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Review Article

Recent Advances on Genotoxicity of Engineered Nanoparticles

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Abstract

Industrial, commercial, agricultural and medical practices in the new millennium have been reported to employ engineered nanoparticles or nanomaterials at a large scale. They are widely used in plastic ware, cosmetics, clothing, electrical appliances, medicines and food products. They are generally classified as carbon based materials, metal based materials, dendrimers and composites. With the increasing development of nanotechnology, demand for nanosafety and health risk assessment studies has also increased. In recent years, several methods have been developed to study their cytotoxicity and genotoxicity. Nano-genotoxicity testing employs *in vitro* and *in vivo* approaches viz. comet assay, micronucleus test, 8 –hydroxy deoxyguanosine (8-OHdG) analysis and Ames test. Genotoxic mechanisms of nanoparticles have been mainly attributed to reactive oxygen species and oxidative stress.

Present review discusses recent researches made to record the genotoxicity of carbon nanotubes, fullerenes, silver and gold nanoparticles, titanium dioxide nanoparticles, quantum dots, silica nanoparticles and zinc oxide nanoparticles using *in vitro* and *in vivo* models. Perspectives of using alternate models in genotoxicity testing have also been discussed.

***Keywords**: Genotoxicity; DNA Damage; Nanoparticles; Carbon Nanotubes; Quantum Dots*

Introduction

Nanoparticles or nanomaterials are now being widely used in diverse sectors viz. agriculture, commerce, cosmetics, electronics, energy, industry and medicine [1]. Economic benefits received from nanotechnology, according to an estimate, reached $ 1 trillion in 2012 [2]. Prevalence of these materials in environment has also increased exponentially [3]. As such, growing production and use of nanomaterials, in conjunction with their unique physicochemical properties and the possible risk of unwanted exposures, renders them to be hazardous from human and ecosystem heath point of view. The paucity of toxicological information and lack of standardized protocols make the health risk assessment of NPs, a difficult venture [4,5].

Dire need to understand their adverse biologic effects has been emphasized in earlier reports [6,7]. By virtue of their small size, NPs are translocated into the circulatory and lymphatic systems, tissues and organ systems. Ultimately, NPs are able to enter cells and interact with organelles like nucleus, mitochondria, endoplasmic reticulum etc. NPs can penetrate cells through passive endocytosis initiated

by Van der Waals forces, electrostatic charges, steric interactions or interfacial tension without forming any vesicles [8,9]. Very small nanoparticles can penetrate cells via mechanisms different than phagocytosis probably through ion channels or pores in the cell membrane [10]. After uptake, nanoparticles are not necessarily located within a phagosome albeit non-phagocytic uptake can facilitate their entry in outer cell membrane and cytoplasm [11], mitochondria [12], lipid vesicles [13] or within the nucleus [12]. It is their bioaccumulation/bioconcentration in the cell that manifests into diverse cell death signalling pathways.

Mechanistic paradigms of cell death caused by NPs include, the generation of reactive oxygen species (ROS), oxidative stress, modulation of intracellular calcium concentration, activation of transcription factors and cytokine secretion [10]. ROS damage cellular lipids, proteins and DNA altering signalling pathways and gene transcription factors [14]. Oxidative stress occurs due to their effects on cell respiration, metabolism, ischemia/reperfusion and inflammation [15].

Conversely, oxidative stress induced by nanoparticles may be the result of reactive oxygen species; altered mitochondrial function and/or activation of inflammatory cells. NPs are known to generate higher amount of ROS than the bulk particles due to their higher surface area [16]. ROS can overwhelm the cell by shifting redox balance and destroy the biomolecules i.e. DNA leading to heritable mutations [8]. *In vitro* and *in vivo* experimental studies show that nanoparticles express xenotoxic effects in man and animals. Present review discusses these effects caused especially by engineered nanoparticles (ENPs).

Genotoxicity caused by carbon nanotubes

Carbon nanotubes (CNTs) were first described by Iijima [17]. They are further classified as single walled (SWCNT), double walled (DWCNT) and multiwalled (MWCNT) CNTs. They exhibit unique properties like strength, hardness, thermal conductivity, microwave absorption, electrical and catalytic properties [18]. They can be manipulated for specific applications in electronic devices, waste water treatment and drug delivery systems [19,20]. Nonetheless, CNTs are known to cause reproductive, developmental and pulmonary toxicity in mice [21]. They possess genotoxic potential that varies considerably due to their heterogeneity. Genotoxic effects of CNTs resembled those of asbestos fibres [22]. The genetic alterations in MWCNT induced rat mesothelioma were also found to be similar to those induced by asbestos [23]. Intriguingly, genotoxic hazards posed by CNTs varied amongst different types of CNTs. Weight of evidence analysis (WoE) made by Moller and Jacobson [24] revealed that MWCNT-7 demonstrated strongest genotoxicity amongst CNTs. MWCNTs were shown to be genotoxic both *in vitro* and *in vivo* tests. They induced micronuclei formation in A549 cells and enhanced sister chromatid exchange (SCE) in CHO AAB cells. DNA damage in lungs of ICR mice intratracheally instilled with a single dose (0.05 or 0.2 mg/animal) analysed by comet - assay increased in a dose dependent manner [25]. Later on, Snegin., *et al*. [26] confirmed that orally introduced MWCNTs damage DNA in different organs of male mice.

Genetic toxicity potential of SWCNTs (diameter: 1 - 1.2 nm, length- 20 µm) was evaluated in *Salmonella typhimurium* or *Escherichia coli* using standard battery of genotoxic tests suggested by Organization of Economic Cooperation and Development (OECD) guidelines 471, 473 and 474 using good laboratory practice (GLP) system by Kim., *et al*. [27]. The study concluded that SWCNTs expressed no genotoxicity.

Genotoxicity induced by asbestos, carbon nano fibres (CNF) and SWCNTs has also been compared. Cytotoxicity tests revealed a concentration and time dependent loss of V79 cell viability in the sequence: asbestos > CNF > SWCNT. Strongest effect on DNA and MN induction was found after CNF exposure of RAW264.7 macrophages [28]).

Co-exposure of zebra fish liver cell line (ZFL) to oxidized multiwalled carbon nanotubes (ox-MWCNTs) and cadmium has also been studied. Although both the xenobiotics affected the antioxidant system of ZFL, MWCNTs modulated the effects of cadmium [29]. Recently, Saleemi., *et al*. [30] reported that functionalization and agglomeration of CNTs might induce oxidative stress and toxic signalling pathways. An insight into the molecular mechanisms of their toxicity may open new channels for the mitigation of their harmful effects. Further studies on genotoxicity of CNTs are warranted.

Genotoxicity of fullerenes

Fullerenes are the molecules with 60 or 70 atoms of carbon, denoted as C_{60} or C_{70}. They were first reported by Kroto., *et al*. [31]. They possess a three dimensional structure with unique physical and chemical properties [32]. They are lipophilic in nature and can cross membranes and blood brain barrier [33]. Manipulation of surface chemistry and molecular make up can create diverse population of fullerenes that may exhibit different toxic behaviour [34]. Both *in vitro* and *in vivo* studies have shown that fullerene (C_{60}) could induce micronuclei formation and enhanced frequency of sister chromatid exchanges in cultured mammalian cells. Formation of DNA adducts in the lungs of ICR mice intratracheally instilled four consecutive doses at 0.2 mg/animal/week was monitored by Totsuka., *et al*. [35]. 2 to 5 fold increase in 8-oxodeoxyguanosine and other lipid peroxide related adducts was also recorded. Further, mutation spectrum analysis showed increase in G:C to C:G transversions. These authors suggested the role of oxidative stress and inflammation in the genotoxicity of fullerenes. Two bacterial genotoxicity tests i.e. Rec assay and the umu test at concentrations as low as 0.048 mg/L and 0.43 mg/L respectively performed in *Bacillus subtilis* yielded positive results [36]. These authors suggested that aqu-C_{60} had DNA damaging potential. Intriguingly, a study made in male rats, intratracheally instilled a single dose at 0.5 or 2.5 mg/kg or repeated dose at 0.1 or 0.5 mg/kg once a week for five weeks showed no increase in tail DNA measured in rat lung cells. The study concluded that C_{60} possesses no DNA damaging potential as measured through comet assay.

A few reports have shown protective effects of C_{60} against the toxicity of a few xenobiotics. It protected against the genotoxicity of doxorubicin in human lymphocytes evaluated *in vitro* through comet assay [37]. Prylutska., *et al*. [38] reported that C_{60} fullerene in combination with cisplastin, a most effective therapeutic agent used against cancer diseases, does not affect genotoxicity of cisplastin *in vitro*. The potential effects of C_{60} and virgin olive oil (4 mg/kg dissolved in 1 mL of olive oil) against cyclophosphamide induced cytotoxic and mutagenic effects in hepatic and cardiac tissues of rats were evaluated by Aly., *et al*. [39]. These authors confirmed the protective effects of C_{60} and virgin olive oil against cyclophosphamide induced mutagenicity and toxicity. Protective effects of C_{60} and virgin olive oil against cadmium chloride induced genotoxicity in rats were also reported [40]. Further studies on its protective effects against toxicity caused by a variety of xenobiotics are important from nanomedicine point of view.

Genotoxicity of silver nanoparticles

Till date approximately 450 consumer products are known to contain silver nanoparticles. They are easy to synthesize and possess superior optico-chemical properties. Cyto and genotoxicity induced by AgNPs have been attributed to oxidative stress and inflammation [41,42]. Experimental evidence suggests that AgNPs can induce DNA double strand breaks, chromosomal aberrations, chromosomal fusions and fragmentation [43]. It was shown that DNA repair deficient cells are more prone to AgNP toxicity [44]. The inhibition of DNA phosphokinase Cs (DNA PKcs) renders the cells susceptible to DNA damage and genome instability that leads to cell death. Biogenic AgNPs were found to be less cyto/genotoxic *in vivo* in comparison to chemically synthesized nanoparticles. Furthermore, human cells expressed greater resistance to their toxic effects in comparison to other organisms [45]. Gene expression studies made by Shati and Elsaid [46] suggested that AgNPs might be favourable antibacterial agents especially against multidrug resistant bacteria. Genotoxicity of silver nanoparticles was tested *in vitro* and *in vivo* using human lymphocytes, Swiss albino male mice and a few plant species viz. *Allium cepa* and *Nicotiana tabacum*. Results on comet assay revealed that AgNPs at a concentration of 25 µg/mL can cause genotoxicity in lymphocytes [47]. A novel study was made by Mohamed [48] who showed the combined effects of cadmium chloride (1.5 mg/kg) and single dose of AgNPs (20, 41 and 82 mg/kg) on the sperms of mice. A significant increase in micronucleated polychromatic erythrocytes (MNPCEs) and DNA damage was also reported. Potentiation of AgNPs induced toxicity by cadmium raises serious human health concerns. A review on genotoxicity of AgNPs was recently published by Rodriguez-Garrus., *et al*. [49]. It suggests that further studies on genotoxicity of AgNPs are warranted following the guidelines of ICH, EFSA and OECD.

Genotoxicity of gold nanoparticles

Gold nanoparticles (Au-NPs) are one of the most common nanoparticles. They are now used in several biomedical applications viz. diagnosis, chemical sensing, biological imaging, drug delivery and cancer treatment. They possess peculiar characteristics i.e. biocompatibility, controllable shape and size, ease of synthesis, surface functionalization, chemical stability and tunable optical properties [50]. *In vitro* and *in vivo* toxicity studies on Au-NPs were recently documented by Wang., *et al*. [51]. *In vitro* studies included Ames test, SOS chromotest, comet assay and micro nucleus assay. Human peripheral lymphocytes (PBLs), Chinese hamster ovary (CHO) cells, MRC-5 human fetal lung fibroblast cells, BALB/c 3T3 cells, HepG2 human liver cancer cells showed oxidative DNA damage in a dose, particle size and cell line dependent manner after Au-NP treatment. An earlier study by Xia., *et al*. [52] using HepG2 cells and Chinese hamster lung (CHL) cells presented that size of Au-NPs played an important role in determining the genotoxicity of Au-NPs. Vales., *et al*. [53] investigated the role of surface charge in determining the genotoxic potential of Au-NPs employing comet assay, micronucleus assay in human bronchial epithelial BEAS-2B cells. Their findings suggested that cellular uptake and cytotoxicity of Au-NPs were clearly enhanced by positive surface charge, however, neither functionalization nor size could single handily account for their genotoxic effects. Adewale and colleagues [54] emphasized an integrated approach to study the toxicological behaviour of gold nanoparticles. Physicochemical properties viz. size, shape, surface charge, methods used in the synthesis of gold nanoparticles, models used, route of administration may be the confounding factors in their toxicity. Combined effects of an environmental contaminant, gemfibrozil and Au-NPs on DNA damage in the erythrocytes of a fish, commonly known as gilthead seabream (*Sparus aurata*) were studied by Barreto., *et al*. [55]. The combined exposure produced an antagonistic response. Interaction between Au-NPs and other environmental contaminants opens new channels of research.

Genotoxicity of titanium dioxide nanoparticles

Titanium dioxide (TiO_2) is a natural polymorphic crystalline oxide of titanium. It occurs in different forms viz. rutile, anatase and brookite crystals. TiO_2 has been widely used in paints, pigments, plastics, paper, inks, foods, cosmetics and pharmaceuticals. It can cause inflammation, pulmonary damage, fibrosis, lung tumors, apoptosis and carcinogenicity.

A few studies to monitor its genotoxicity have been made in the past. Ghosh., *et al*. [56] studied its genotoxicity using *Allium cepa*, *Nicotiana tabecum* and human lymphocytes employing comet assay and DNA laddering techniques. They correlated these effects with increased lipid peroxidation caused by TiO_2. A dose dependent effect was noticed in human lymphocytes. A review on the genotoxicity of TiO_2 was presented by Chen., *et al*. [57]. They reported that *in vitro* studies showed greater number of negative effects than *in vivo* systems. Tests for DNA damage and chromosome damage produced more positive results than the tests for gene mutation. The results were attributed to oxidative stress. A dose dependent decrease in mitotic index and increase in the number of chromosomal aberrations and micronuclei formation was observed in the roots of *Allium cepa* after exposure to different concentration (12.5, 25, 50, 100 µg/mL) of TiO_2. The genotoxicity was correlated with reactive oxygen species [58]. Mohamed and Hussien [59] confirmed genotoxicity of TiO_2 nanoparticles in the brain of male Swiss Webster mice after its oral administration (500 mg/kg b.w) for five consecutive days. Comet assay revealed DNA fragmentation whereas direct sequencing showed point mutation of Presenilin gene at exon5, gene linked to inherited forms of the Alzheimer's disease. The study concluded that exposure to TiO_2 might lead to increased incidence of Alzheimer's disease. Increasing use of TiO_2 in consumer products might cause DNA damage in bone marrow and testicular cells. This assumption was drawn by Bakare., *et al*. [60] using mouse bone marrow and sperm morphology assays. In a recent study, four different cell lines viz. A549 (lung cells), HepG2 (liver cells), A172 (glial cells) and SH-SY5Y (neurones) were used by Brandao., *et al*. [61]. A concentration, time and cell type dependent study did not show significant induction of micronuclei. A review of recent *in vitro* and *in vivo* studies on the genotoxicity of TiO_2 was recently published by Wani and Shadab [62]. They concluded that comet assay remains to be commonly used genotoxicity test followed by Ames test and chromosomal aberration tests. However, inconsistency in the results needed to be addressed. Correlation of TiO_2 exposure with Alzheimer's disease is a significant observation from human health point of view. Such correlation with other diseases is yet to be established.

Genotoxicity of quantum dots (QDs)

Quantum dots are now widely used in biological, biomedical and industrial applications. They possess exceptional photo-physiological properties. Cadmium and selenium are the chief components of the majority of quantum dots. They are acutely toxic to cells and organisms [3]. Thus emerges the concept of nanosafety with special reference to human and ecosystem health.

Recent information shows that modification of the core of NPs, the type of the nanocarrier used and shape of the QDs can influence their cytotoxicity and genotoxicity. Non cytotoxic concentrations of a few QDs i.e. dendrimers, mesoporous silica and supermagnetic iron oxide (SPIO) could induce genotoxicity due to their carriers. Negatively charged and neutral carriers exhibited no genotoxicity. However, positively charged cationic liposomes, dendrimers and SPIO NPs induced genotoxicity leading to increased number of micronuclei in cells [63]. In a similar study, maltodextrin - carbohydrate coated cadmium sulphide nanoparticles induced cell death in HepG2 cells at a concentration higher than 4.92 nM. They caused embryotoxicity in chicken embryos in a dose dependent manner. However, no toxicity was observed with doses lower than 3.28 nM neither in cells nor chicken embryos. It was emphasized that coating of NPs with maltodextrin makes NPs suitable for bioimaging applications [64]. A comparative study on the genotoxicity of CdS nanorods (30 - 50 nm diameter, 50 - 110 nm length) and CdS nanodots (3 - 5 nm) in mice was performed by Liu., *et al*. [65]. They attributed the DNA damage and spermatozoon viability and abnormality not to elemental composition or the amount of ions released before receptor intake but to the morphology of QDs. The significance of *in vitro* assays used for testing genotoxicity of CdO NPs in different models was reported by Demir., *et al*. [66]. While results on Ames test showed no genotoxicity, positive results were obtained with micronucleus assay, Comet and mouse lymphoma assays. The results suggested chromosomal damage, single or double strand breaks in DNA and mutations.

Experimental modification of the surface chemistry of QDs could also affect their genotoxicity. Mice treated with CdSe QDs modified with mercaptoacetic acid (MAA), of average size 5.0 ± 0.2 nm and CdSe MMA QDs doped with 1% cobalt, orally at doses of 500, 1000, and 2000 mg/kg b.w. for two and seven days exhibited genotoxicity in bone marrow and liver cells. Increasing DNA damage, frequency of MN formation and generation of DNA adduct -OHdG was observed in mice exposed to seven days. They attributed genotoxicity to free radical induced DNA damage [67]. Effect of negative (carboxyl), neutral (hexadecylamine) and positive (amine) polymer coating of QDs on genotoxicity in lymphoblastoid TK6 cells was studied by Manshian., *et al*. [68]. The study concluded that reactive oxygen species and oxidative stress are not only the mechanisms associated with genotoxicity of QDs. In addition, nanoparticle surface chemistry, colloidal stability, cellular uptake and the intrinsic characteristics of QDs are critical players in their genotoxic effects. CdTe/Cd/Se/ZnSe core double shelled QDs capped with glutathione were also found to be genotoxic in Chinese hamster ovary cells at a concentration of 50 and 100 μg ml^{-1} [69]. Genotoxicity of 30 nm sized citrate stabilized Cd based semiconductor QDs was determined using fluorometric γ-H2AX assay by GeiBler., *et al*. [70]. These authors concluded that genotoxicity of Cd-QDs was correlated with the shielding of their Cd containing core/shell/ shell architecture preventing genotoxic risks.

Genotoxicity of QDs in nonconventional models i.e. haemolymph of *Mytilus*, rainbow trout cell lines and hemocytes of *Drosophila melanogaster* have also been investigated. Rocha., *et al*. [71] determined genotoxicity of CdTe QDs using micronucleus test, nuclear abnormalities assay and comet assay. The results indicated that genotoxicity of CdTe QDs and Cd (2^{+}) are mediated by different mechanisms. CdS quantum dots coated with methylpolyethylene glycol (M-PEG) were also found to be genotoxic in RTG-2 cell lines of rainbow trout [72]. A concentration dependent (10 and 50 μg/ml) genotoxicity was observed after 24h exposure. *Drosophila melanogaster* was reported to be a suitable model to detect *in vivo* genotoxicity of QDs. Alaraby., *et al*. [73] showed that CdSe QDs can penetrate the intestinal barrier of the larvae reaching the hemolymph, interacting with hemocytes and inducing dose/time dependent genotoxic effects as determined by the comet assay. Significant disturbance in the antioxidant and p53 genes were also observed.

Genotoxicity of zinc oxide nanoparticles

Zinc oxide nanoparticles are most commonly used nanomaterials in industrial applications. Consumer products like antimicrobial and antifungal agents, paints, textiles, plastics, glass, ceramics, rubber, cosmetics and food products contain ZnONPs. Therefore, impact of

these nanoparticles on human health needs to be investigated. A few studies on their genotoxicity are available in literature. Genotoxicity of ZnONPs (15 - 18 nm in diameter) at different concentrations i.e. 0.1, 10, and 100 µg/mL was investigated in A 549 cell line. Foci analyses showed DNA double strand breaks with increased generation of reactive oxygen species. Treatment of ZnONPs treated cells with diethylene triamine pentaacetic acid (DTPA) resulted into loss of their toxicity [74]. A comparative study between the cytotoxicity and genotoxicity of ZnONPs and TiO_2 NPs was made in human erythrocytes and lymphocytes at different concentrations (50, 100, 250, 500 ppm) by Khan., *et al*. [75]. Both were found to be cytotoxic to human RBCs. Both induced oxidative stress. DNA damage measured by comet assay was dose dependent and significantly greater than respective ionic forms. Genotoxicity of ZnONPs was greater than TiO_2NPs. Mild genotoxicity of ZnONPs was demonstrated in Swiss mice after oral administration at dose levels of 300 and 2000 mg/kg b.w. for two days [76]. DNA damage was evaluated after 24, 48, 72hr post treatment using a randomly amplified polymorphic DNA (RAPD) assay. Morphological changes in sperms were also reported. They attributed these effects to ROS. A review on molecular mechanisms of ZnONP induced toxicity was presented by Scherzad., *et al*. [77]. They concluded that ZnONPs possess the potential to damage DNA in acute exposure conditions, however, DNA repair mechanisms need to be investigated. ZnONPs were found to be protective against dimethylnitrosamine induced hepatic and renal toxicity in rat (Varsha., *et al*. 2018, 2021). A unique study on the genotoxicity of ZnONPs was made by Singh., *et al*. [78] in *Deinococcus radiodurans*, an extremophilic bacterium known as radiation resistant organism. These authors showed that ZnONPs are internalized into the bacterial cells and induce significant DNA damage. They could generate ROS and concomitant thiol depletion. Genes related to DNA repair and metabolic pathway were down regulated. Genotoxicity of ZnONPs in morning glory plant, *Ipomoea obscura* (L.) has also been studied by Murali., *et al*. [79]. They showed that phytofabricated ZnONPs could arrest the cell division in HT-29 cells, as early as in the G0/G1 phase with 73.14% cells showing apoptosis after 24hr incubation.

Genotoxicity of silica nanoparticles

Nanosilica or silica nanoparticles (SiNPs) possess distinct physico-chemical properties**.** They are widely used in biomedical applications and consumer products. They are used in processed food, in pharmaceutical products, electronics, glass and anticancer drugs [80]. An update on toxicology of silica nanoparticles was published by Murugadoss., *et al*. [81]. These authors reported that SiNPs synthesized through wet route exhibited different biological effects compared to those synthesized through thermal route. Acute effects were severe than the chronic effects. Strategies available for evaluation of genotoxicity of SiNPs have been discussed by Yazdimamaghani., *et al*. [82]. Particle mediated inflammation, oxidative stress and formation of intranuclear inclusions might contribute to its cytotoxicity and genotoxicity [83,84]. However, Sayes., *et al*. [85] have suggested that short term exposures expressed no significant inflammatory or genotoxic effects on mouse lungs. Health risks posed by SiNPs require further investigations.

Genotoxicity of other nanoparticles

In addition to the genotoxicity of nanoparticles described above, a few reports are available on aluminium, cobalt and chromium nanoparticles also. Aluminium oxide nanoparticles are also called as nanoceramics. They are used in industrial and orthopaedic products. Increased micronucleus frequencies and chromosomal loss was noticed in an *in vitro* study made by Tsaousi., *et al*. [86]. Aluminium oxide nanoparticles were found to be cytotoxic and genotoxic in Chinese hamster ovary (CHO-K1) cells [87]. Kim., *et al*. [88] studied genotoxicity of aluminium oxide nanoparticles in mammalian cell lines i.e. L5178Y cells BEAS-2B cells. They used comet assay and mouse lymphoma thymidine kinase (tk(+/-)) gene mutation assay(MLA). The study concluded that Al_2O_3 could cause primary DNA damage and cytotoxicity but no mutagenicity in cultured mammalian cells. De., *et al*. [89] evaluated genotoxicity caused by Al_2O_3NPs in *Allium cepa*. It was found to be higher in NP treated roots than the bulk treated roots. A recent study made by Zalili., *et al*. [90] in rats showed that Al_2O_3NPs could induce genotoxicity in bone marrow and colon as determined by the micronucleus assay. However, no chromosomal mutations were observed.

A few reports are available on the genotoxicity of cobalt nanoparticles. Cobalt based NPs are now used as pigments, catalysts, sensors, magnetic contrast agents and in energy storage devices [91]. Cobalt and its alloys are also used in hip joint replacements that may lead to

its dispersion in surrounding tissues and even lymphocytes [92]. Vales., *et al*. [93] administered CoNPs and ionic cobalt chloride to third instar larvae of *Drosophila melanogaster* through food at the concentration of 0.1 to 10 mM. Both were able to increase the frequency of mutant clones. CoNPs were able to induce genotoxicity as observed through wing spot assay. Exposure of mice to CoNPs (50 µg/ mouse) for 28 days through intra tracheal instillation resulted into lung inflammation, oxidative stress, cell proliferation and DNA damage [94]. Different mechanisms responsible for the genotoxicity of CoNPs were discussed by Cappellini., *et al*. [95]. A summary of these reports is presented by table 1.

Type of ENP	Effect	Reference
SWCNT	No genotoxicity	Kim., *et al*. [27]
MWCNT-7	Strongest genotoxicity	Moller and Jacobson [24]
CNF	MN induction	Kisin., *et al*. [28]
CNT	Agglomeration and oxidative stress	Saleemi., *et al*. [30]
C-60 fullerene	Protection against doxorubicin toxicity	Alfanasieva., *et al*. [37]
C-60 fullerene + cisplastin	No genotoxicity of cisplastin	Prylutska., *et al*. [38]
AgNPs	Genotoxicity in lymphocytes	Ghosh., *et al*. [47]
AgNPs + CdCl2	DNA damage	Mohamed., *et al*. [48]
AuNPs	Oxidative stress	Wang., *et al*. [51]
TiO_2	DNA and chromosome damage	Chen., *et al*. [57]
TiO_2	DNA damage in bone marrow and testicular cells	Bakare., *et al*. [60]
TiO_2	DNA damage	Shadeb [2020]
Quantum dots	Genotoxicity	Shah., *et al*. [63]
Cd nanodots and nanorods	DNA damage	Liu., *et al*. [65]
CdONPs	MN induction	Demir., *et al*. [66]
CdSeQDs	Genotoxicity	Alarby., *et al*. [73]
ZnONPs	DNA damage	Schergad., *et al*. [77]
ZnONPs	Genotoxicity in morning glory	Singh., *et al*. [78]
SiNPs	Genotoxicity	Yazdimamaghani., *et al*. [82]
AlNPs	Increased MN formation	Tsaousi., *et al*. [86]
AlNPs	Genotoxicity in bone marrow	Zalili., *et al*. [90]
CoNPs	DNA damage	Wan., *et al*. [94]

Table 1: *Recent reports on the genotoxicity of ENPs.*

Conclusion and Perspectives

Our knowledge on the toxicological profile of nanomaterials has increased significantly in the recent past. Nevertheless, certain issues still remain to be addressed. Insufficient epidemiological studies make it difficult to assess the real time potential health risk involved in the workplace exposure to NPs. There remains inconsistency in the parameters, models and methodologies used to determine their genotoxicity. Authors have used NPs of different sizes and metallic compositions. No comparative studies are available on the genotoxic effects between bioinspired and synthesized NPs. Optimization of biomarkers of DNA damage is the need of hour. Further, genotoxicity of the carriers of NPs also remains unknown. Long term studies are needed to study their role in tumourogenesis and cancer. Close monitoring

of the emissions of NPs from industries is essential to protect public health and environment. NPs do promise advanced applications in agriculture, commerce, medicine and energy but doubts regarding their safety would require further investigations. Regulatory agencies should issue suitable guidelines on the usage and toxicity testing of nanomaterials.

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