

Original Research

In vivo effects of quantum dot on organs development before maturity

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Abstract

Objective(s): The field of nanotechnology is rapidly expanding. The development quantum dots quantum dot (QDs), show great promise for treatment and diagnosis of cancer and targeted drug delivery little data on the toxicity of QDs, especially for in vivo applications, are available. As a result, concerns exist over their toxicity for in vivo applications. Then, cytotoxic effects of cadmium selenide (CdSe) quantum dots on organs development before maturity were studied in this study.

Materials and Methods: One month old male Mice treated by injection of CdSe at the doses of 10, 20 and 40 mg/kg.

Structural and optical properties of quantum dots were studied by XRD, UV-Vis absorption spectrum and Scanning Tunneling Microscopy and the number of cells in seminiferous tubes of various groups were analyzed using SPSS 16 program (one way ANOVA test).

Results: Histological studies of testis tissue showed high toxicity of cdse in the dose of 40 mg/kg which followed by decrease in lamina propria thickness, destruction in interstitial tissue, deformation of seminiferoustubes, and reduction in number cells. Also histological study of lung tissue showed in 20 and 40 mg/kg doses destruction in interstitial and epithelium tissues.

Conclusion: On the whole, this study showed high toxicity of cdse on development of testis and lung tissues, even in low doses considering lack of literature review in this field, this study can be an introduction to researches about toxicity effect of quantum dots on development of organs.

Keywords: Cytotoxicity, In vivo, Organs development, Quantum dots

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Introduction

Nanomaterials have found wide applications in biomedicine and biotechnology because of their novel physicochemical properties (1, 2). Among them, quantum dots (QDs) have proven to be ideal optical probes for biological imaging (3) and attracted great attention since the pioneering work of Alivisatos and Nie (4, 5). In comparison with traditional organic fluorophores, QDs possess a number of advantages including high quantum yields, broad absorption spectra, narrow symmetric, size-tunable emission spectra, and exceptional resistance to photo and chemical degradation (4–9).

QDs are nanoparticles which have numerous applications in biology and medicine including markers diagnosis, imaging, drug delivery, gene technology, fluorescent protein labeling in living cells, cell tracking, photodynamic treatment, diagnosis of pathogens and toxins, in vivo imaging of animals, and early diagnosis of cancer because of their special optical and electrical properties and, in case of using these particles in medicine, great changes will occur in curing incurable diseases like cancer and diabetes (14–21, 23, 24, 30). Semiconductor QDs have been considered lately from scientific technological aspects considering their small size, zero dimension structure, unique physical and chemical properties.

Successful use of QDs has been reported in various medical fields but the important point is the high toxicity of core compounds of these nanoparticles which are composed of heavy metals such as cadmium and thallium (15, 21, 25).

Therefore, the study of the toxic effect of QDs is very important for their biological use and it is a decisive factor in their wide use in medicine; hence much attention has been paid to them in recent years (12, 22). If it would determine that the combination of heavy metal has a minor role in the cytotoxicity of QDs, they have a good chance for being used as contrast agents in clinical use (21).

Materials and Methods

Methods of producing CdSe quantum dots

CdSe nanoparticles were synthesized by chemical precipitation method. For this purpose, three solutions of cadmium chloride ($\text{CdCl}_2 \cdot 4\text{H}_2\text{O}$), mercaptoethanol (ME) and sodium selenite ($\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$) were prepared in the distilled deionized water, under vigorous stirring (all from Merck Company). At first, CdCl_2 solution was poured into a three spout balloon container and in the meanwhile, ME solution was added to the same balloon. Finally, sodium selenite solution was added to the balloon by the same way under nitrogen (N_2) atmosphere control condition. The resulting solution was mixed with deionized water and then was centrifuged in order to remove any impurity aggregate. Then, the precipitated sample was dried at room temperature.

All processes were done at room temperature (10). The crystal structure and optical properties of CdSe QDs were characterized by XRD (X-Ray Diffraction, Bruker D8 ADVANCE $\lambda = 0.154$ nm Cu $\text{K}\alpha$ radiation) and UV-Vis spectrophotometer (Ultra Violet – Visible, UV-2600 Shimadzu, Japan). STM (Scanning Tunneling Microscope, NATS-ICO Iran) were used for investigation of particle size distribution. The optical properties of the CdSe nanoparticles were also investigated at different temperatures: 10–70 °C.

Breeding and treatment of animals

Some male mice (about 20 days old) were kept for 10 days in natural day light and temperature 22–24 °C in order to adapt their life cycle to this environment. Then, one month old mice were divided in four groups: control, and treated with 10, 20, and 40 mg/kg doses of CdSe QDs. CdSe nanoparticles were prepared in normal saline solution and were injected intraperitoneally in 10, 20, and 40 mg/kg doses. Control group received only normal saline solution. In this study work with

laboratory animals was approved by the ethics committee of the University.

Tissue preparation

One month after CdSe injection, both control and treatment groups were anesthetized and testis and lung organs were rapidly cut, and preserved in formaldehyde fixative. Five micron slides were dehydrated and prepared in paraffin. Then, the slides were stained using hematoxylin-eosin staining method.

Morphological structure of seminiferous tubes, and average number of spermatogonia, spermatocytes, spermatids, and matured sperms in testis were studied, and epithelial height and morphology and structure of tissue were measured in lung.

Statistical analysis

The data (number of cells in seminiferous tubes of various groups) were analyzed using SPSS 16 program by one way ANOVA followed by Tukey post hoc test. Statistical analysis Data were represented as means \pm SEM. Differences was considered significant at * $p < 0.05$, ** $p < 0.01$.

Results

The results of XRD, STM and UV-Vis absorption spectrum

The structure of the CdSe QDs was investigated by XRD. Fig. 1 put in evidence the XRD pattern of the CdSe QDs. It can be seen that, the sample has a single phase and also a cubic crystal structure.

According to the standard JCPDS (Joint Committee on Powder Diffraction Standards) card No. 19-0191, the diffraction peaks correspond to the (111), (220) and (311) crystal planes.

The mean size of the particles was determined by Debye-Scherrer formula. It was calculated as being of 2.4 nm for CdSe QDs.

Basically, the electronic state is one of the most important properties of a semiconductor and can be described in

terms of valence and conductivity bands and of the gap between these bands. However, as the particles become smaller, the wavelength of the electrons is closer to the range of the particle sizes and the laws of classical physics have to be substituted by quantum confinement or quantum size effect (QSE).

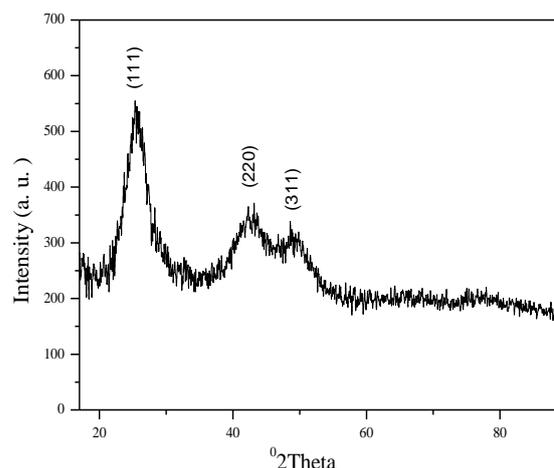


Figure 1. XRD patterns of the CdSe QDs.

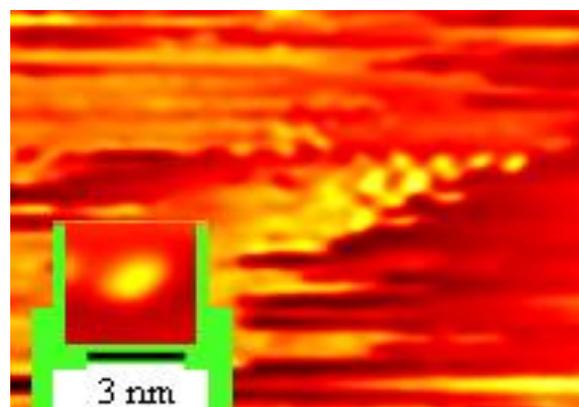


Figure 2. Scanning Tunneling Microscopy of CdSe QDs.

Moreover, many studies have reported the QSE in direct-gap semiconductors such as a shift of the optical absorption edge to higher energies with decreasing size of QDs.

UV-Vis absorption spectra were measured with an UV-visible spectrophotometer.

The UV-Vis absorption spectrum at 10 °C showed that the absorption peak of the CdSe QDs, in aqueous solution, is 420 nm (2.95 eV) whereas for bulk cubic CdSe it is 698 nm (1.78 eV) (1). Therefore,

absorption peak was shifted from red to blue by decreasing the size from bulk to nano-dimension. It is clearly demonstrated the effect of QSE.

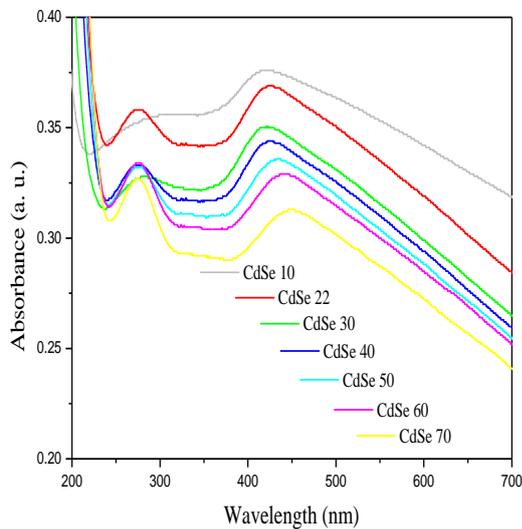


Figure 3. UV-Vis absorption spectrum of CdSe QDs at different temperatures from the range, 10-70 °C.

The CdSe QDs absorption peak, obtained by UV-visible spectrophotometer, is slowly shifted by the temperature increase. It reaches 448 nm (2.76 eV) at 70 °C which represents 28 nm raise in comparison with that recorded at 10 °C. It is probably because of the increase in nanoparticle size which is happened due to the temperature increase. The details of results are listed in Table 1.

The estimated particle size was about 2-3 nm, according to Brus-Equation:

$$\Delta E_g = E_g^{QD} - E_g^{bulk} = \frac{\hbar^2 \pi^2}{2MR^2}$$

where E_g^{QD} and E_g^{bulk} are the energy gap of nanoparticle and bulk respectively, \hbar is the reduced Planck constant, R is the nanoparticle radius, and M is the reduced mass of the electron mass, m_e and of the hole mass, m_h :

$$\frac{1}{M} = \frac{1}{m_e} + \frac{1}{m_h}$$

The Energy band diagram of nanocrystalline CdSe and bulk material are schematically shown in Figure 4.

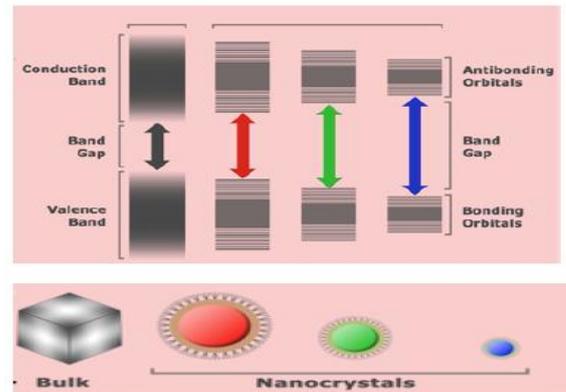


Figure 4. Energy band diagram of nanocrystalline CdSe and bulk materials (11).

When CdSe nanoparticles prepared in different sizes are suspended in a liquid and irradiated with white light each test tube emits light of a different color depending on the size of the nanoparticle as shown in the Fig. 5.

This clearly indicates that the band gap of CdSe changes depending on the size of the nanoparticle.

In fact, smaller sizes have the larger band gaps. These are completely compatible with our results.

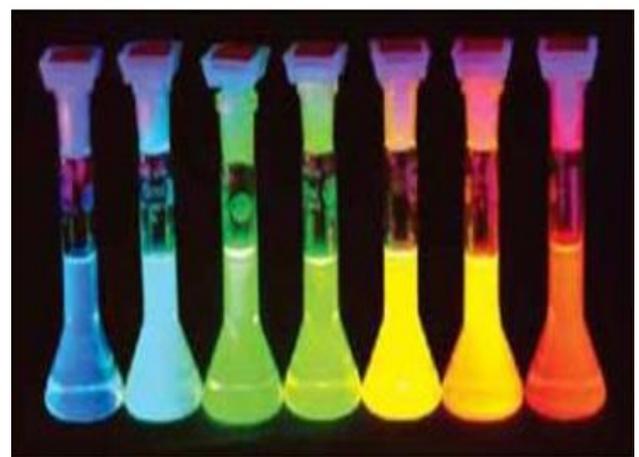


Figure 5. Fluorescence in different-sized CdSe quantum-dots (11).

Histological study of testis

The seminiferous tubules are in different spermatogenic stages in control group

Table 1. The physical properties of the CdSe nanoparticles at different temperatures from 10 to 70 °C.

Temperature °C	Sample	λ_{max} (nm)	E(eV)	Estimated particle size (Brus-Equation) (nm)	Crystal size (nm)
10	CdSe10	420	2.95	2.78	---
22	CdSe22	421	2.94	2.78	---
30	CdSe30	421	2.94	2.78	2.24
40	CdSe40	425	2.91	2.8	---
50	CdSe50	433	2.86	2.9	---
60	CdSe60	440	2.81	2.96	---
70	CdSe70	448	2.76	3.03	---

and in the case of mice treated with 10 and 20 mg/kg QDs, spermatozooids being observed in lumen tubules, but in the group treated with 40 mg/kg QDs, abnormal growth of seminiferous tubes, impaired spermatogenesis, reduction in number of spermatogonia, spermatocyst,

spermatides and obvious decrease in matured sperms of lumen were noticed. Fig 7, 8 and 9 shows these results. On the other hand, degeneration of the interstitial tissue and blood vessels and reduction in thickness of the lamina propria can also be seen (Figure 6).

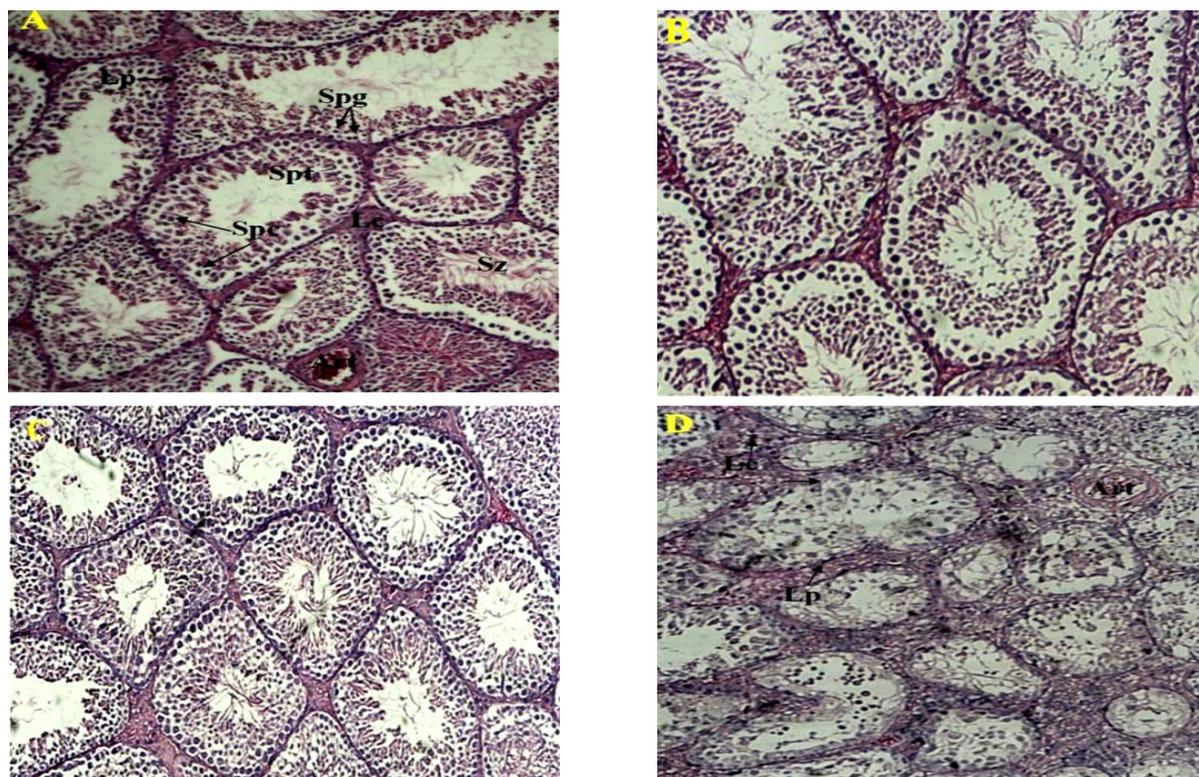


Figure 6. Microscopic images of testis slides, one month after injection (H & E, 400×) (A) Control group and B, C and D treated groups with doses: 10, 20, 40 mg/kg CdSe. (Sz: spermatozoa, Art: artery vessel, Lc: Leydig cells, Lp: lamina propria, Spg: spermatogoni, Spc: spermatocyte, Spt: spermatid).

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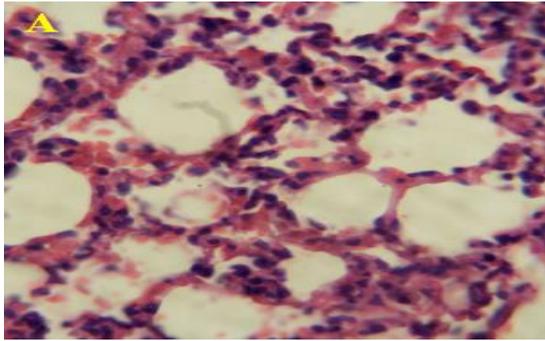


Figure 7. Average and mean comparison of espermatogonia numbers in one tubule one month after injection (One way ANOVA test, Values are means \pm SD, * $p < 0.01$).

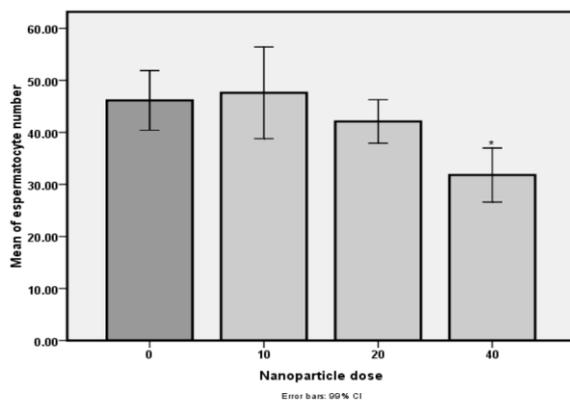


Figure 8. Average and mean comparison of numbers espermatocyte in one tubule one month after injection (One way ANOVA test, Values are means \pm SD, * $p < 0.01$).

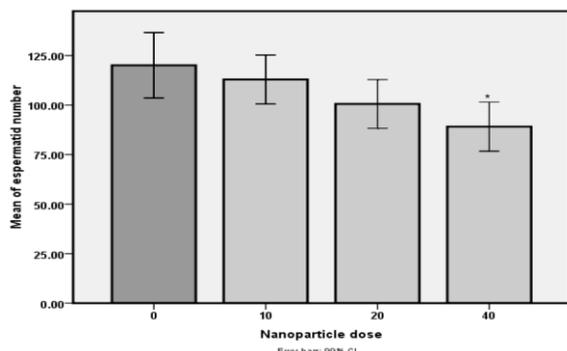


Figure 9. Average and mean comparison of numbers espermatid in one tubule one month after injection (One way ANOVA test, Values are means \pm SD, * $p < 0.01$).

Histological study of lung

To observe the histopathological changes, the lung tissues after one month treatment were stained with HE methods. In the normal group, there were no signs of septal

oedema and inflammation, but in treatments at doses of 20 and 40 mg/kg significantly reduced the infiltration of inflammatory cells and collagen deposition, thickening of the interstitium, and thickening and tearing of basement membrane also a disordered structure of pulmonary alveoli were observed (Figure 10).

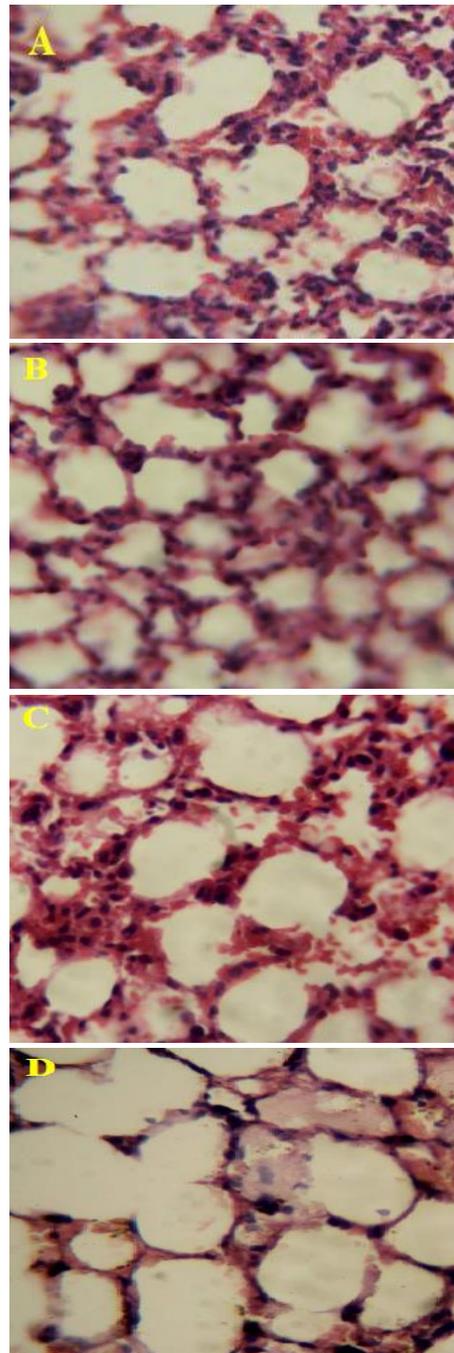


Figure 10. Microscopic images of lung slides, one month after injection (H & E, 400 \times) (A) Control group and B, C and D treated groups with doses: 10, 20, 40 mg/kg CdSe.

Discussion

The researches show that QDs have many applications by conjugation to organic dyes especially because of their unique optical properties (12, 25, 31).

On the other hand, considering their cytotoxic effect of this matter, their high permeability power, connected with high specificity, and high destruction power under UV, they can be used as efficient factors in cancer medication. The interesting point about QDs is their cytotoxicity highly affected by their size ranges, core compositions, and surface coverage (12, 20, 27- 29).

It seems that in vivo synthesis of various types of QDs with high and low toxicities are necessary for different applications, which have been scarcely studied. In this study, the cytotoxicity of uncoated CdSe QDs with 2-3 nm size, synthesized by in vivo sedimentation method was studied. Histopathology studies of testis tissue showed toxicity effect of these nanoparticles in dose of 40 mg/kg. According to these studies, the number of spermatogonia, spermatocytes, spermatids, and matured sperms were decreased, interstitial tissue was degenerated, and Leydig cell number was reduced. Also, the histology study of lung tissue showed a high degeneration in lung epithelium, in the case of 40 mg/kg dose of CdSe QDs.

Cytotoxic effect of CdSe QDs on lung tissue and testis of animals has been studied for the first time in this research. Considering these results, we can say that nanoparticles are able to cross blood barrier-testicular and cause intensive direct destruction in germinal cells and spermatogenesis and lung tissue of mice. However, more studies are necessary in this field in order to identify effective background mechanism of QDs cytotoxicity.

Results of other studies about effect of other different nanoparticles on reproduction system have showed also a cytotoxic effect. Treatments with TiO₂, Gold, and C60 nanoparticles for pregnant

women is one of the previous studies incriminating cytotoxic effects on spermatogenesis and histopathology changes of testis in their male children. In vitro studies showed also cytotoxic effect of TiO₂ and carbon black (CB) nanoparticles on living power of mice Leydig cells. Gold nano-particles decrease movement of matured sperms, silver and aluminum nanoparticles being toxic for stem cells of rat spermatogonia (26).

Conclusion

In vivo high throughput toxicity of quantum dots in this study are worrying also show long way Existence in biological use of them in spite of their obvious advantages in medicine. Lately, quantum dots have been considered for photodynamic therapy of cancer. As a result, it seems that more research is required on quantum dot toxicity synthesized by different methods and covering a variety. Further research is required to study the toxicity of quantum dot synthesized by different methods.

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