Chapter-6

Biodistribution of MNPs

Biodistribution and kinetics of MNPs were assessed in *Caenorhabditis elegans* with use of fluorescein isothiocyanate (FITC)-labeled ZnO NPs (100 nm, 50 nm and 10 nm) in different concentrations (1 to 10 mg/L). Their translocation and biodistribution mapping were observed under fluorescence microscope equipped with peltier cooled-charge coupled camera. Both differential interference contrast (DIC) and epi-fluorescence imaging were observed in FITC-ZnO NPs treated *C. elegans* at 24 hrs, 48 hrs and 72 hrs of exposure.

The mean percentage of *in-vivo* biodistribution of 100 nm at 24 hrs of exposure varied at large scale (15-100%) (fig 6.1.1) while, with exposure of 2 mg/L remained restricted to 15%. Although, exposure of 4 mg/L and 6 mg/L distributed for 35 and 60%. Exposure of 8 mg/L and 10 mg/L were found 65 and 100% respectively (fig 6.1.1). At 48 hrs of exposure biodistribution varied 60% to 90% (fig 6.1.2). At exposure of 2 mg/L and biodistribution was quite high (60%) and at exposure of 4 mg/L and 6 mg/L biodistribution was recorded 75 and 90% respectively. Similarly 90% biodistribution was also observed at exposure of 8 mg/L at 48 hrs. At 72 hrs for 2 mg/L particles were not found in their body and 5% particles were found with exposure of 4 mg/L (fig 6.1.3). The particles were distributed for 7 and 15% at (fig 6.2) exposure of 6 mg/L and 8 mg/L respectively.

Exposure of 50 nm ZnO NPs (2 mg/L) distributed for 70% and treatment of 4 mg/L and 6 mg/L distributed for 35 and 50 percent after 24 hrs of exposure (fig 6.4.1). However, 75 and 90 percent particles were found at exposure of 8 mg/L and 10 mg/L respectively. After 48 hrs of exposure at dose of 2 mg/L and 4 mg/L 80% particles were found in their body. And 80-85 percent particles were also found in 6 and 8 mg/L treated *C. elegans*. But at the dose of 10 mg/L only 5% percent excreted out and 95% were remained in their
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Findings revealed that after 72 hrs of exposure only 5 - 10% particles remained in their body for 2 mg/L and 4 mg/L treated *C. elegans*. But 35 and 80 percent particles were found in 6 and 8 mg/L treated worms. And, 65% particles remained in their body after 72 hrs of exposure in 10 mg/L treated worms respectively (fig 6.4.3).

Findings revealed 50 nm particles was distributed maximum at 48 hrs and least at 72 hrs of exposure in all treatments (fig 6.3). It may concluded biodistribution was time dependent, it attained highest distribution at 48 hrs and released the excess nanoparticles at 72 hrs, therefore low kinetics of biodistribution were observed at 72 hrs almost in all treatments.

Fig.6.1.1. *In-vivo* distribution of ZnO NPs (100 nm) in *C. elegans* at 24 hrs; (a) control, (b) 4 mg/L, (c) 6 mg/L, (d) 8 mg/L, (e) 10 mg/L.

Fig.6.1.2. *In-vivo* distribution of ZnO NPs (100 nm) in *C. elegans* at 48 hrs; (f) control, (g) 4 mg/L, (h) 6 mg/L, (i) 8 mg/L, (j) 10 mg/L.

Fig.6.1.3. *In-vivo* distribution of ZnO NPs (100 nm) in *C. elegans* at 72 hrs; (k) control, (l) 4 mg/L, (m) 6 mg/L, (n) 8 mg/L, (o) 10 mg/L.
Fig. 6.2. Biodistribution (%) of ZnO NPs (100 nm) at different time interval in *C. elegans*.

Fig. 6.3. Biodistribution of ZnO NPs (50 nm) at different time interval in *C. elegans*.

The smaller nanoparticles (10 nm) were found 40 and 70 percent at the dose of 2 mg/L and 4 mg/L respectively. With 10 mg/L particles were uniformly distributed at 24 hrs of exposure (fig 6.5.1).
At 72 hrs of exposure significant decline in kinetics of distribution were recorded (fig 6.6). Only 10, 15, 30, 70 percent particles were found with 2 mg/L, 4 mg/L, 6 mg/L and 8 mg/L respectively in treated worms. In contrast to 10 nm nanoparticles, 80% remained in their body after 72 hrs of exposure (fig 6.5.3). Biodistribution of 10 nm ZnO NPs had shown very different kinetics of distribution in comparison to 50 and 100 nm at different time of interval. A very high accumulation of ZnO NPs were found at 48 hrs in comparison to other nanoparticles at various interval of time (fig 6.5.2). Initially at 24 hrs, the uptake rate of ZnO NPs was quite high for 2 mg and 4 mg/L doses and at 48 hrs increases upto the 65 - 80% respectively (fig 6.5.1).

![Fig.6.4.1. In-vivo distribution of ZnO NPs (50 nm) in C. elegans at 24 hrs; (a) control, (b) 4 mg/L, (c) 6 mg/L, (d) 8 mg/L, (e) 10 mg/L.](image)

![Fig.6.4.2. In-vivo distribution of ZnO NPs (50 nm) in C. elegans at 48 hrs; (f) control, (g) 4 mg/L, (h) 6 mg/L, (i) 8 mg/L, (j) 10 mg/L.](image)

![Fig.6.4.3. In-vivo distribution of ZnO NPs (50 nm) in C. elegans at 72 hrs; (k) control, (l) 4 mg/L, (m) 6 mg/L, (n) 8 mg/L, (o) 10 mg/L.](image)
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Fig. 6.5.1. *In-vivo* distribution of ZnO NPs (10 nm) in *C. elegans* at 24 hrs; (a) control, (b) 4 mg/L, (c) 8 mg/L, (d) 10 mg/L.

Fig. 6.5.2. *In-vivo* distribution of ZnO NPs (10 nm) in *C. elegans* at 48 hrs; (e) control, (f) 4 mg/L, (g) 8 mg/L, (h) 10 mg/L.

Fig. 6.5.3. *In-vivo* distribution of ZnO NPs (10 nm) in *C. elegans* at 72 hrs; (i) control, (j) 4 mg/L, (k) 8 mg/L, (l) 10 mg/L.

Significant gonadal permeation (80 and 90%) of ZnO NPs was observed in *C. elegans* at exposure of 4 mg and 6 mg/L (fig 6.4.2). Moreover, at 72 hrs highest localization and distribution was recorded with 10 mg/L exposure that was followed with thrashed, bend and distorted body. Exposure of 8 mg/L also induced deformed body. Study revealed uptake and distribution of NPs depends on size and shape of ZnO NPs as suggested by Dam *et al.* (2014). It may be concluded that ingested NPs, translocated along with gut and intestinal epithelial cells. Uptake rate was highest at initial 24 hrs and NPs were found along the edge of mouth, pharynx followed with gut and further...
assimilated and internalized by various cell signaling pathways as well ion-gated channels.

![Graph showing biodistribution of ZnO NPs](image1)

**Fig.6.6.** Biodistribution of ZnO NPs (10 nm) at different time interval in *C. elegans*.

![Graph showing in-vivo size dependent translocation and biodistribution](image2)

**Fig.6.7.** *In-vivo* size dependent translocation and biodistribution of ZnO NPs in *C.elegans*. 

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Rate of uptake of NPs also depends on phase of cell cycle as recorded by Kim et al. (2012). Biodistribution and localization mapping in present study advocates that smaller size ZnO NPs (10 nm ZnO NPs) biodistributed all over the body at 4 mg/L - 10 mg/L dose. It was found 100 nm ZnO NPs biodistributed in C. elegans but not permeated in gonads of worms and excreted out from body in next 24 hrs. The study demonstrated application of 6 mg/L to 8 mg/L (100 nm ZnO NPs) significantly thrashed worms body while ROS generations were not recorded. Exposure of 10mg/L particles shown adverse effect on metabolism of C. elegans and 50% NPs were observed at 72 hrs that localized throughout the body length in the form of endosomes. Additionally, at higher dose (10 mg/L) 100 nm ZnO causes toxicity and found to be harmful for C. elegans. Exposure of lower concentration (2 mg/L) of all sized particles distributed in intestine and dispersed to gonads. However, their concentration was increased at 6mg/L from anterior most part to middle length of intestine. Traces of particles were also observed at posterior length of the body. Biodistribution was not found significant at 72 hrs of exposure and was recorded to reduce by 35% (fig 6.7). It was observed that primary route of uptake and internalization of FITC-ZnO NPs occurred via oral route. At 48 hrs of post exposure the migration of nanoparticles from mouth to gut and to other remote tissues was also found. The acquired fluorescence images were obtained and compared with control (K-medium) that advocates and confirmed uptake and translocation of FITC-ZnO NPs in worm’s body. Present study revealed that uptake process was fastidious during 24 hrs and localized at anterior region. Afterwards, particles migrated towards middle and posterior part of the worm’s body and later excreted out. Findings corresponds to observation of Gao et al. (2008) who observed biodistribution of ZnO NPs is assimilated and accumulated by worm’s body remote from the portal of entry i.e. in-vivo translocation from intestine to gonads and excretory tissues that induced the reactive oxygen species (ROS) generation and oxidative stress. Dubey et al. (2015) observed ROS generation is directly proportional to internalization and biodistribution of NPs. The generation of reactive species and oxidative stress were toxic in C. elegans and caused cytotoxicity, genotoxicity, DNA damage, and change in membrane potential of
mitochondria, thrashed and deformed body if exposure was prolonged for 48 hrs and 72 hrs. The smaller size ZnO NPs retained in *C. elegans* long lastly and penetrates deep inside the tissue including worm’s eggs. Tate *et al.* (2011) studied potential of smaller size nanoparticles and their long last circulation in mice and recorded vascular tissue received more nanoparticles dispersion and accumulation. Similarly, it was found that 10 nm ZnO NPs shown more penetration, distribution and retention in comparison to 50 nm and 100 nm size. While, 100 nm and 50 nm found to be low penetration rate and easily excreted out from body with in defined time frame as observed by Khlebtsov and Dykman (2011), Oberdorster *et al.* (2007) and Lewinski *et al.* (2008). The main route of uptake of metal nanoparticles may be via receptor-mediated endocytosis, metal ion dissociated and pH of lysosome or endosome as cation form (Behra *et al.*, 2013; Zhang *et al.*, 2006). Cho *et al.* (2011) reported that cellular internalization of ZnO NPs occurs in the form of phagosomes and was found pH dependent with rapid dissolution of ZnO NPs into Zn$^{2+}$ ion that mainly responsible for lung injuries in mice. Garter *et al.* (2000) reported biodistribution were directly related to induce mortality followed by apoptosis.