Cytotoxicity effects of CdSe quantum dots on testis development of laboratory mice

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Background: There are many promising progresses in biological diagnosis and using quantum dots in nanostructures and multipurpose tools. Nevertheless, *in vivo* cytotoxicity of these nanoparticles has not been highly considered. For this reason, the cytotoxic effects of CdSe quantum dots on testis development before maturity are presented in this study.
Materials and Methods: In this work, 10, 20 and 40 mg/kg doses of CdSe quantum dots were injected to some one month old male mice. 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 programme (one way Anova test).
Results: Histological studies of testis tissue showed a high toxicity of CdSe in 40 mg/kg dose followed by a decrease in *lamina propria*, destruction in interstitial tissue, deformation of seminiferous tubes, and reduction in number of spermatogonia, spermatocytes, spermatides, and matured sperms. Although histological study of epididymis tissue showed no significant effect of quantum dots on morphology and structure of tube and its covering epithelium there was a considerable reduction in the lumen content.
Conclusion: This study showed a high toxicity of CdSe quantum dots on development of testis tissue, even in lower doses and considering lack of literature review in this field, this study can be an introduction to researches of toxicity effect of quantum dots on development of male reproduction system.

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Keywords: CdSe, Quantum dots, Testis development, Cytotoxicity

1. Introduction

QDs (Quantum dots), also known as colloidal semiconductor nanocrytals, are generally composed of II-VI and III-V groups of table of elements. Their optical and electrical properties are strongly size dependent [1-4]. High quality semiconductor nanocrystals have many applications such as thin film light-emitting devices, non-linear optical devices, solar cells and life science [5-11]. On the other hand, most of chemical materials used for their production are toxic, expensive, and even explosive.

With the advancement of nanotechnology many nanoparticles have been widely used in biology and medicine [12, 13]. 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 especial optical and electrical properties and, in case of using these particles in medicine, great changes will occurr 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].

2. Materials and methods

2.1 Methods of producing CdSe quantum dots

CdSe nanoparticles were synthesized by chemical precipitation method. For this purpose, three solutions of cadmium chloride ($CdCl_2.4H_2O$), mercaptoethanol (ME) and sodium selenite ($Na_2SeO_3.5H_2O$) 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 andin 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 λ = 0.154 nm Cu Kα radiation) and UV-Vis spectrophotometer (Ultra Violet – Visible, UV-2600 Shimadzu, Japan). STM (Scanning Tunneling Microscope, NATSICO 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.

2.2 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 to adapt their life cycleto this environment. Then, one month old mice were divided in three 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 of CdSe. Control group received only normal saline solution.

2.3 Tissue preparing

One month after CdSe injection, both control and treatment groups were anesthetized and testis and epididymis organs were rapidly cut, weighted, and preserved in formaldehyde fixative. Five micron slides were dehydrated and prepared in paraffin. Then, the slides were coloured 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 sperm density were measured in epididymis.

2.4 Statistical analysis

The data (the number of cells in seminiferous tubes of various groups) were analyzed using SPSS 16 program (one way Anova test).

3. Results

3.1 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-Scherer 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).

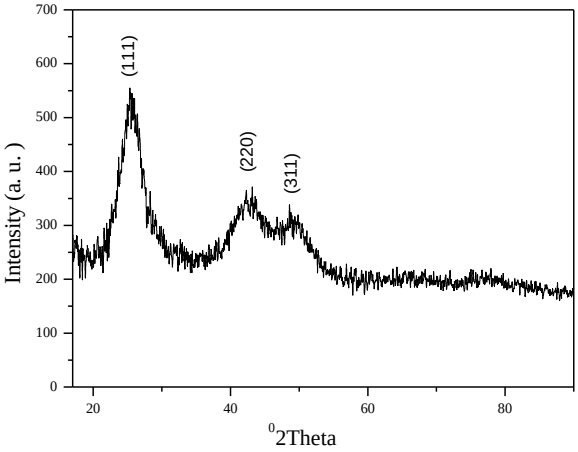

Fig. 1. XRD patterns of the CdSe QDs.

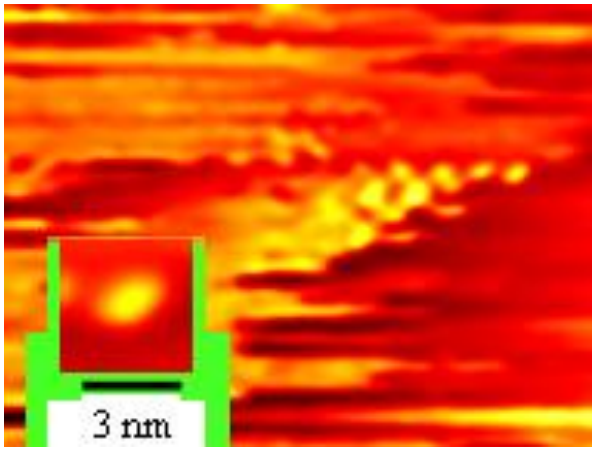

Fig. 2. Scanning Tunneling Microscopy of CdSe QDs.

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 sizeof QDs.

UV-Vis absorption spectra were measured with an UV-visible spectrophotometer. Fig. 3 indicates the absorption spectrum of CdSe QDs at different temperatures from 10 to 70 °C. 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.

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 massof the electron mass, m_e and of the hole mass, m_h:

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

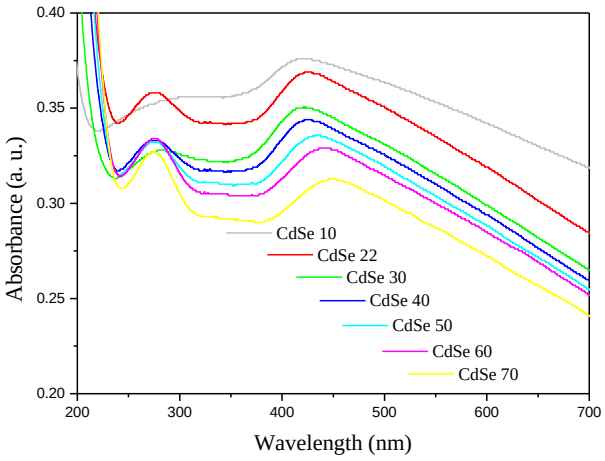

Fig. 3. UV-Vis absorption spectrum of CdSe QDs at different temperatures from the range, 10-70 °C.

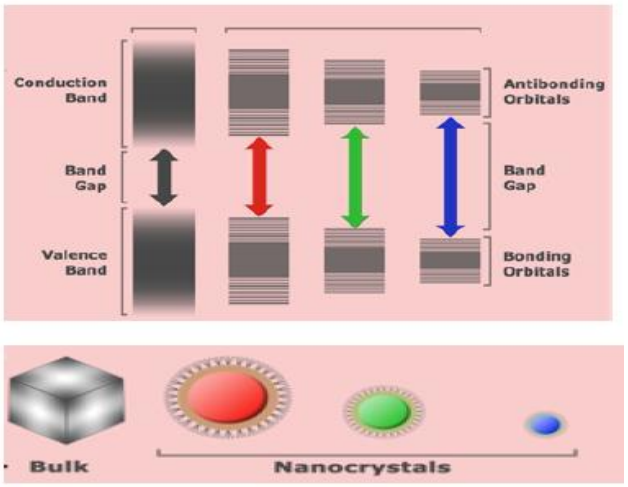

Fig. 4. Energy band diagram of nanocrystalline CdSe and bulk materials [11].

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

Fig. 5. Fluorescence in different-sized CdSe quantum-dots [11].

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	---

3.2. Histological study of testis

The seminiferous tubules are in different spermatogenic stages in groups control, and in the case of mice treated with 10 and 20 mg/kg QDs, spermatozoids being observed in lumen tubules, but in the group treated with 40 mg/kgQDs, abnormal growth of seminiferous tubes, impaired spermatogenesis, reduction in number of spermatogonia, spermatocyst 1, spermatides, and obvious decrease in matured sperms of lumen were noticed. Table 2 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 (Fig. 6).

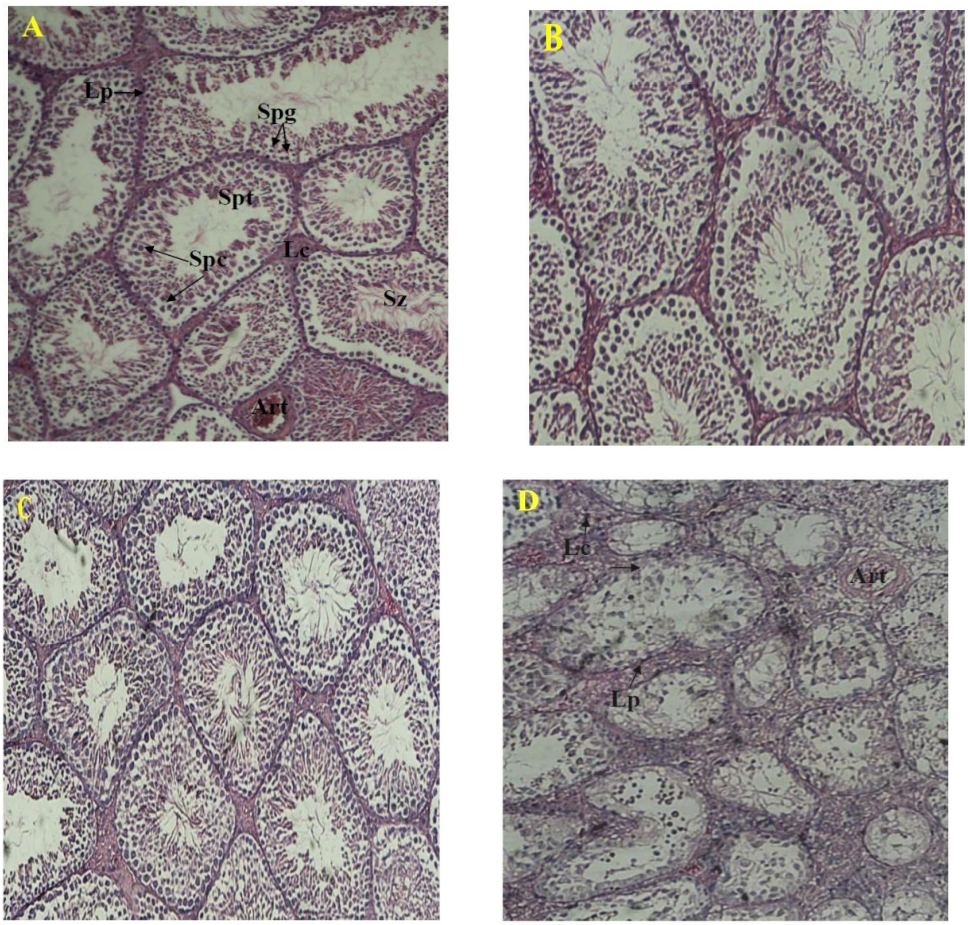

Fig. 6. Microscopic images of testis slides, one month after injection (H & E, 400×) (A) Control group and B, C,and D treated groups with doses: 10, 20, 40 mg/kg CdSe. (Sz: spermatozoa, Art: artery vessel, Lc: Leydig cells, Lp: lamina propria, Spg: espermatogoni, Spc: espermatocyte, Spt: espermatid).

Qualitative studies using optical microscope showed that epithelia of epididymis were similar in treated groups and control, but spermatozoa volume in lumen epididymis was obviously decreased in mice treated with 40 mg/kg of CdSe doses (Fig. 7).

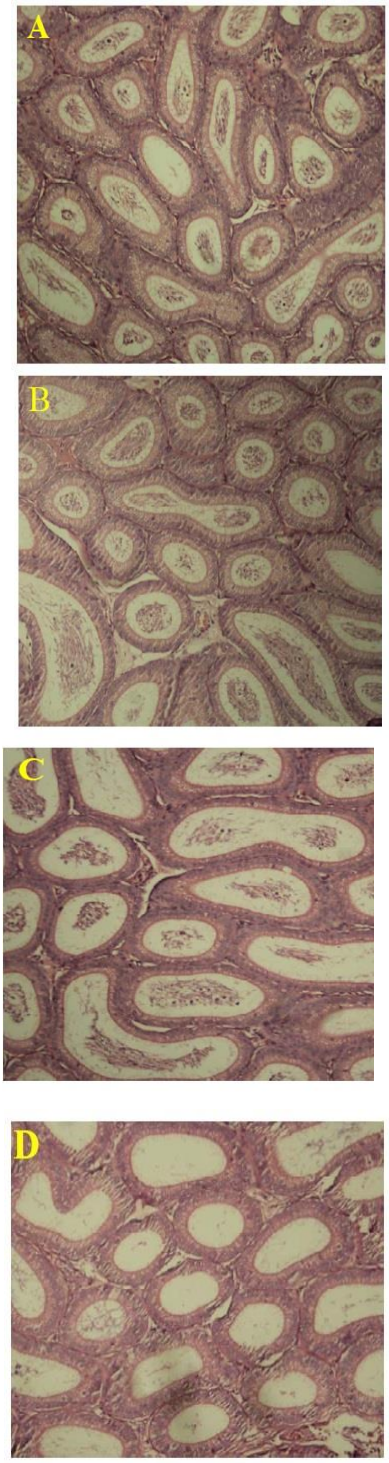

Fig. 7. Microscopic images of slides of kidney tissues, one month after injection (H & E, 400×) (A) control group and B, C, D treated groups (with doses: 10, 20, 40 mg/kg CdSe).

Table 2. Average and mean comparison of sperm stem cell numbers in one tubule one month after injection ($p < 0.01$).

Parameter	Groups (n =12 mice)			
	Control	10 mg/kg	20 mg/kg	40 mg/kg
Mean spermatogonia	35 ± 7.4	37 ± 7.10	32 ± 7.32	20* ± 5.41
Mean spermatocyte I	46 ± 8.9	47 ± 9.70	42 ± 6.53	31* ± 8.10
Mean spermatid	120 ± 25.0	112 ± 19.31	100 ± 19.20	89* ± 19.40

4. 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, s which has been scarcely studied. In this study, thye citotoxicity 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 40 mg/kg dose. According to these studies, the number of spermatogonia, spermatocytes, spermatids, and matured sperms in seminiferous tubes were decreased, interstitial tissue was degenerated, and Leydig cell number was reduced. Also, the histology study of epididymis tissue showed a high reduction in sperm volume in lumen epididymis, in the case of 40 mg/kg dose of CdSe QDs.

Cytotoxic effect of CdSe QDs on epididymis tissue and testis of animals has been studied for the first time in this research. Results of other studies about effect of other different nanoparticles on reproduction system have showedalso a cyctotoxic effect. Treatments with Tio2, Gold, and C60 nanoparticles for pregnant women is one of the previous studies incriminating cyctotoxic effects on spermatogensis and histopathology changes of testis in their male children. *In vitro* studies showed also cyctotoxic effect of TiO_2 and carbon black (CB) nanoparticles on living power of mice Leydig cells. Gold nanoparticles decrease movement of matured sperms, silver and

aluminum nanoparticles being toxic for stem cells of rat spermatogonia [26].

4. Conclusion

Considering these results, we can say that nanoparticles are able to cross blood barrier-testicular and impose severe toxicity effects on the reproductive system. In exchange, the epithelium epididymis did not show any special histopathology changes in CdSe ODs treatments,but the lumen content explains many testis problems. Histopathology results of this study showed that CdSe nanoparticles can cross blood barrier-testicular and cause intensive direct destruction in germinal cells and spermatogenesis of mice. However, more studies are necessary in this field in order to identify effective background mechanism of QDs cytotoxicity.

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