

Research Paper

Toxicity and Teratogenic Effects of Zinc Sulfide Nanoparticles on Chick Embryo and Chick Fibroblast Cell Culture



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Citation Asadi A, Abdolmaleki A. [Evaluation of Toxic and Teratogenic Effects of Zinc Sulfide Nanoparticles on Chicken Embryos and Fibroblastic Cells Culture (Persian)]. Quarterly of "The Horizon of Medical Sciences". 2019; 25(4):270-281. <https://doi.org/10.32598/hms.25.4.270>

<https://doi.org/10.32598/hms.25.4.270>



Received: 27 Jan 2019
Accepted: 23 Jul 2019
Available Online: 01 Oct 2019

Key words:

Nanoparticles, Toxicity, Malformation, Chick embryo, Zinc sulfide

ABSTRACT

Aims Nanoparticles (particles with a diameter of 10-500 nm) are currently used in the cosmetics industry as well as for pharmaceuticals, diagnostic imaging, and tissue engineering. Since these nanoparticles are used in industry and drug delivery, they can also be used by pregnant women. Thus, the current study investigated the teratogenic and cytotoxic effects of Zinc Sulfide (ZnS) nanoparticles on the embryo and their fibroblastic cell culture.

Methods & Materials Zinc sulfide (ZnS) nanoparticles were synthesized. Then, nanoparticles at the concentrations of 5, 10, 15, 30, and 40 mg/mL/egg were injected into the air sac of the eggs in three replicates on the third day of incubation. Next, the treated and control eggs, on day 19 of incubation were opened, and embryos were weighted, and the relevant mortality rate was recorded. Fibroblast cells were isolated, cultured, and treated from the control embryo, and morphological changes and cell survival percentages were recorded.

Findings The obtained results revealed that the embryos' survival rate depends on the nanoparticle concentration. As a result, at the highest concentration, only 36.32% of the embryos survived, and the lethal dose 50% (LD50) was equal to 32.47 mg/egg. Morphological study of the treated embryos club foot and skeletal staining suggested the deletion of caudal vertebrae. The cytotoxicity study results of ZnS nanoparticles on fibroblastic cells indicated the survival fractions of 88.45%, 68.75%, and 49.32%, respectively, and its IC50 value was measured as 1460 µM.

Conclusion The present study results suggested that ZnS nanoparticles had no significant toxic effects on the embryos and culture of chicken fibroblastic cells at low concentrations.

Extended Abstract

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1. Introduction

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2. Methods

Asadi et al. evaluated the effects of nano polymer BDP18, as a carrier with slow drug-release property used in cancer treatment on chick embryos. Their results indicated that BDP18 has low toxic and teratogenic effects only at high doses; therefore, it can be used at low concentrations as an effective drug release system [1]. In another study, the toxic and physiological effects of platinum nanoparticles widely used in the treatments were estimated on chick embryo development. The relevant results reflected that these nanoparticles induce apoptosis in the brain tissues [2].

Ninety Ross hatching eggs were obtained from a local company (Arta Jujeh). The research was carried out in the research laboratory of the Biology Department of Mohaghegh Ardabili University in Summer 2018.

Sampling

In this study, a random sampling method was used. Moreover, for each concentration, on average, 3 groups of 5 samples were selected.

Tools

Alizarin red staining, culture medium, Dimethyl Sulfoxide (DMSO), Multi-and Table Tournament (MTT) colorimetric assay were used in the present research.

3. Results

The morphological examination of embryos suggested low abnormalities in the treated specimens; no abnormality was observed up to the concentration of 30 mg/mL per egg. The assessment of the relationship between treatment concentration and frequency of survived embryos revealed a lethal dose 50% (LD50) of 32.47 mg/egg. The results of the effect of 500, 1000, and 1500 μ M ZnS concentrations on chick embryo fibroblastic cells after 16 hours reported the survival fractions of 88.45%, 68.75%, and 49.32% respectively. Therefore, it can inhibit cell growth with an LC50 of 1460 μ M.

4. Discussion

Studies have indicated that embryonic mortality after injection into the egg can be due to the destruction and disruption of embryonic homeostasis, as well as the susceptibility of embryos to their developmental stage [3]. Many researchers have demonstrated the teratogenic effect of antibiotics and growth factors when injected into the egg during the first week of pregnancy [4]. Various nanopar-

ticles have been generated and used as contrast agents in diagnosis and imaging, as well as drug delivery [5].

The effect of ZnS nanoparticles on chick embryos resulted in the skeletal malformations of the caudate vertebrae. This may be due to its direct effect on the loss of embryonic cells growing in the caudate vertebra. In this regard, evaluating the effect of methotrexate in rats suggested that most of the abnormalities in rats were due to the injections of methotrexate confined to caudal vertebrae [6]. Previous studies have indicated that the bones of the lower extremities are more prone to be affected by teratogenic drugs [7]. Barnes et al. (1996) argued that valproic acid reduces the transcription rate of Pax1 in chicken somites [8].

Studnicka et al. examined the effect of nanoparticles of silver/palladium alloy on chicken embryo development; they concluded that these nanoparticles do not affect embryo growth and weight [9]. Bavanilatha and Stanley assessed the effect of silver nanoparticles on chicken embryo. Heart, eye, and liver tissues were examined after chickens were injected on day 18 of incubation. Their results reflected no changes in heart, eye, and liver tissue cells [10]. In another study, teratogenic and cytotoxic effects of salen-vanadium complex on chicken embryos, hepatic, and fibroblastic-cell cultures were evaluated. According to their results, at high concentrations in the culture medium, fibroblastic cells became dense, and their interconnections were loosed [28].

Recommendations

It is recommended to investigate the chemical properties of nanoparticles and provide further information in this respect.

Limitations

There were no limitations to the current study.

5. Conclusion

The threshold effect of ZnS nanoparticles in this study was 32.47 mg/mL per egg. It had no significant effects on the chick embryo at lower doses and no morphological and skeletal teratogenic effects at doses lower than the threshold value. Therefore, when using ZnS in various industries, such as pharmacy, cosmetics production, and other related industries, its threshold level of toxicity and malformations for embryo should be considered.

Ethical Considerations

Compliance with ethical guidelines

All animal experiments were carried out in accordance with local University of Mohaghegh Ardabili (UMA) committee for Human and Animal ethics.

Funding

University of Mohaghegh Ardabili support the financial of this study.

Authors' contributions

Participated in research design: Asadollah Asadi, Conducted experiments: Arash Abdolmaleki; Performed data analysis, wrote or contributed to the writing of the manuscript: All authors.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors would like to thank the Research Council of University of Mohaghegh Ardabili for the financial support of this study.