashi, S., Dalai, S., Humayun, A., Chakravarty, S., Chandrasekaran, N., Mukherjee, A., 2013. *Ceriodaphnia dubia* as a potential bio-indicator for assessing acute aluminum oxide nanoparticles toxicity in fresh water environment. *PLoS One.,* 8(9): e74003.

315. Pallauf, K., Bendall, J.K., Scheiermann, C., Watschinger, K., Hoffmann, J., Roeder, T., Rimbach, G., 2013. Vitamin C and lifespan in model organisms. *Food and Chemical Toxicology,* 58, 255-263.

316. Pandurangan, M., Kim, H.D., 2015. *In vitro* toxicity of zinc oxide nanoparticles : A review. *Journal of Nanoparticle Research,* 17, 158-163.

317. Panieri, E., Santoro, M.M., 2016. ROS homeostasis and metabolism: a dangerous liason in cancer cells. *Cell Death and Diseases,* 7(6), e2253.

318. Parveen, N., Ahmad, S., Shadab, G.G.H.A., 2014. Iron induced genotoxicity: Attenuation by Vitamin C and its optimization. *Interdisciplinary Toxicology*, 7(3), 154-158.

319. Patel, V., 2011. *Global carbon nanotubes market-industry beckonsn.* Available at https://www.nanowerk.com/spotlight/spotid=23118.php [accessed 10 january 2015].

320. Pati, R., Das, I., Mehta, R.K., Sahu, R., Sonawane, A., 2016. Zinc-oxide nanoparticles exhibit genotoxic, clastogenic, cytotoxic and actin depolymerization effects by inducing oxidative stress responses in macrophages and adult mice. *Toxicology Sciences*, 150(2), 454-472.

321. Paunovic, M.G., Ognjanovic, B.I., Matic, M.M., Stajn, A.S., Saicic, Z.S., 2016. Protective effects of quercetin and vitamin C against nicotine-induced toxicity in the blood of Wistar rats. *The Journal of Institute for Medical Research and Occupational Health*, 67(4), 304-310.

322. Pavan, A.R., Silva, G.D., Jornada, D.H., Chiba, D.E., Fernandes, G.F.S., Man Chin, C., dos Santos, J.L., 2016. Unraveling the anticancer effect of curcumin and resveratrol. *Nutrients,* 8(11), 1-50.

323. Peng, C., Zhan,g W., Gao H., Li Y., Tong X., Li K., Zhu X., Wang Y., Chen Y., 2017. Behavior and potential impacts of metal-based engineered nanoparticles in aquatic environments. *Nanomaterials(Basel),* 7(1), 1-33.

324. Peng, X., Palma, S., Fisher, N.S., Wong, S.S., 2011. Effect of morphology of ZnO nanostructures on their toxicity to marine algae. *Aquatic Toxicology*, 102 (3-4), 186-196.

325. Petrarca, C., Clemente, E., Amato, V., Pedata, P., Sabbioni, E., Bernardini, G., Iavicoli, I., Cortese, S., Niu, Q., Otsuki, T., Paganelli, R., Di Gioacchino, M., 2015. Engineered metal based nanoparticles and innate immunity. *Clinical and Molecular Allergy*, 3(1), 1-12.

326. Piechulek, A., von Mikecz, A., 2017. Life span resolved nanotoxicology enables identification of age associated neuromuscular vulnerabilities in the nematode *Caenorhabditis elegans*. *Environmental Pollutution,* 233, 1095-1103.

327. Praetorius, A., Scheringer, M., Hungerbuhler, K., 2012. Development of environmental fate models for engineered nanoparticles- A case study of TiO_2 nanoparticles in the Rhine River. *Environmental Science and Technology,* 46(12), 6705-6713.

328. Prasad, R., Kumar, M., Trivedi, S.P., 2017. Antigenotoxic effect of turmeric powder extract curcumin against chromium trioxide induced genotoxicity in fish *Channa punctatus*. *Journal of Entomology and Zoology Studies,* 5(1), 89-94.

329. Qin, X., Cao, M., Lai, F., Yang, F., Ge, W., Zhang, X., Cheng, S., Sun, X., Qin, G., Shen, W., Li, L., 2015. Oxidative stress induced by zearalenone in porcine granulosa cells and its rescue by curcumin *in vitro*. *PLoS One*, 10(6), e0127551.

330. Qin, G., Tang, S., Li, S., Lu, H., Wang, Y., Zhao, P., Li, B., Zhang, J., Peng, L., 2017. Toxicological evaluation of silver nanoparticles and silver nitrate in rats following 28 days of repeated oral exposure. *Environmental Toxicology,* 32(2), 609-618.

331. Rahmouni, F., Hamdaoui, L., Badraoui, R., Rebai, T., 2017. Protective effects of Teucrium polium aqueous extract and ascorbic acid on hematological and some biochemical parameters against carbon tetrachloride (CCl_4) induced toxicity in rats. *Biomedicine Pharmacotherapy*, 91, 43-48.

332. Ramachandraiah, K., Han, S.G., Chin, K.B., 2015. Nanotechnology in meat processing and packaging: Potential applications - A review. *Asian-Australas Journal of Animal Sciences,* 28 (2), 290-302.

333. Ramsden, C.S., Smith, T.J., Shaw, B.J., Handy, R.D., 2009. Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (Oncorhynchus mykiss): no effect on growth, but subtle biochemical disturbances in the brain. *Ecotoxicology,* 18(7), 939-951.

334. Rasheed, N.A., Abdel Baky, N.A., Shebly, W., Ahmed, A.M., Faddah, L. M., 2012. Effect of vitamin E and α-lipoic acid on nano zinc oxide induced renal cytotoxicity in rats. *African Journal of Pharmacy and Pharmacology*, 6(29), 2211-2223.

335. Ratnasekhar, Ch., Sonane, M., Satish, A., Mudiam, M.K., 2015. Metabolomics reveals the perturbations in the metabolome of *Caenorhabditis elegans* exposed to titanium dioxide nanoparticles. *Nanotoxicology,* 9(8), 994-1004.

336. Ravindran, J., Prasad, S., Aggarwal, B.B., 2009. Curcumin and cancer cells: how many ways can curry kill tumor cells selectively? *The American Association of Pharmaceutical Scientists journal,* 11 (3), 495-510.

337. Ravi Ram, K., Ji, S., Wolfner, M.F., 2005. Fates and targets of male accessory gland proteins in mated female *Drosophila melanogaster*. *Insect Biochemistry and Molecular Biology*, 35(9), 1059–1071.

338. Reckziegel, P., Dias, V.T., Benvegnú, D.M., Boufleur, N., Barcelos, R.C.S., Segat, H.J., Pase, C.S., Dos Santos, C.M.M., Flores, E.M.M., Bürgerg, M.E., 2016. Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats. *Toxicology Reports,* 3, 351-356.

339. Research and Markets, 2011. T*he Global Market for Metal and Metal Oxide Nanoparticles 2010-2027.* Future market, Inc.

340. Research and Market, 2013. *The global market for metal oxide nanoparticles to 2020*. https://www.prnewswire.com/news-releases/the-global-market-for-metal-oxide-nanoparticles-to-2020-210803631.html [accessed 12 May 2016].

341. Rim, K.T., Song, S.W., Kim, H.Y., 2013. Oxidative DNA damage from nanoparticles exposure and its application to workers' health: A literature review. *Safety and Health at Work,* 4(4), 177-186.

342. Rizk, M.Z., Ali, S.A., Hamed, M.A., El-Rigal, N.S., Aly, H.F., Salah, H.H., 2017. Toxicity of titanium dioxide nanoparticles: Effect of dose and time on biochemical disturbance, oxidative stress and genotoxicity in mice. *Biomedicine Pharmacotherapy*, 90, 466-472.

343. Rocheleau, S., Arbour, M., Elias, M., Sunahara, G.I., Masson, L., 2014. Toxicogenomic effects of nano- and bulk-TiO_2 particles in the soil nematode *Caenorhabditis elegans*. *Nanotoxicology,* 9(4), 502-512.

344. Roco, M.C., 2011. The long view of nanotechnology development: The national nanotechnology initiative at 10 years. *Journal of Nanoparticle Research,* 13, 427-445.

345. Rogers, S., Rice, K.M., Manne, N.D., Shokuhfar, T., He, K., Selvaraj, V., Blough, E. R., 2015. Cerium oxide nanoparticle aggregates affect stress response and function in *Caenorhabditis elegans. SAGE Open Medicine,* 3, 1-9.

346. Roh, J.Y., Park, Y.K., Park, K., Choi, J., 2010. Ecotoxicological investigation of CeO_2 and TiO_2 nanoparticles on the soil nematode *Caenorhabditis elegans* using gene expression, growth, fertility, and survival as endpoints. *Environmental Toxicology and Pharmacology,* 29, 167-172.

347. Roh, J.Y., Park, Y.K., Choi, J., 2008. Ecotoxicological effects of CeO_2 nanoparticles on soil nematode *Caenorhabditis elegans. Journal of EnvironmentalToxicology,* 23(2), 87-91.

348. Roy, P., Giri, S., Mukherjee, A., 2014. Vitamin C pretreatment mitigates the genotoxic effects of sodium arsenite in human lymphocytes *in-vitro*. *Journal of Appllied Biology and Biotechnology*, 2 (4), 16-20.

349. Rudrappa, T., Bais, H.P., 2008. Curcumin, a known phenolic from Curcuma longa, attenuates the virulence of Pseudomonas aeruginosa PAO1 in whole plant and animal pathogenicity models. *Journal of Agriculture and Food Chemistry,* 56(6), 1955-1962.

350. Rui, Q., Zhao, Y., Wu, Q., Tang, M., Wang, D., 2013. Biosafety assessment of titanium dioxide nanoparticles in acutely exposed nematode *Caenorhabditis elegans* with mutations of genes required for oxidative stress or stress response. *Chemosphere,* 93(10), 2289-2296.

351. Sabat, D., Patnaik, A., Ekka, B., Dash, P., Mishra, M., 2016. Investigation of titania nanoparticles on behaviour and mechanosensory organ of *Drosophila melanogaster*. *Physiology Behavior,* 167, 76-85.

352. Sabella, S., Carney, R.P., Brunetti, V., Malvindi, M.A., Al-Juffali, N., Vecchio, G., Janes, S.M., Bakr, O.M., Cingolani, R., Stellacci, F., Pompa, P.P., 2014. A general mechanism for intracellular toxicity of metal-containing nanoparticles. *Nanoscale*, 6(12), 7052-7061.

353. Saber, T.M., Reda, M.A., 2016. Curcumin ameliorates mancozeb-induced neurotoxicity in rats. *Japanese Journal of Veterinary Research,* 64(2), S197-S202.

354. Saddick, S., Afifi, M., Abu Zinada, O.A., 2015. Effect of Zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zillii. Saudi Journal of Biological Sciences*, 24(7), 1672-1678.

355. Sadiq, I.M., Dalai, S., Chandrasekaran, N., Mukherjee, A., 2011. Ecotoxicity study of titania (TiO_2) NPs on two micro algae species: *Scenedesmus sp. and Chlorella sp. Ecotoxicology and Environment Safety,* 74(2011), 1180–1187.

356. Sahijpaul, Y., 2015. A review on recent developments in nano particle based cutting fluids in machining processes. *International Journal for Ressearch in Mechanical and civil Engineering*, 1(2), 1-3.

357. Sahu, D., Kannan, G.M., Tailang, M., Vijayaraghavan, R., 2016. *In vitro* cytotoxicity of nanoparticles : A comparison between particle size and cell type. *Journal of Nanoscience,* 2016, 1-9.

358. Sahu, D., Kannan, G.M., Vijayaraghavan, R., Anand, T., Khanum, F., 2013. Nanosized zinc oxide induces toxicity in human lung cells. *ISRN Toxicology,* 2013, 1-8.

359. Salem, M., Rohani, S., Gillies, E.R., 2014. Curcumin, a promising anti-cancer therapeutic: A review of its chemical properties, bioactivity and approaches to cancer cell delivery. *RSC Advances*, 2014, 4, 10815-10829.

360. Saliani, M., Jalal, R., Kafshdare Goharshadi, E., 2016. Mechanism of oxidative stress involved in the toxicity of ZnO nanoparticles against eukaryotic cells. *Nanomedicine Journal,* 3, 1-14.

361. San Miguel, S.M., Opperman, L.A., Allen, E.P., Zielinski, J.E., Svobod, K.K., 2 0 1 3. Antioxidant combinations protect oral fibroblasts against metal-induced toxicity. *Archives Oral Biology,* 58(3), 299-310.

362. Sangartit, W., Kukongviriyapan, U., Donpunha, W., Pakdeechote, P., Kukongviriyapan, V., Surawattanawan, P., Greenwald, S.E., 2014. Tetrahydrocurcumin protects against cadmium-induced hypertension, raised arterial stiffness and vascular remodeling in mice. *PLoS One,* 9(12), e114908.

363. Sanna, A. A., Maha, Z. R., Samy, A. A., Hebatallah, A. D., Mai, O. K., 2015. Role of potent antioxidants in regulation of SMAD-2 transcription and TGF-β1 signaling in nano sized titanium dioxide-induced oxidative injury in mice liver. *International Journal of Pharmacotherapy*, 5(1), 17-26.

364. Saptarshi, S.R., Feltis, B.N., Wright, P.F., Lopata, A.L., 2015. Investigating the immunomodulatory nature of zinc oxide nanoparticles at sub-cytotoxic levels *in vitro* and after intranasal instillation *in vivo*. *Journal of Nanobiotechnology*, 13(6), 1-11.

365. Saquib, Q., Al-Khedhairy, A.A., Siddiqui, M.A., Abou-Tarboush, F.M., Azam, A., Musarrat, J., 2012. Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells. *Toxicology In Vitro,* 26(2), 351-61.

366. Sardarodiyan, M., Sani, A.M., 2016. Natural antioxidants: sources, extraction and application in food systems. *Nutrition and Food Science,* 46 (3), 363-373.

367. Sarkar, A., Sil, P.C., 2014. Iron oxide nanoparticles mediated cytotoxicity via PI3K/AKT pathway: Role of quercetin. *Food and Chemical Toxicology,* 71, 106-115.

368. Sarkozi, K., Papp, A., Horvath, E., Mate, Z., Hermesz, E., Kozma, G., Zomborszki, Z.P., Kalomista, I., Galbacs, G., Szabo, A., 2017. Protective effect of green tea against neuro-functional alterations in rats treated with MnO_2 nanoparticles. *Journal of the Science of Food and Agriculture,* 97(6), 1717-1724.

369. Savoly, Z., Hracs, K., Pemmer, B., Streli, C., Zaray, G., Nagy, P.I., 2016. Uptake and toxicity of nano-ZnO in the plant-feeding nematode, *Xiphinema vuittenezi*: the role of dissolved zinc and nanoparticle-specific effects. *Environmental Science and Pollution Resesrch International,* 23(10), 9669-9678.

370. Scharf, A., Guhrs, K.H., von Mikecz, A., 2016.Anti-amyloid compounds protect from silica nanoparticle-induced neurotoxicity in the nematode *C. elegans*. *Nanotoxicology*, 10(4), 426-435.

371. Schieber, M., Chandel, N.S., 2014. ROS function in redox signaling and oxidative stress. *Current Biology*, 24(10), R453-462.

372. Schlich, K., Terytze, K., Hund Rinke, K., 2012. Effect of TiO_2 nanoparticles in the earthworm reproduction test. *Environmental Science Europe,* 24(5), 1-10.

373. Seitz, F., Lüderwald, S., Rosenfeldt, R.R., Schulz, R., Bundschuh, M., 2015. Aging of TiO_2 nanoparticles transiently increases their toxicity to the pelagic microcrustacean *Daphnia magna*. *PLoS One*, 10(5), e0126021.

374. Sewelam, N., Kazan, K., Schenk, P.M., 2016. Global Plant Stress Signaling: Reactive oxygen species at the cross-road. *Frontiers in Plant Science,* 7(187), 1-21.

375. Sha, B., Gao, W., Cui, X., Wang, L., Xu, F., 2015. The potential health challenges of TiO_2 nanomaterials. *Journal of Applied Toxicology,* 35(10), 1086-1101.

376. Shah, S.N., Shah, Z., Hussain, M., Khan, M., 2017. Hazardous effects of titanium dioxide nanoparticles in ecosystem. *Bioinorgnic Chemistry and Application*, 2017, 1-12.

377. Shahidi, F., Zhong, Y., 2010. Novel antioxidants in food quality preservation and health promotion. *European Journal of Lipid Science and Technology,* 112, 930-940.

378. Shalaby, S., Abdel-Latif, A., Farrag, A.R., 2018. Comparative toxicity of two nanoparticles and two insecticides against albino rats. *Research Journal of Environmental Sciences*, 12(1), 41-52.

379. Sharma, D.N., Bhattacharya, L., 2010. Role of some antioxidants on mercury chloride induced spermatogenesis in Swiss albino mice during prepubertal phase of life. *Indian Journal of Science Research,* 1, 19-25.

380. Sharma, B., Singh, S., Siddiqi, N.J., 2014. Biomedical implications of heavy metals induced imbalances in redox systems. *BioMed Research International,* 2014, 1-26.

381. Sharma, A., Sharma, S., Meena, M., 2016. Antioxidants in dentistry: A review. *EC Dental Science*, 3.4 (2016), 580-584.

382. Shaye, D.D., Greenwald, I., 2011. OrthoList: a compendium of *C. elegans* genes with human orthologs. *PLoS One*, 6(5), e20085.

383. Shi, H., Magaye, R., Castranova, V., Zhao, J., 2013. Titanium dioxide nanoparticles: A review of current toxicological data. *Particle and Fibre Toxicology,* 10(15), 1-33.

384. Shim, K.H., Hulme, J., Maeng, E.H., Kim, M.K., An, S.S., 2014. Analysis of zinc oxide nanoparticles binding proteins in rat blood and brain homogenate. *International Journal of Nanomedicine*, 9(Supp 2), 217-224.

385. Shin, S.W., Song, I.H., Um, S.H., 2015. Role of physicochemical properties in nanoparticle toxicity. *Nanomaterials(Basel),* 5(3), 1351-1365.

386. Shinde, A., Tsai, C., 2015. Toxicity mechanism in fetal lung fibroblast cells for multi-walled carbon nanotubes defined by chemical impurities and dispersibility. *Toxicology Research,* 5, 248-258.

387. Shrivastava, R., Raza, S., Yadav, A., Kushwaha, P., Flora, S.J., 2014. Effects of sub-acute exposure to TiO_2, ZnO and Al_2O_3 nanoparticles on oxidative stress and histological change s in mouse liver and brain. *Drug and Chemical Toxicology*, 37(3), 336-347.

388. Shukla, R.K., Sharma, V., Pandey, A.K., Singh, S., Sultana, S., Dhawan, A., 2011. ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. *Toxicology In Vitro,* 25(1), 231-241.

389. Siddiqui, M.A., Ahamed, M., Ahmad, J., Majeed Khan, M.A., Musarrat, J., Al-Khedhairy, A.A., Alrokayan, S.A., 2012. Nickel oxide nanoparticles induce cytotoxicity, oxidative stress and apoptosis in cultured human cells that is abrogated by the dietary antioxidant curcumin. *Food and Chemical Toxicology,* 50(3-4), 641-647.

390. Singal, A.K., Jampana, S.C., Weinman, S.A., 2011. Antioxidants as therapeutic agents for liver disease. *Liver International,* 31(10), 1432-1448.

391. Singh, R., Sharma, P., 2011. Hepatoprotective effect of curcumin on lindane-induced oxidative stress in male Wistar rats. *Toxicology International,* 18(2), 124-129.

392. Snousy, M.G., Zawrah, M.F., 2017. Nanoparticles Restrictions in environmental cleanup. *Nano Research and Application,* 3(1), 1-5.

393. Somayeh, B., Mohammad, F., 2014. Vitamin C can reduce toxic effects of nano zinc oxide. *International Research Journal of Biological Sciences,* 3(3), 65-70.

394. Sonane, M., Moin, N., Satish, A., 2017. The role of antioxidants in attenuation of *Caenorhabditis elegans* lethality on exposure to TiO_2 and ZnO nanoparticles. *Chemosphere*, 187, 240-247.

395. Song, G., Gao, Y., Wu, H., Hou, W., Zhang, C., Ma, H., 2012. Physiological effect of anatase TiO_2 nanoparticles on *Lemna minor*. *Environmental Toxicology and Chemistry*, 31(9), 2147-2152.

396. Song, S., Zhang, X., Wu, H., Han, Y., Zhang, J., Ma, E., Guo, Y., 2015. Molecular basis for antioxidant enzymes in mediating copper detoxification in the nematode *Caenorhabditis elegans*. *PLoS One,* 9(9), e107685.

397. Song, B., Zhang, Y., Liu, J., Feng, X., Zhou, T., Shao, L., 2016. Is neurotoxicity of metallic nanoparticles the cascades of oxidative stress? *Nanoscale Research Letter*, 11(1), 1-11.

398. Song, B., Zhou, T., Liu, J., Shao, L., 2016. Involvement of programmed cell death in neurotoxicity of metallic nanoparticles: Recentadvances and future perspectives. *Nanoscale Research Letter*, 11(1), 1-12.

399. Soni, D., Gandhi, D., Tarale, P., Bafana, A., Pandey, R.A., Sivanesan, S., 2017. Oxidative stress and genotoxicity of zinc oxide nanoparticles to pseudomonas species, human promyelocytic leukemic (hl-60), and blood cells. *Biological Trace Element Research*, 178(2), 218-227.

400. Soria, C., Coccini, T., De Simone, U., Marchese, L., Zorzoli, I., Giorgetti, S., Raimondi, S., Mangione, P.P., Ramat, S., Bellotti, V., Manzo, L., Stoppini, M., 2015. Enhanced toxicity of silver nanoparticles in transgenic *Caenorhabditis elegans* expressing amyloidogenic proteins. *Amyloid,* 22(4), 221-228.

401. Srinivas, A., Rao, P.J., Selvam, G., Murthy, P.B., Reddy, P.N., 2011. Acute inhalation toxicity of cerium oxide nanoparticles in rats. *Toxicology Lett*er*,* 205(2), 105-115.

402. Srivastava, V., Gusain, D., Sharma, Y.C., 2015. Critical review on the toxicity of some widely used engineered nanoparticles. *Indrustrial and Engineering Chemical Research,* 54, 6209-6233.

403. Srivastava, S., Pant, A., Trivedi, S., Pandey, R., 2016. Curcumin and β-caryophellene attenuate cadmium quantum dotsinduced oxidative stress and lethality in *Caenorhabditis elegans* modelsystem. *Environmetal Toxicology and Pharmacol*ogy, 42, 55-62.

404. Stark, W.J., Stoessel, P.R., Wohlleben, W., Hafner, A., 2015. Industrial applications of nanoparticles. *Chemical Society Reviews,* 44(16), 5793-5805.

405. Strambeanu, N., Demetrovici, L., Dragos, D., 2014. *Nanoparticles' Promises and Risks: Characterization, Manipulation, and Potential Hazards to Humanity and the.* New York CitySpringer International Publishing.

406. Subramani, T., Yeap, S.K., Ho, W.Y., Ho, C.L., Omar, A.R., Aziz, S.A., Rahman, N.M., Alitheen, N.B., 2014. Vitamin C suppresses cell death in MCF-7 human breast cancer cells induced by tamoxifen. *Journal of Cellular and Molecular Medicine,* 18(2), 305-313.

407. Suganthi, P., Murali, M., Bukhari, A.S., Mohamed, H.E.S., Basu, H., Singhal, R.K., 2015. Behavioural and histological variations in *Oreochromis mossambicus* after exposure to ZnO nanoparticles. *Internatiional Journal of Applied Research,* 1(8), 524-531.

408. Sun, T.Y., Gottschalk, F., Hungerbühler, K., Nowack, B., 2014. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environmental Pollution,* 185, 69-76.

409. Syama, S., Sreekanth, P.J., Varma, H.K., Mohanan, P.V., 2014. Zinc oxide nanoparticles induced oxidative stress in mouse bone marrow mesenchymal stem cells. *Toxicology Mechanism amd Methods,* 24(9), 644-653.

410. Szymanska, R., Pospisil, P., Kruk, J., 2016. Plant-derived antioxidants in disease prevention. *Oxidative Medicine and Celullular Longevity,* 2016, 1-2.

411. Tagliafierro, L., Officioso, A., Sorbo, S., Basile, A., Manna, C., 2015. The protective role of olive oil hydroxytyrosol against oxidative alterations induced by mercury in human erythrocytes. *Food and Chemical Toxicology,* 82, 59-63.

412. Tawfik, S.S., Abouelella, A.M., Shahein, Y.E., 2013. Curcumin protection activities against γ-Rays-induced molecular and biochemical lesions. *BMC Research Notes*, 6(375), 1-10.

413. Taylor, N.S., Merrifield, R., Williams, T.D., Chipman, J. K., Lead, J. R., Viant, M. R., 2016. Molecular toxicity of cerium oxide nanoparticles to the freshwater alga *Chlamydomonas reinhardtii* is associated with supra-environmental exposure concentrations. *Nanotoxicology*, 10(1), 32-41.

414. Thakkar, M., Mitra, S., Wei, L., 2016. Effect on growth, photosynthesis, and oxidative stress of single walled carbon nanotubes exposure to marine alga *Dunaliella tertiolecta*. *Journal of Nanomaterials,* 2016, 1-17.

415. Tian, J.H., Hu, J.S., Li, F.C., Ni, M., Li, Y.Y., Wang, B.B., Xu, K.Z., Shen, W.D., Li, B., 2016. Effects of TiO_2 nanoparticles on nutrition metabolism in silkworm fat body. *Biology Open,* 5(6), 764-769.

416. Titus, D., Samuel, E.J., Mohana Roopan, S., 2016. Current scenario of biomedical aspect of metal-based nanoparticles on gel dosimetry. *Applied Microbiology and Biotechnology,* 100(11), 4803-4816.

417. Tiwari, M., Sharma, N.C., Fleischmann, P., Burbage, J., Venkatachalam, P., Sahi, S.V., 2017. Nanotitania exposure causes alterations in physiological, nutritional and stress responses in tomato (*Solanum lycopersicum*). *Frontiers in Plant Sciences,* 8, 1-12.

418. Tizabi, Y., Hurley, L.L., Qualls, Z., Akinfiresoye, L., 2014. Relevance of the anti-inflammatory properties of curcumin in neurodegenerative diseases and depression. *Molecules,* 19(12), 20864-20879.

419. Trouiller, B., Reliene, R., Westbrook, A., Solaimani, P., Schiestl, R.H., 2009. Titanium dioxide nanoparticles induce DNA damage and genetic instability *in vivo* in mice. *Cancer Research,* 69(22), 8784-8789.

420. Trujillo, J., Chirino, Y., Molina-Jijon, E., Anderica-Romero, A.C., Tapia, E., Pedraza-Chaverri, J., 2013. Renoprotective effect of the antioxidant curcumin: Recent findings. *Redox Biology*, 1, 448-456.

421. Truppi, A., Petronella, F., Placido, T., Striccoli, M., Agostiano, A., Curri, M., Comparelli, R., 2017. Visible-light-active TiO_2-based hybrid nanocatalysts for environmental applications. *Catalysts*, 7(4), 100, 1-33.

422. Tsalik, E.L., Hobert, O., 2003. Functional mapping of neurons that control locomotory behavior in *Caenorhabditis elegans. Journal of Neurobiology,* 56(2), 178-197.

423. Turkez, H., Sonmez, E., Aydin, E., 2016. Choosing the right antioxidant supplement for protecting liver from toxicity of engineered nanoparticles : A comprehensive *in vitro* screening. *Applied Mechanics and Materials*, 835, 57-62.

424. Tvermoes, B.E., Boyd, W.A., Freedman, J.H., 2010. Molecular characterization of *numr-1* and *numr-2:* genes that increase both resistance to metal-induced stress and lifespan in *C. elegans*. *Journal of Cell Science*, 123(Pt 12), 2124-2134.

425. Uikey, P., Vishwakarma, K., 2016. Review of zinc oxide (ZnO) nanoparticles applications and properties. *International Journal of Emerging Technology in Computer Sciences and Electronics*, 21(2), 239-242.

426. Undre, S.B., Pandya, S.R., Kumar, V., Singh, M., 2016. Dendrimers as smart materials for developing the various applications in the field of biomedical sciences. *Advanced Materials Letter,* 7(7), 502-516.

427. Unnikrishnan, M.K., Rao, M.N., 1995. Inhibition of nitrite induced oxidation of hemoglobin by curcuminoids. *Pharmazie,* 50(7), 490-492.

428. Usatii, A., Chiseliţa, N., Efremova, N., 2016. The evaluation of nanoparticles ZnO and TiO_2 effects on *Saccharomyces cerevisiae* CNMN-Y-20 yeast strain. *Acta University Cibiniensis. Series E: Food Technology,* 20(1), 85-92.

429. van der Reest, J., Gottlieb, E., 2016. Anti-cancer effects of vitamin C revisited. *Cell Research,* 26(3), 269–270.

430. Vance, M.E., Kuiken, T., Vejerano, E.P., McGinnis, S.P., Hochella, M.F.Jr., Rejeski, D., Hull, M.S., 2015. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein Journal of Nanotechnology,* 6, 1769-1780.

431. Vasantharaja, D., Ramalingam, V., Aadinaath Reddy, G., 2015. Oral toxic exposure of titanium dioxide nanoparticles on serum biochemical changes in adult male Wistar rats. *Nanomedicine Journal,* 2(1), 46–53.

432. Vergauwen, H., Tambuyzer, B., Jennes, K., Degroote, J., Wang, W., De Smet, S., Michiels, J., Van Ginneken, C., 2015. Trolox and ascorbic acid reduce direct and indirect oxidative stress in the IPEC-J2 cells, an *in vitro* model for the porcine gastrointestinal tract. *PLoS One.,* 10(3), e0120485.

433. Vicario-Pares, U., Castanaga, L., Lacave, J. M., Oron, M., Reip, P., Berhanu, D., Valsami-Jones, E., Cajaraville, M.P., Orbea, A., 2014. Comparative toxicity of metal oxide nanoparticles (CuO, ZnO and TiO_2) to developing *zebrafish embryos*. *Journal of Nanoparticle Research,* 16, 1-16.

434. Vigneshkumar, B., Pandian, S.K., Balamurugan, K., 2011. Catalase activity and innate immune response of *Caenorhabditis elegans* against the heavy metal toxin lead. *Environment Toxicology,* 28(6), 313-321.

435. Villanueva, C., Kross, R.D., 2012. Antioxidant-induced stress. *International Jourrnal of Molecular Sciences*, 13(2), 2091-2109.

436. Vinardell, M.P., Llanas, H., Marics, L., Mitjans, M., 2017. *In vitro* comparative skin irritation induced by nano and non-nano zinc oxide. *Nanomaterials (Basel),* 7(3), 1-8.

437. Walczynska, M., Jakubowski, W., Wasiak, T., Kadziola, K., Bartoszek, N., Kotarba, S., Siatkowska, M., Komorowski, P., Walkowiak, B., 2018. Toxicity of silver nanoparticles, multiwalled carbon nanotubes, and dendrimers assessed with multicellular organism *Caenorhabditis elegans*. *Toxicology Mechanism and Methods*, 28(6), 432-439.

438. Wang, J., Fan, Y., 2014. Lung injury induced by TiO_2 nanoparticles depends on their structural features: size, shape, crystal phases, and surface coating. *International Journal of Molecular Sciences,* 15(12), 22258-22278.

439. Wang, C., Lu, J., Zhou, L., Li, J., Xu, J., Li, W., Zhang, L., Zhong, X., Wang, T., 2016. Effects of long-term exposure to zinc oxide nanoparticles on development, zinc metabolism and biodistribution of minerals (Zn, Fe, Cu, Mn) in mice. *PLoS One.,* 11(10), e0164434.

440. Wang, J., Sun, P., Bao, Y., Dou, B., Song, D., Li, Y., 2012. Vitamin E renders protection to PC12 cells against oxidative damage and apoptosis induced by single-walled carbon nanotubes. *Toxicology In Vitro*, 26(1), 32-41.

441. Wang, J., Deng, X., Zhang, F., Chen, D., Ding, W., 2014. ZnO nanoparticle-induced oxidative stress triggers apoptosis by activating JNK signaling pathway in cultured primary astrocytes. *Nanoscale Research Letter,* 9(1), 1-12.

442. Wang, H., Wick, R.L., Xing, B., 2009. Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode *Caenorhabditis elegans*. *Environment Pollution,* 157 (4), 1171-1177.

443. Wang, J., Wang, L., Fan, Y., 2016. Adverse biological effect of TiO_2 and hydroxyapatite nanoparticles used in bone repair and replacement. *International Journal of Molecular Sciences,* 17(6), 1-14.

444. Wang, Y., Cui, H., Zhou, J., Li, F., Wang, J., Chen, M., Liu, Q., 2014. Cytotoxicity, DNA damage, and apoptosis induced by titanium dioxide nanoparticles in human non-small cell lung cancer A549 cells. *Environmental Science and Pollution Research International,* 22(7), 5519-5530.

445. Watson, E., MacNeil, L.T., Ritter, A.D., Yilmaz, L.S., . Rosebrock, A.P., Caudy, A.A., Walhout, A.J.M., 2014. Interspecies systems biology uncovers metabolites affecting *C. elegans* gene expression and life history traits. *Cell,* 156(4), 759-770.

446. Wehmas, L.C., Anders, C., Chess, J., Punnoose, A., Pereira, C.B., Greenwood, J.A., Tanguay, R.L., 2015. Comparative metal oxide nanoparticle toxicity using embryonic *zebrafish*. *Toxicology Reports,* 2, 702-715.

447. Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., von Goetz, N., 2012. Titanium dioxide nanoparticles in food and personal care products. *Environmental Science and Technology,* 46(4), 2242-2250.

448. Williams, D., 2014. *Advanced ceramics and nanoceramic powders*. https://www.bccresearch.com/market-research/nanotechnology/advanced-ceramics-nanoceramic-powders-nan015g.html [accessed 22 May 2015].

449. Wu, Q., Nouara, A., Li, Y., Zhang, M., Wang, W., Tang, M., Ye, B., Ding, J., Wang, D., 2013. Comparison of toxicities from three metal oxide nanoparticles at environmental relevant concentrations in nematode *Caenorhabditis elegans*. *Chemosphere*, 90 (3), 1123-1131.

450. Wu, Q, Zhao, Y, Li, Y, Wang, D., 2014. Susceptible genes regulate the adverse effects of TiO_2-NPs at predicted environmental relevant concentrations on nematode *Caenorhabditis elegans*. *Nanomedicine*, 10(6), 1263-1271.

451. Xiang, L., Nakamura, Y., Lim, Y.M., Yamasaki, Y., Kurokawa-Nose, Y., Maruyama, W., Osawa, T., Matsuura, A., Motoyama, N., Tsuda, L., 2011. Tetrahydrocurcumin extends life span and inhibits the oxidative stress response by regulating the FOXO forkhead transcription factor. *Aging (Albany NY)*, 3(11), 1098-1109.

452. Xie, Y., Wang, Y., Zhang, T., Ren, G., Yang, Z., 2012. Effects of nanoparticle zinc oxide on spatial cognition and synaptic plasticity in mice with depressive-like behaviors. *Journal of Biomedical Sciences,* 19(1), 1-11.

453. Xiong, D., Fang, T., Yu, L., Sima, X., Zhu, W., 2011. Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. *Science of Total Environment,* 409(8), 1444-1452.

454. Xiong, Z.E., Dong, W.G., Wang, B.Y., Tong, Q.Y., Li, Z.Y., 2015. Curcumin attenuates chronic ethanol-induced liver injury by inhibition of oxidative stress via mitogen-activated protein kinase/nuclear factor E2-related factor 2 pathway in mice. *Pharmacognosy Magazine*, 11(44), 707–715.

455. Yan, Y., Warren, S.C., Fuller, P., Grzybowski, B.A., 2016. Chemoelectronic circuits based on metal nanoparticles. *Nature Nanotechnology,* 11(7), 603-608.

456. Yang, X.X., Li, C.M., Huang, C.Z., 2016. Curcumin modified silver nanoparticles for highly efficient inhibition of respiratory syncytial virus infection. *Nanoscale,* 8(5), 3040-3048.

457. Yang, X., Jiang, C., Hsu-Kim, H., Badireddy, A. R., Dykstra, M., Wiesner, M., Hinton, D.E., Meyer, J.N., 2014. Silver nanoparticle behavior, uptake, and toxicity in *Caenorhabditis elegans*: Effects of natural organic matter. *Environmental Science and Technology*, 48(6), 3486-3495.

458. Yang, H.C., Chen, T.L., Wu, Y.H., Cheng, K.P., Lin, Y.H., Cheng, M.L., Ho, H.Y., Lo, S.J., Chiu, D.T., 2013. Glucose 6-phosphate dehydrogenase deficiency enhances germ cell apoptosis and causes defective embryogenesis in *Caenorhabditis elegans*. *Cell Death and Diseases,* 4(5), 1-10.

459. Yang, H., Liu, C., Yang, D., Zhang, H., Xi, Z., 2009. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. *Journal of Applied Toxicol*ogy, 29(1), 69-78.

460. Yang, X., Shao, H., Liu, W., Gu, W., Shu, X., Mo, Y., Chen, X., Zhang, Q., Jiang, M., 2015. Endoplasmic reticulum stress and oxidative stress are involved in ZnO nanoparticle-induced hepatotoxicity. *Toxicology Letter*, 234(1), 40-99.

461. Yin, N., Yao, X., Zhou, Q., Faiola, F., Jiang, G., 2015. Vitamin E attenuates silver nanoparticle-induced effects on body weight and neurotoxicity in rats. *Biochemical and Biophysical Research Communications*, 458(2), 405-410.

462. Yoon, S.J., Kwak, J.I., Lee, W.M., Holden, P.A., An, Y.J., 2014. Zinc oxide nanoparticles delay soybean development: a standard soil microcosm study. *Ecotoxicology and Environmental Safety,* 100, 131-137.

463. Younes, N.R., Amara, S., Mrad, I., Ben-Slama, I., Jeljeli, M., Omri, K., El Ghoul, J., El Mir, L., Rhouma, K.B., Abdelmelek, H., Sakly, M., 2015. Subacute toxicity of titanium dioxide (TiO_2) nanoparticles in male rats: Emotional behavior and pathophysiological examination. *Environmental Science and Pollution Research Internationl,* 22(11), 8728-8737.

464. Youngman, M.J., Rogers, Z.N., Kim, D.H., 2011. A Decline in p38 MAPK signaling underlies immunosenescence in *Caenorhabditis elegans*. *PLoS Genetics*, 7(5), e1002082.

465. Yousef, J.M., Mohamed, A.M., 2015. Prophylactic role of B vitamins against bulk and zinc oxide nanoparticles toxicity induced oxidative DNA damage and apoptosis in rat livers. *Pakistan Journal of Pharmaceutical Sciences,* 28(1), 175-184.

466. Yu, C.W., Liao, V.H., 2014. Arsenite induces neurotoxic effects on AFD neurons via oxidative stress in *Caenorhabditis elegans*. *Metallomics*, 6(10), 1824-1831.

467. Yu, C.W., Wei, C.C., Liao, V. H., 2014. Curcumin-mediated oxidative stress resistance in *Caenorhabditis elegans* is modulated by *age-1, akt-1, pdk-1, osr-1, unc-43, sek-1, skn-1, sir-2.1*, and *mev-1*. *Free Radical Research,* 48(3), 371-379.

468. Yu, Q., Liu, Z., Xu, H., Zhang, B., Zhanga, M., Mingchun, L., 2015. TiO_2 nanoparticles promote the production of unsaturated fatty acids (UFAs) fighting against oxidative stress in *Pichia pastoris*. *RSC Advances*, 5, 41033-41040.

469. Yuan, W., Zhang, Y., Cheng, L., Wu, H., Zheng, L., Zhao, D., 2016. The applications of carbon nanotubes and graphene in advanced rechargeable lithium batteries. *Jiurnal of Materials Chemistry A.,* 4 (23), 8932-8951.

470. Zafar, H., Ali, A., Ali, J.S., Haq, I.U., Zia, M., 2016. Effect of ZnO nanoparticles on *Brassica nigra* seedlings and stem explants: Growth dynamics and antioxidative response. *Frontiers in Plant Science,* 7, 1-8.

471. Zayerzadeh, E., Fardipour, A., Shabanian, M., Koohi, M.K., 2016. Evaluation of cardiopulmonary toxicity following oral administration of multi-walled carbon nanotubes in Wistar rats. *Nanomedicine Ressearch Journal,* 1(1), 47-51.

472. Zhai, Q., Narbad, A., Chen, W., 2015. Dietary strategies for the treatment of cadmium and lead toxicity. *Nutrients,* 7(1), 552-571.

473. Zhang, H., He, X., Zhang, Z., Zhang, P., Li, Y., Ma, Y., Kuang, Y., Zhao, Y., Chai, Z., 2011. Nano-CeO_2 exhibits adverse effects at environmental relevant concentrations. *Environmental Science and echnology,* 45(8), 3725-3730.

474. Zhang, J., Wang, X., Vikash, V., Ye, Q., Wu, D., Liu, Y., Dong, W., 2016. ROS and ROS-mediated cellular signaling. *Oxidative Medicine and Cellular Longevity,* 2016, 1-18.

475. Zhao, Y., Jia, R., Qiao, Y., Wang, D., 2016. Glycyrrhizic acid, active component from glycyrrhizae radix, prevents toxicity of graphene oxide by influencing functions of microRNAs in nematode *Caenorhabditis elegans*. *Nanomedicine*, 12(3), 735-744.

476. Zhao, Y.L., Wu, Q.L., Li, Y.P., Wang, D.Y., 2013. Translocation, transfer, and *in vivo* safety evaluation of engineered nanomaterials in the non-mammalian alternative toxicity assay model of nematode *Caenorhabditis elegans. RSC Advances*, 3, 5741-5757.

477. Zhao, Y., Zhi, L., Wu, Q., Yu, Y., Sun, Q., Wang, D., 2016. p38 MAPK-SKN-1/Nrf signaling cascade is required for intestinal barrier against graphene oxide toxicity in *Caenorhabditis elegans*. *Nanotoxicology*, 10(10), 1469-1479.

478. Zhu, X., Tian, S., Cai, Z., 2012. Toxicity assessment of iron oxide nanoparticles in zebrafish (*Danio rerio*) early life stages. *PLoS One,* 7(9), e46286.

479. Zhu, X., Chang, Y., Chen, Y., 2010. Toxicity and bioaccumulation of TiO_2 nanoparticle aggregates in *Daphnia magna*. *Chemosphere,* 78(3), 209-215.

480. Zijno, A., De Angelis, I., De Berardis, B., Andreoli, C., Russo, M.T., Pietraforte, D., Scorza, G., Degan, P., Ponti, J., Rossi, F., Barone, F., 2015. Different mechanisms are involved in oxidative DNA damage and genotoxicity induction by ZnO and TiO_2 nanoparticles in human colon carcinoma cells. *Toxicology In Vitro*, 29(7), 1503-1512.

Annexure I
List of the publication and Paper presented

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List of publications and papers presented

Publications:

1. **Madhavi Sonane**, Nida Moin, Aruna Satish **(2017)**. **The role of antioxidants in attenuation of *Caenorhabditis elegans* lethality on exposure to TiO_2 and ZnO nanoparticles.** ***Chemosphere***, 187(2017): 240–247, doi:10.1016/j.chemosphere.2017.08.080.

IF. 4.506

2. Priyanka Khare, **Madhavi Sonane**, Yogendra Nagar, Nida Moin, Shakir Ali, Kailash C. Gupta and Aruna Satish **(2015)**. **Size dependent toxicity of zinc oxide nano-particles in soil nematode *Caenorhabditis elegans*.** ***Nanotoxicology***, 9(4): 423–432, doi: 10.3109/17435390.2014.940403. **IF. 6.428**

3. Ratnasekhar Ch, **Madhavi Sonane**, Aruna Satish, Mohana Krishna Reddy Mudiam **(2015)**. **Metabolomics reveals the perturbations in the metabolic profiles of *Caenorhabditis elegans* exposed to titanium dioxide nanoparticles.** ***Nanotoxicology***, 9(8): 994–1004, doi: 10.3109/17435390.2014.993345. **IF. 6.428**

4. **Madhavi Sonane,** Ritu Goyal, Debopratim K. Chowdhuri, Kristipati Ravi Ram and Kailash C. Gupta **(2013)**. **Enhanced efficiency of P-element mediated transgenesis in Drosophila: Microinjection of DNA complexed with nanomaterial.** ***Scientific Reports***, 3(3408), 1-5, DOI: 10.1038/srep03408. **IF. 4.259**

5. Priyanka Khare, **Madhavi Sonane**, Rakesh Pandey, Shakir Ali, Kailash C. Gupta and Aruna Satish **(2011)**. **Adverse effects of TiO_2 and ZnO nanoparticles in soil nematode, *Caenirhabditis elegans*.** ***Jounal of biomedical nanotechnology***, 7(1), 116-117.

IF. 4.521

Conferences:

Abstracts

1. Yogendra Nagar, **Madhavi Sonane** and Aruna Satish. "**Toxicity assessment of parabens in soil nematode *Caenorhabditis elegans".*** Presented in Annual conference of society of toxicology, Nov 19th-21st, 2015, held at SVS medical college, Hyderabad, India.

2. Priyanka Khare, **Madhavi Sonane**, Rakesh Pandey, Shakir Ali, Kailash Chand Gupta and Aruna Satish. "Adverse effects of TiO_2 and ZnO nanoparticles in soil nematode, *Caenorhabditis elegans*" Presented in International symposium on the safe use of Nanomaterials & workshop on Nanomaterial safty: status, procedures, policy and ethical concern, Feb 1st-3rd, 2011, Lucknow, India.
3. Priyanka Khare, **Madhavi Sonane**, Kailash Chand Gupta and Aruna Satish. "Gene expression studies to evaluate the size releted toxixity of ZnO nanoparticles on the soil nematode, *Caenorhabditis elegans*" Presented in 19th International *C. elegans* Meeting June 26th -30th, 2013 at the University of California, Los Angeles.

Posters presented

4. **Madhavi Sonane,** Aruna Satish. "**Protective role of ascorbic acid in amelioration of titanium dioxide nanoparticles-induced toxicity in *Caenorhabditis elegans".*** Presented in International toxicology conclave, Nov 5th-6th, 2015, held at CSIR-Indian Institute of Toxicology Research Lucknow, India.
5. Yogendra Nagar, **Madhavi Sonane** and Aruna Satish."**Toxicity assessment of parabens in soil nematode *Caenorhabditis elegans".*** Presented in Annual conference of society of toxicology, Nov 19th-21st, 2015, held at SVS medical college, Hyderabad, India.

6. **Madhavi Sonane** , Aruna Satish, K. C. Gupta, and Rishi Shanker. "**Adverse effect of ZnO NPs in soil nematode, *Caenorhabditis elegans".*** Presented in XXXI annual conference of Society Of Toxicology, [STOX] India & Symposium on Current trends in Environmental Toxicology during Dec 22nd- 24th, 2011, Jaipur, india.

7. Priyanka Khare, **Madhavi Sonane**, Rakesh Pandey, Shakir Ali, Kailash Chand Gupta and Aruna Satish. "**Adverse effects of TiO_2 and ZnO nanoparticles in soil nematode, *Caenorhabditis elegans*"** Presented in International symposium on the safe use of Nanomaterials & workshop on Nanomaterial safty: status, procedures, policy and ethical concern during Feb 1st-3rd, 2011, Lucknow, India.
8. Priyanka Khare, **Madhavi Sonane**, Kailash Chand Gupta and Aruna Satish. **"Gene expression studies to evaluate the size releted toxixity of ZnO nanoparticles on the soil nematode, *Caenorhabditis elegans*".** Presented in 19th International *C. elegans* Meeting June 26th -30th, 2013 at the University of California, Los Angeles.

Conferences attended

1) **1st International Toxicology Conclave** organised by CSIR- Indian Institute of Toxicology Research, Lucknow, Nov 5th-6th, 2015.
2) **XXXI annual conference of Society Of Toxicology**, [STOX] India & Symposium on Current trends in Environmental Toxicology, Dec 22nd- 24th, 2011.

Workshops Attended

- **Internatinal workshop on comprehensive toxicology**, Depatment of pharmacology and toxicology, Veternity College, Begalure, July 27th to 31st, 2015.
- Bioinformatics workshop on **Genomic Data Analysis** from Bioinformatics sub-centre, D.A.V.V. Indore (M.P.), Oct 29th to Nov 2nd, 2007.

Awards and Distinction

- **Consolation price for Poster Presentation** at XXXI annual conference of Society Of Toxicology, [STOX] India & Symposium on Current trends in Environmental Toxicology, Dec 22nd- 24th, 2011.