

A comparative study about toxicity of CdSe quantum dots on reproductive system development of mice and controlling this toxicity by ZnS coverage

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ABSTRACT:

Objective(s): Medicinal benefits of quantum dots have been proved in recent years but there is little known about their toxicity especially in vivo toxicity. In order to use quantum dots in medical applications, studies on their in vivo toxicity is important.

Materials and Methods: CdSe:ZnS quantum dots were injected in 10, 20, and 40 mg/kg doses to male mice 10 days later, mice were sacrificed and five micron slides were prepared structural and optical properties of quantum dots were evaluated using XRD.

Results: Histological studies of testis tissue showed high toxic effect of CdSe:ZnS in 40 mg/kg group. Histological studies of epididymis did not show any effect of quantum dots in terms of morphology and tube structure. Mean concentration of LH and testosterone and testis weight showed considerable changes in mice injected with 40 mg/kg dose of CdSe:ZnS compared to control group. However, FSH and body weight did not show any difference with control group.

Conclusion: Although it has been reported that CdSe is highly protected from the environment by its shell, but this study showed high toxicity for CdSe:ZnS when it is used in vivo which could be suggested that shell could contribute to increased toxicity of quantum dots. Considering lack of any previous study on this subject, our study could potentially be used as a basis for further extensive studies investigating the effects of quantum dots toxicity on development of male sexual system.

Keywords: CdSe:ZnS, In vivo toxicity, Quantum dots, ZnS shell

INTRODUCTION

Nowadays, nanotechnology is widely known and has many applications including in medicine [3]. With advances in nanotechnology, many nanoparticles have been used widely in biology and medicine. Cytotoxicity of these particles is an important factor in their use in

medicine and hence has received a great deal of attention in recent years [2, 27].

Among nanoparticles, quantum dots (QDs) are considered as extremely interesting materials for the development of photovoltaic devices because of their potential in medical diagnosis, cell imaging, and as biological tracers in human diseases diagnosis [3, 19, 27]. But, in spite of obvious advantages of QDs, concerns regarding human exposure to these particles and its effect on human health have increased.

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Therefore, studies have been conducted on reaction of QDs in vivo and in vitro but there are still limited data on this category, which has led to not using dots in medications as therapeutic or diagnostic tools. So, further studies are needed to investigate QDs in the environments similar to human body [4, 9, 18]. Among semiconductor nanocrystal QDs, the CdSe core nanocrystals QDs have shown the best optical properties in light stability and radiative quantum efficiency in optical tests [4, 7, 19] Coolen et al study demonstrated that CdSe:ZnS nanocrystal QDs are the most widely used QDs [7].

Most studies reported CdSe, which is a semiconductor matter, as core of nanocrystals QDs covered by another semiconductor matter (mainly ZnS). On the other hand, in vitro studies have shown an approximately complete control of CdSe-induced cytotoxicity by ZnS coverage [7, 11, 12, 23, 29].

Therefore, CdSe and CdSe:ZnS nanocrystal QDs were selected for this study. Because of their importance as semiconductor and in biology and medicine, which has been highlighted in different studies, the synthesized QDs were characterized and their in vivo toxicity effects on male sexual system were also investigated.

MATERIALS AND METHODS

Materials

All chemicals and solvents were purchased from Merck and were of analytical grades.

Preparation of CdSe and CdSe:ZnS QDs

Quantum dots were synthesized by chemical precipitation method. Briefly, 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. At first, CdCl_2 solution was poured into a three-spout balloon container and simultaneously ME solution was added to the same balloon. Finally, Na_2SeO_3 solution was added under nitrogen. The resulting solution was mixed with deionized water and then centrifuged in order to remove any impurity aggregate. Then, the precipitated sample was dried at room temperature. All processes were done at room temperature [1].

The crystal structure and optical properties of CdSe:ZnS QDs were characterized by XRD (X-Ray Diffraction, Bruker D8 ADVANCE $\lambda = 0.154$ nm Cu K radiation) and UV-Vis spectrophotometer (Ultra Violet Visible, UV-2600 Shimadzu, Japan). Scanning Tunneling

Microscope (STM) (NATSICO, Iran) was used to investigate particle size distribution.

Breeding and treatment of animals

Male Balb/C mice were purchased from Razi Vaccine and Serum Research Institute (Hesarak, Iran). Some male mice (about 20 days old) were kept for 10 days in natural day light and at temperature 22-24°C. Then, male mice were divided in 7 groups of 6 mice: control, three groups injected with CdSe at 10, 20, and 40 mg/kg and three groups injected with CdSe:ZnS QDs at 10, 20, and 40 mg/kg doses. Either CdSe or CdSe:ZnS nanoparticles were prepared in normal saline solution and single-dose was injected intraperitoneally at 10, 20, and 40 mg/kg doses. Control group received only normal saline solution. The protocol of study was approved by the Ethics Committee of the University.

Tissue preparing

10 Days after CdSe and CdSe:ZnS injection, all control and treatment groups were anesthetized and testis and epididymis organs were rapidly removed, weighed, and preserved in formaldehyde as fixative agent. Five micron slides were dehydrated and prepared in paraffin. Then, the slides were stained using hematoxylin-eosin. Morphological structure of seminiferous tubes, and mean number of spermatogonia, spermatocytes, spermatids, and matured sperms in testis were studied, and epithelial height, connective tissue, smooth muscle and sperm density were measured in epididymis.

Hormones measurements

Serum FSH and LH were measured by double antibody radioimmunoassay as previously described [20, 21]. Serum testosterone was assayed using a competitive chemiluminescent immunoassay kit (DRG Co, Germany; Pishtaz Teb, Iran).

(testis and body weight and the number of cells in seminiferous tubes of various groups) were analyzed using SPSS 16 (by one way ANOVA followed by Tukey's post hoc test.). Data were represented as means \pm standard error. Differences were considered significant at $p < 0.05$ and $p < 0.01$.

RESULTS

QDs characterization by XRD, STM and UV-Vis analysis

The structure of the CdSe QDs was investigated by XRD. Fig 1 illustrates the XRD pattern of the CdSe

QDs. As seen, the sample has a single phase and also a cubic crystal structure.

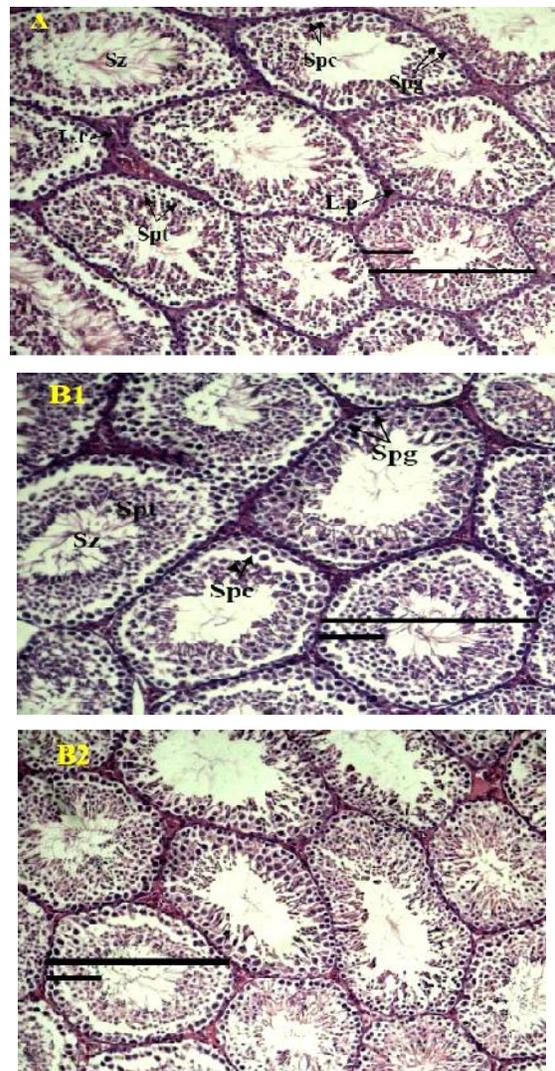
According to the standard Joint Committee on Powder Diffraction Standards card No. 19-0191, the diffraction peaks correspond to the 111, 220 and 311 crystal planes. The mean size of the particles was determined by Debye-Scherrer formula. It was calculated as 2.4 nm for CdSe QDs [1].

Dose-finding study

there was no study on in vivo toxicity of QDs, we selected three doses of 125, 250, and 500 mg/kg according to an in vitro study conducted by Ming-Shu Hsieh [13]. In the first stage, CdSe and CdSe:ZnS nanoparticles were prepared in normal saline solution and were intraperitoneally injected into 36 mice. All mice died in less than 24 hours after the injection. The remarkable finding was that in vivo administration of ZnS as the shell increased toxicity of CdSe and mice showed greater responses to CdSe:ZnS compared to CdSe. These results were contrary to what has been reported for the cytotoxicity of CdSe:ZnS where the ZnS shell would minimize the cell toxicity of the CdSe core. The CdSe:ZnS-treated mice died within a short time after ip injection of the QDs and even in some cases mice died almost immediately after the injection. In the next step, DMSA-coated CdSe nanoparticles were injected to groups of 8 mice each intraperitoneally at 125 and 250 mg/kg doses. The results showed that all mice injected with DMSA-coated CdSe at 250 mg/kg dose and 80% of mice injected with DMSA-coated CdSe at 125 mg/kg dose died within one week post-injection. Then, CdSe and CdSe:ZnS were intraperitoneally injected to groups of 16 mice each at 75 and 100 mg/kg doses. In CdSe:ZnS groups at 75 and 100 mg/kg doses 50% and 83% of mice died, respectively whereas in CdSe groups at 75 and 100 mg/kg doses 50% and 66% of mice died within one week, respectively. Finally, lower doses of CdSe and CdSe:ZnS were selected and injected to group of 4 mice each. In CdSe groups, no mortality was observed during 20 days post-injection at all three doses of 10, 20 and 40 mg/kg. However, in CdSe:ZnS groups, while no mortality was observed at 10 mg/kg group but 12.5 and 20% mortality was observed, respectively. According to the obtained results in dose-finding study, 10 mg/kg dose was determined as a safe dose and 20 and 40 mg/kg doses were determined as doses with toxicity effects.

Histological study of testis

The seminiferous tubules are in different spermatogenic stages in control group, and in mice treated with all three doses of CdSe and CdSe:ZnS QDs. Spermatozooids were observed in lumen tubules of the control group, but in the group treated with 40 mg/kg CdSe:ZnS QDs, abnormal growth of seminiferous tubes, impaired spermatogenesis, reduction in number of spermatogonia, spermatocyst 1, spermatides and an obvious decrease in matured sperms of lumen were observed (Table 2). On the other hand, degeneration of the interstitial tissue and blood vessels and reduction in thickness of the lamina propria were observed as illustrated in Fig 1.



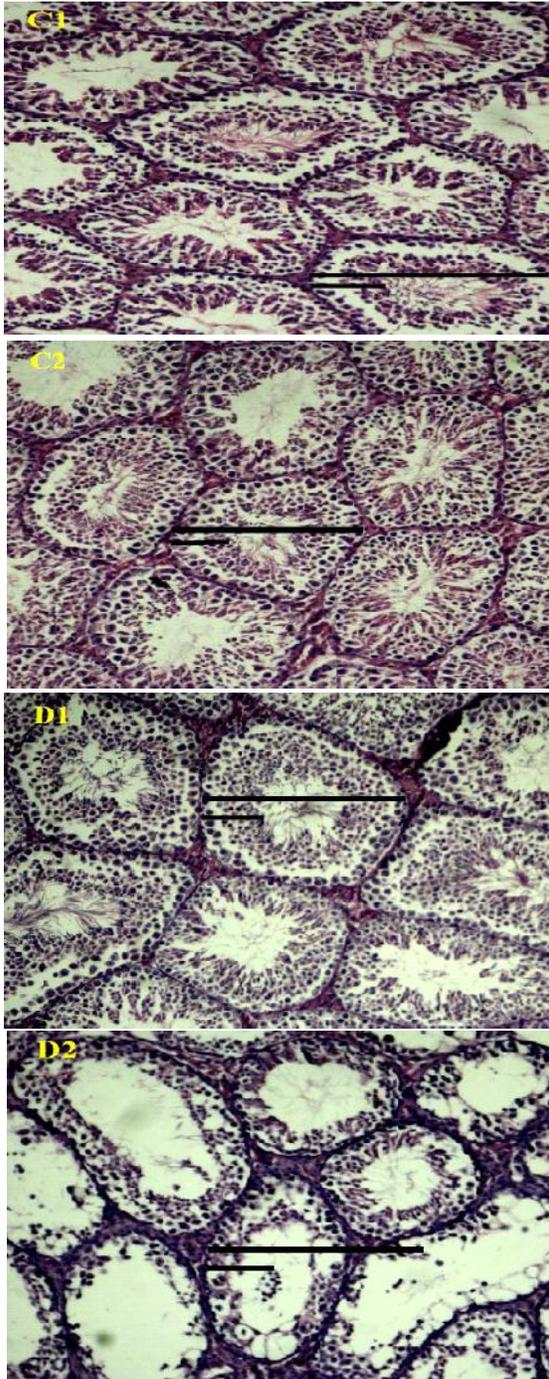
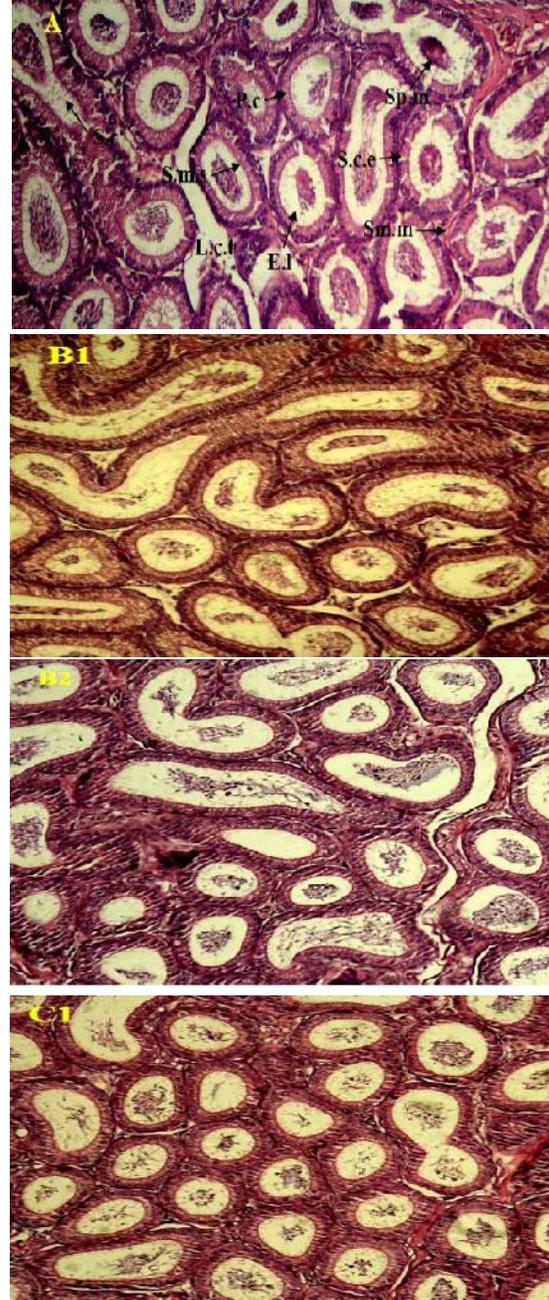


Fig. 1. Microscopic images of testis slides, 10 days post-injection (H & E, 400×). A control group; B1, C1 and D1 groups treated with doses of 10, 20, 40 mg/kg CdSe, respectively; B2, C2 and D2 groups treated with doses of 10, 20, 40 mg/kg CdSe:ZnS, respectively. Sz: spermatozoa, Lc: Leydig cells, Lp: Lamina propria, Spg: Espermatogoni, Spc: Espermatocyte, Spt: Espermatid

Histological study of epididymis

Qualitative studies using optical microscope showed that epithelia, lumen, connective tissue and smooth muscle of epididymis were similar in groups treated with the QDs and control, but spermatozoa volume in lumen epididymis obviously decreased in mice treated with CdSe:ZnS at 40 mg/kg (Fig 2).



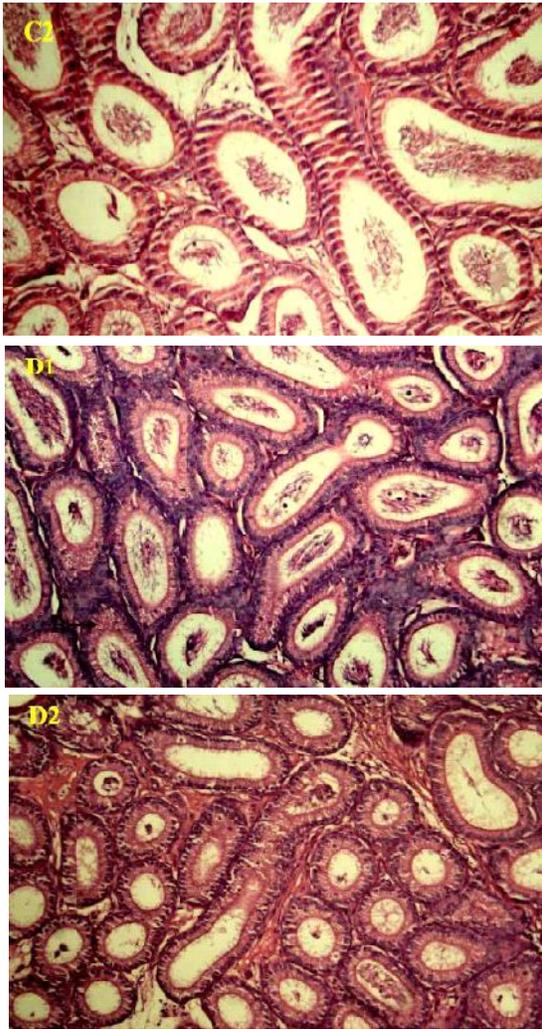


Fig. 2. Microscopic images of epididymis slides, 10 days post-injection (H & E, 400×). A control group; B1, C1 and D1 groups treated with doses of 10, 20, 40 mg/kg CdSe, respectively; B2, C2 and D2 groups treated with doses of 10, 20, 40 mg/kg of CdSe:ZnS, respectively. SPM: Sperm mass, SMM: Smooth muscle, SC: Stereocilia, PC: Principal cells, SCE: Stereociliated simple columnar epithelium, LE: Lumen epididymis, LCT: Loose connective tissue

Changes in body and testis weight, and hormones level

Body weight did not change significantly in treatment groups but testis weight decreased significantly ($P < 0.01$) by CdSe:ZnS at 40 mg/kg. FSH did not change by treatments with QDs (Table 2) but testosterone decreased significantly and LH increased significantly in mice treated with CdSe:ZnS at 40 mg/kg (Fig 3, 4, 5).

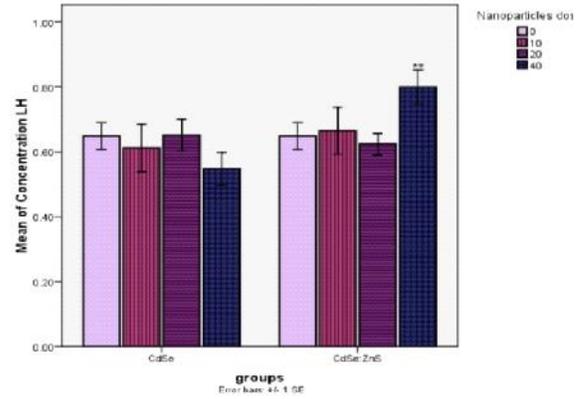


Fig. 3. Mean of concentration LH in 10 days post-injection (** $p < 0.01$, * $p < 0.05$)

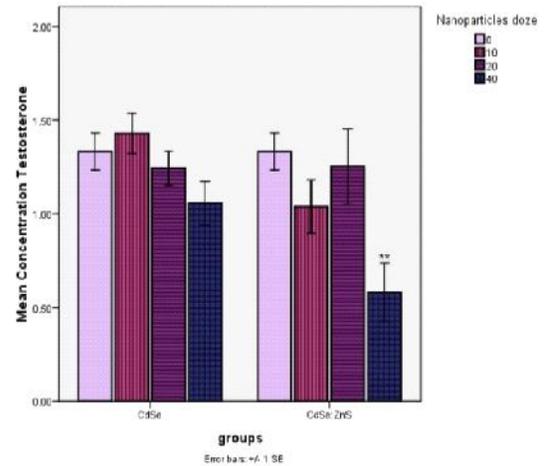


Fig. 4. Mean of concentration testosterone in 10 days post-injection (** $p < 0.01$, * $p < 0.05$)

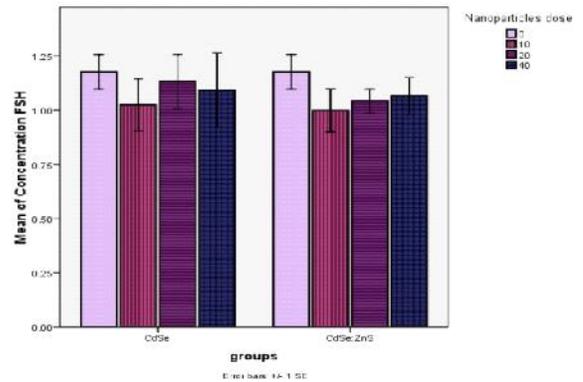


Fig. 5. Mean of concentration FSH in 10 day post-injection (** $p < 0.01$, * $p < 0.05$)

Table 1. Average and mean comparison of sperm stem cell numbers in one tubule 10 days post-injection ($p < 0.01$)

| groups | Nanoparticle doses | Mean of spermatogonia | Mean of spermatocyte I | Mean of spermatid |
|----------|--------------------|-----------------------|------------------------|-------------------|
| Control | 0 mg/kg | 34.55 ± 6.39 | 44.15 ± 9.35 | 111.95 ± 33.63 |
| | 10 mg/kg | 35.50 ± 7.55 | 45.05 ± 9.76 | 100.45 ± 18.64 |
| CdSe | 20 mg/kg | 35.95 ± 6.76 | 46.10 ± 8.58 | 100.85 ± 24.96 |
| | 40 mg/kg | 34.30 ± 7.55 | 45.70 ± 7.01 | 99.10 ± 24.68 |
| | 10 mg/kg | 33.60 ± 8.94 | 45.25 ± 8.45 | 113.65 ± 23.29 |
| CdSe:ZnS | 20 mg/kg | 32.80 ± 6.67 | 43.80 ± 8.04 | 109.15 ± 20.72 |
| | 40 mg/kg | 18.85** ± 6.94 | 29.60** ± 10.76 | 83.00** ± 23.44 |

Table 2. Average and mean comparison of body and testis in 10 days post-injection (** $p < 0.01$, * $p < 0.05$)

| groups | QDs doses | Mean of body weight | Mean of testis weight |
|----------|-----------|---------------------|-----------------------|
| Control | 0 mg/kg | 27.17 ± 1.3 | .093 ± .01 |
| | 10 mg/kg | 25.98 ± 1.6 | .087 ± .01 |
| CdSe | 20 mg/kg | 27.67 ± 3.9 | .087 ± .01 |
| | 40 mg/kg | 26.83 ± 1.4 | .083 ± .02 |
| | 10 mg/kg | 27.33 ± 2.8 | .087 ± .01 |
| CdSe:ZnS | 20 mg/kg | 26.83 ± 2.1 | .107 ± .02 |
| | 40 mg/kg | 27.67 ± 1.3 | .055** ± .01 |

Discussion

Recently, there are a lot of reports on the use of nanoparticulate systems in biomedical applications [14, 30]. Among these nanoparticles, crystals with nanometer size, which are called mainly QDs, have been studied extensively for molecular imaging and targeted therapy. Because QDs are fluorescent nanoparticles with high sensitivity using them as probe is a promising technique for biological applications. Due to large area provided by QDs, they are appropriate as targeting ligand to direct them to site of action [13, 21]. Moreover, because of high light stability which provides long time observation of biological molecules, QDs could be the main hope as label for biological systems imaging. Therefore, the use of QDs as bright and photostable probes for long-term fluorescence imaging is gaining more interest [13, 27]. Studies about the physicochemical properties of QDs and their use in vivo are important. There are a few studies about the effects of QDs on testis. In some studies, exposing young mice to DE (Diesel Exhaust) and silver induced degeneration of Leydig cells action and hence fluctuation of testosterone level [11, 15, 32]. Guo et al reported reduction in density and movement of spermatozooids, increase in spermatozooids abnormalities, and apoptosis of cells in mice injected by TiO₂ nanoparticles. Histopathological studies of testis and epididymis did not show any change [12]. In another study by Wen-Qing, gold nanoparticles (14 nm) with two -methoxy and -aminothyl polyethylene glycol covers passed testis blood barrier, entered to germ cells and increased testosterone [33]. Recently, effects

of nanoparticles smaller than 100 nm (like DEP) (Diesel Exhaust Particles) have been studied. Results indicated separation of cells of seminiferous epithelium [26, 33].

Sleiman et al examined the adverse productive toxic effects of AgNPs in male Wistar rats exposed to AgNP (Silver Nanoparticles) during the prepubertal period and sacrificed at postnatal days 53 and 90. The weight of the animals at the postnatal day 90 did not change markedly, but growth was less in the group treated with AgNP at 50 µg/kg from the postnatal day 34 to 53. AgNP exposure produced a delay in puberty in both treated groups. Decreased sperm reserves in the epididymis and diminished sperm transit time were observed at the postnatal day 53, while a reduction in sperm production occurred at the postnatal day 90. The morphology of the seminiferous epithelium was markedly altered and data demonstrated that prepubertal exposure to AgNP altered reproductive development in prepubertal male Wistar rats, as evidenced by impairment in spermatogenesis and a lower sperm count in adulthood [27].

Mathias et al showed AgNPs reduced the acrosome and plasma membrane integrities, reduced the mitochondrial activity and increased the abnormalities of the sperm in both treatment groups. AgNP exposure also delayed the onset of puberty, although no changes in body growth were observed in the treatment groups. The animals did not show changes in sexual behavior or serum hormone concentrations

[18]. In another study, AgNPs (Silver Nanoparticles) at low dose (1 mg/kg) were intravenously injected into male CD1 mice over 12 days. Treatment resulted in no changes in body and testis weights, sperm concentration and motility, fertility indices, or follicle-stimulating hormone and luteinizing hormone serum concentrations; however, serum and intratesticular testosterone concentrations were significantly increased 15 days after initial treatment. Histologic evaluation revealed significant changes in epithelium morphology, germ cell apoptosis, and Leydig cell size. Additionally, gene expression analysis revealed that *Cyp11a1* and *Hsd3b1* mRNA were up regulated significantly in treated animals. These data suggest that AgNPs do not impair spermatogonial stem cells in vivo since treatment did not result in significant decreases in testis weight and sperm concentrations. However, AgNPs appear to affect Leydig cell function, leading to increase in testicular and serum testosterone levels [11]. In vitro studies revealed high concentrations of TiO_2 nanoparticles affected the viability and proliferation of mouse Leydig cells, but not the gene expression associated with spermatogenesis [20]. And gold nanoparticles decreased the motility of human sperm. Also, silver, aluminum, and MoO_3 nanoparticles were toxic to mouse spermatogonia stem cells. Silica nanoparticles inhibited the differentiation of mouse ES cells and C60 -based nanoparticles inhibited the differentiation of mouse midbrain cells, CB (Carbon Black) decreased the viability of mouse Leydig cells and CdSe QDs inhibited the pre- and post-implantation development of mouse embryos [13, 23, 31, 32]. In vitro studies have shown cytotoxic effect of TiO_2 and CB (Carbon Black) nanoparticles on viability of mice Leydig cells, as well [9, 12, 15, 33]. Also injection of TiO_2 , gold, diesel exhaust (DE) and C60 nanoparticles to pregnant mothers caused cytotoxic effects on spermatogenesis and testis tissue in their male children [20, 26, 30, 32, 33]. In the present study, CdSe and CdSe:ZnS with 2-3 nm size which were prepared by sedimentation method was investigated in vivo. Histopathological studies of testis tissue showed toxic effect of these nanoparticles at 40 mg/kg dose. Moreover, study of the testis slides showed reduction in spermatogonia, spermatocytes, spermatid, and mature sperm in seminiferous tubular, degeneration of interstitial tissue and reduction in Leydig cells number. Histological study of epididymis tissue showed intensive reduction in sperm volume of epididymis at 40 mg/kg. According to Table 4, FSH level did not change by treatments with QDs but

testosterone decreased significantly and LH increased significantly in group received CdSe:ZnS at 40 mg/kg dose, which is in agreement with the findings of testis weight and histopathology of mature group which include significant reduction in weight, degeneration of the interstitial tissue and Leydig cells by CdSe:ZnS at 40 mg/kg. Significant increase in LH could be due to a feedback mechanism in response to degeneration of interstitial tissue and considerable reduction of testosterone. Obviously, considering small size of these particles which provide them with easy passing from blood barrier of testis, we could infer that passing of this nanoparticles from brain blood barrier and enforcing direct toxicity on brain tissue and hypothalamus is possible but the first former suggestion is more probable because FSH level was not affected. In this study, cytotoxic effect of CdSe and the role of ZnS cover in controlling the degree of toxicity on sexual hormones, testis, and body weight of animals was investigated. In view of the results obtained in the present study, QDs can pass testis blood barrier and enforce effects on reproduction system. Although epithelium epididymis did not show any special histopathological changes in CdSe-treated mice by optical microscope but lumen content showed many failures occurring in testis tissue.

CONCLUSIONS

According to histopathological results of this study, CdSe:ZnS nanoparticles can pass testis blood barrier and enforce direct destruction of germinal cells and spermatogenesis. It seems that despite in vitro findings, including the role of ZnS for controlling the toxicity as the shell in embryonic culture environment, in vivo use of ZnS has increased toxicity of CdSe, which can be due to the increase in CdSe:ZnS stability or independent in vivo toxicity of ZnS itself. Although most of in vitro studies have offered cadmium ions release as the main reason for QDs toxicity, other mechanisms seem to contribute mainly to this toxicity, among which QDs stability plays an important role.

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