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Assessment of nano selenium effect in developing zebra fish embryos

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Abstract
Nano particles explicitly need to be non toxic not only for biomedical applications but for other areas that ends up in environmental exposures. Various platforms are being used from *in vitro* cell culture to higher vertebrate models. However, small efficient, more informative but cost effective well established Zebrafish models are the choice for preclinical studies due to the advantage of sharing not less than 85 percent homology with human genome biology. Systematic vision gained by toxicity assessment of food using various models from *in vitro* cell-based assays, biochemical assays, *in vivo* animal to clinical settings paved way for a better food safety. The present study was designed to find out the comparison and toxicity between Nano Selenium (NaSe) and Inorganic Selenium (Inorg Se) on the embryonic development of Zebrafish. Different concentration of dietary Se was used on developing Zebra fish embryos. The results indicated that with the increased NaSe and Inorg Se concentration, different observable deformities such as growth retardation, shrinkage of chorion, yolk sac edema, lack of pigmentation; tail deformities and scoliosis in developing embryos were observed. The NaSe at 0.1mg/L concentration showed the highest hatchability and healthy embryo development. This study is conducted to identify the optimal NaSe concentration that could be used for food fortification through supplementation in developing zebra fish embryos.

Keywords: Zebra fish – toxicity, NaSe and Inorg Se, embryonic development

Introduction
Selenium was found in the year 1950 and was used as a vital micronutrient for the survival of plants and for other biological organisms including humans [7, 15]. Selenium is important for various aspects of human health which includes cardiovascular health and also plays an important role in several cellular processes including thyroid hormone production and in oxidative stress [12]. Selenium nanoparticles reveals a strong anti-oxidative [8], anti-leishmanial [13] and anti-bacterial effects [17]. The toxicity of selenium ions is reduced by changing the redox state of the selenium ions and in the process leading to the formation of well-defined nanoscale particles [4, 16].

Modern research of nanotechnology mainly focuses on the synthesis and development of metal nanoparticles. Green synthesis of nanoparticles is eco-friendly and economical, that has the added advantage over the physical and chemical synthesis of not using toxic chemicals [14]. The use of bacteria, yeast, fungi and plant extracts are the sources for the green synthesis method of nanoparticle production [4, 16]. In the present study Nano Selenium (NaSe) was green synthesized by a biological procedure using the reducing power of fenugreek seed extract and inorganic source (sodium hydrogen selenite) from Hi-media were used.

Nanoparticles available in commercial form lack in safety regulations and toxicology data. Some reports have concluded that ultrafine particles could cause more damage than larger particles at the same concentration [18]. The recent research was mostly focused on the nano toxicology studies on *in vitro* models. A small number of research groups are dealing with aquatic *in vivo* systems [9]. A toxicology study in *in vivo* systems has better implication in physiology and anatomy.

NaSe could be used for treating as a therapeutic agent for human; but the toxicity of those and the concentration to be used should be evaluated before using in the food industry. To evaluate the toxicity and the dosage of use of NaSe, Zebrafish embryos were selected as a lower animal experimental model in this research.

Zebrafish has not less than 85 percent homology to human genome with more similarity to the human tissue physiology, which leads to additional advantage for toxicological studies in zebra fish embryos [10] with handling and no ethical issues. This makes the zebrafish model as an ideal candidate for toxicology research with the objective to identify adverse effects of chemical exposure.

2. Materials and Method

2.1 Fish Medium and Selenium preparation

Fish medium was made from 0.1 g of sea salt in 1 L of Milli-Q water. Nano Selenium (NaSe) was green synthesized by biological procedure using the reducing power of fenugreek seed extract with minor modification [11]. Inorganic source of dietary selenium (sodium hydrogen selenite) was purchased (Hi-media) and used for the study.

2.2 Embryo collection

Zebrafish embryos were purchased from the certified fishery farm, Chennai. The fertile and unfertile embryos were screened using stereo microscope for further investigation.

2.3 Treatment of zebrafish embryos

Varying doses of NaSe and Inorg Se was treated in embryos of early blastula stage 24 hours post fertilization (hpf) for toxicity studies. The fertilized healthy embryos chosen for trail are grown in petri plate at 10 per plate with fish medium in triplicate. Different dosage level of NaSe 0.15mg/1L, 0.2mg/1L and 0.3mg/1L and Inorg Se 0.2mg/1L 0.3mg/1L and0.4mg/1L was added along with fish medium and incubated for 72 h at 28.5°C. Fresh medium was replaced once in two days. During the exposure period the development of the zebra fish from embryos till hatching were captured under a stereo zoom microscope (Labomed).

2.4 Morphological observation

The percentage mortality, heart rate, edema and malformation were recorded at 24, 48, 72, 96 and 120 hpf using a stereomicroscope. The developmental deformities of the embryos and larvae such as malformations like tail deformity and edema, the rate of hatchability were observed [5].

3. Result and Discussion

In the present study, comparison and toxicity between NaSe and Inorg Se on the embryonic development of zebrafish was analyzed. The result of the study indicates that with the increased amount of NaSe and Inorg Se, different observable deformities are formed in zebrafish embryos. At the minimum concentration, the treatments showed no such changes and malformation in embryos. However, at high concentrations there were distinguishable negative alterations such as growth retardation, shrinkage of chorion, yolk sac edema, and lack of pigmentation, tail deformities and scoliosis in developing embryos were observed. The embryonic development of the zebrafish has seven broad periods of embryogenesis as the zygote, cleavage, blastula, gastrula, segmentation, pharyngula, and hatching periods as reported [6]. Figure 1 shows the stages of development of embryo at 96hpf. In the zygote stage one cell stage newly fertilized egg showed a swelling in the chorion. During the segmentation the rudiments of the primary organs and the tail bud becomes more prominent and visible with the elongation of the embryo. In the pharyngula period an organized bilateral structure, with a well-developed notochord and a newly completed set of somites that extend to the long post-anal tail which coincides with the earlier report [6].

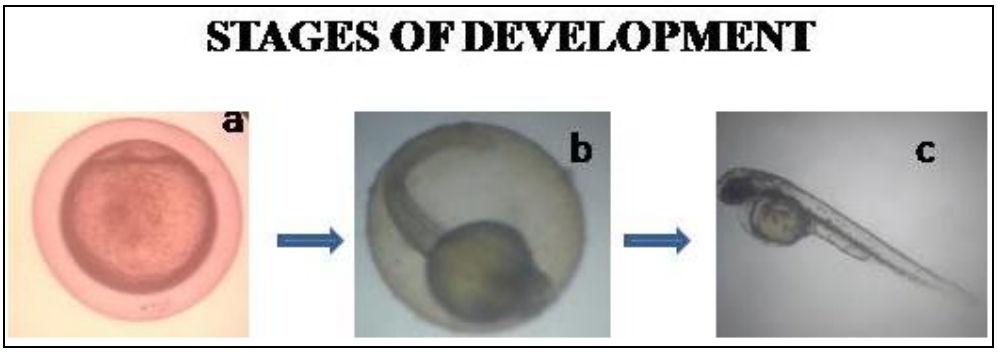

Fig 1: Stages of development of zebra fish a. Zygote stage b. Segmentation C. Pharyngula period

3.1 Effect of selenium nanoparticles on zebra fish embryo

Zebrafish embryos have been used as an *in vivo* model to find out the effect of selenium nanoparticles which could be further used as preclinical drug development for humans and also in the food industry as an additive, for food fortification and as antimicrobial food packaging. The homology of Zebra fish and humans are nearly 85 percent similar in genomic level [1].

The compared toxicity analysis between NaSe and Inorg Se on the embryonic development of zebrafish was analyzed. The effect of NaSe on the viability of zebra fish embryo was also observed (Figure 2) and the percentage over all hatchability was described in Figure 3. There was a minimum number of mortality noted in lower concentrations than with the higher concentrations of NaSe [4]. There was no significant malformation (such as pericardial edema and tail malformation) found in the lower concentrations of NaSe while the both pericardial edema and malformation of the tail were observed at higher concentration of NaSe treated embryos which coincides with [3], as in figure 2. Whereas in inorganic selenium treatment with a lowest dosage (0.2mg/L) showed the delayed hatchability with pericardial edema and tail malformation while at the higher dose of Inorg Se it was observed that the embryos gets coagulated.

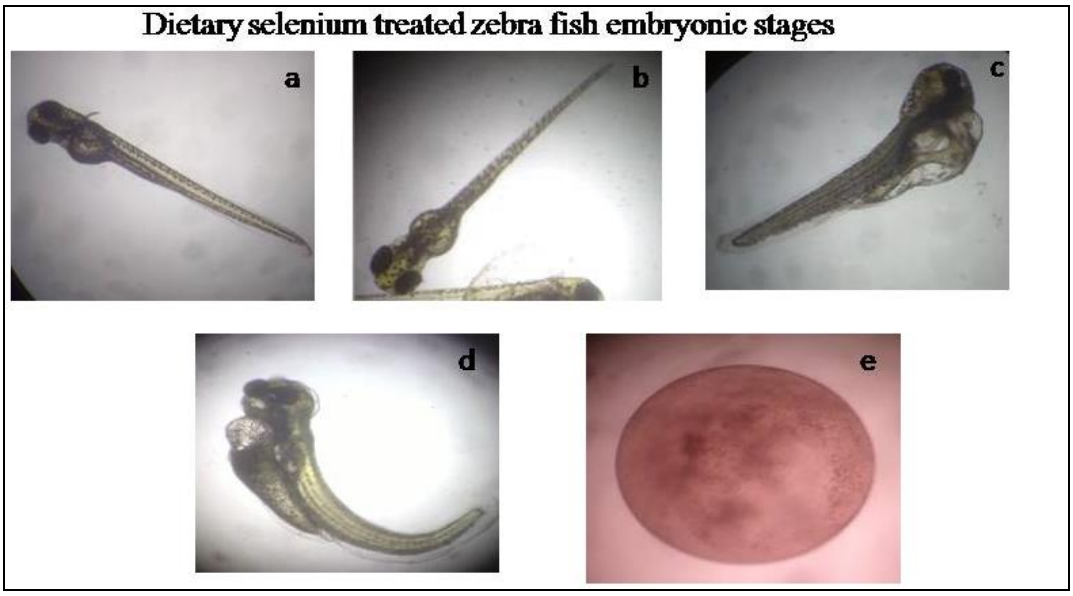

Fig 2: The different concentration of naneselenium dosage in treated zebra fish embryes a) 0.1 mg/L b) 0.2 mg/L c) 0.3 mg/L, concentration of inorganic selenium dosage in treated zebra fish embryes d) inorganic selenium 0.2 mg/L e) inorganic selenima 0.3 mg/L and above

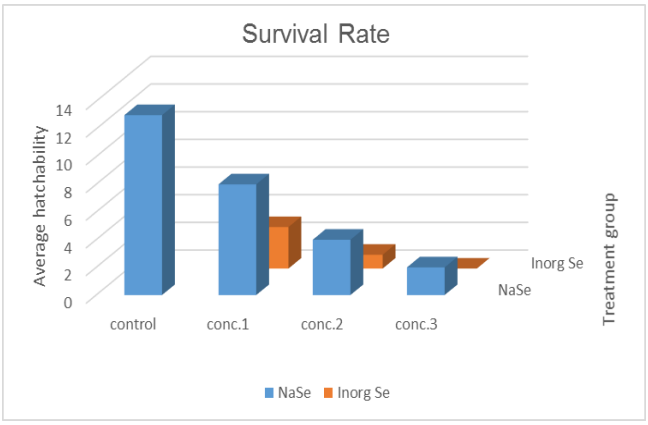

Fig 3: Shows the overall embryo hatchability

3.2 Morphological observations

The NaSe treated embryos exhibited a dose-dependent toxicity under laboratory conditions. The NaSe and Inorg Se showed many variations in our findings. The control groups seem to be normal with an overall mortality of less than 5 percent throughout our study. The Morphological observations such as survival, hatching and heart beat rate of the treated zebrafish embryos were noted from 48hpf to 72 hpf. The heart beat and time taken for hatching were normal in control and NaSe treated groups at the concentration of 0.1mg/L and 0.2mg/L and with the Inorg se of 0.2mg/L dosage. At the higher dose of NaSe the hatching was delayed with low heart beat and edema which correlates with [2] In Inorg Se the eggs chorion was turbid. The malformation of the zebra fish embryos were described in fig 4.

Fig 4: Malformation of zebra fish embryo

4. Conclusion

Green synthesized NaSe using fenugreek seeds showed a significant stability and constant dispersion throughout the test period. Our results suggested that the toxicity of green synthesized NaSe to the developing zebra fish embryos is concentration-dependent. Higher concentration of NaSe and Inorg Se showed a drop in heart rate, high mortality rate and hatching delays were observed in zebrafish embryos. The zebra fish embryos treated with the concentration of upto 0.2mg/L did not show any changes in the percent hatchability and no morphological developmental abnormalities that are observed in inorganic selenium in the same level of incorporation in the embryo culture medium. Hence it is concluded that upto 0.2mg/L dosage is considered to be safe to use further in food industry as a additive or for fortification in foods.

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