Int. J. Bio-Inorg. Hybr. Nanomater., Vol. 4, No. 2 (2015), 65-71

ISSN Print: 2251-8533
ISSN Online: 2322-4142

International Journal of Bio-Inorganic Hybrid Nanomaterials

Toxicity Comparative of CdSe:ZnS Quantum Dots on Testis, and Liver in Adult Mice

Akram Valipoor[1*], Gholamreza Amiri[2], Jafar Taheri[3], Mehdi Abasi[4], Amin Mirzakhani[5]

[1] Physiology Department, Basic Sciences Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

[2] Department of Physic, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

[3] Department of Chemistry, Islamic Azad University, Shahrekord, Iran

[4] Department of Statistics, Islamic Azad University, Shahrekord, Iran

[5] Department of Mechanical Engineering, Payame Noor University, PO BOX 19395-3697 Tehran, Iran

Received: 21 January 2015; Accepted: 25 March 2015

ABSTRACT

Quantum dots are new types of fluorescent materials for biological labeling. As a result, QDs toxicity study is an essential requirement for future clinical applications. Therefore, the cytotoxic CdSe:ZnS quantum dots effects on some organs in mice are presented in this study. In this work, 10, 20 and 40 mg/kg doses of CdSe:ZnS quantum dots were injected to 32 adult male mice. Structural and optical properties of quantum dots were studied by XRD. The testis and liver weight of various groups were analyzed using SPSS 16 program (one way ANOVA test) and histological changes in testis, liver tissues were analyzed by Light microscopy. Testis tissue showed high toxic effect in 40 mg/kg dose. Also histological study of liver tissue showed degeneration of hepatocyte cytoplasm, nuclear matters and sinusoidal dilation in dose-dependent manner in comparable to control groups but the lobular architecture is largely maintained in10, 20 and 40 mg/kg doses. The body weight did not change significantly in any of the CdSe:ZnS treated groups. The testis weight (TW) decreased significantly in mice that received 40 mg/kg CdSe:ZnS QDs and liver weight in the case of mice treated with 20, 40 mg/kg CdSe:ZnS QDs were increased significantly. According to the differences the toxicity of quantum dot on testis and liver tissues in adult, it seems that various organs have different responses to quantum dots toxicity.

Keywords: Quantum dots; CdSe:ZnS; Testis; Liver; Toxicity.

1. INTRODUCTION

Organic dyes have been widely used as fluorophores in biomedical imaging and detection. However, organic dyes are generally vulnerable to the physiological environment and are quickly photobleached under normal imaging conditions. Also they are not good for multicolor imaging because of two inherent properties (1) organic dyes have relatively broad emission spectra and hence result in the signal overlap from different dyes; and (2) one organic dye can only be suitably excited by the lights within a certain narrow wavelength range and it thus needs nearly the same numbers of excitation light sources as the dyes used. [1-3], But semi-

(*) Corresponding Author - e-mail: Valipoor.akram@gmail.com

conductor quantum dots (QDs) are tiny light-emitting particles on the nanometer scale, and are emerging as a new class of fluorescent labels for biology and medicine, in comparison with organic dyes and fluorescent proteins [1, 3, and 4].

Inorganic quantum dots are usually bright (20–80% quantum efficiency) and stable under relative harsh environments [5-7]. The absorption spectra of quantum dots are continuous, and the emissions spectra are narrow (typically 20–30 nm for FWHM, full width at half maximum of the emission spectrum). Excitation–emission matrix (EEM) reveals that quantum dots always emit the same lights no matter what excitation wavelength used. Therefore, the entire different emission colors from quantum dots can be seen at the same time by only one laser excitation source. The emission intensity of quantum dots could also be used as a variant for imaging because of their excellent levels could determine (106-1) nucleic acid or protein sequences [7-9]. The long-term multiplexed biomedical imaging has recently become one of the hottest research topics [7-10].

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 [7, 11, and 12]. 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 [13, 14]. 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 [11].

Considering at present there is relatively little work on toxicity of QDs especial in vivo and lack of any previous study in this category, In this study, cyctoxic effect of CdSe:ZnS has been studied for first time on testis, epididymis, liver tissues histopathology and testis , liver weight of animals.

2. MATERIALS AND METHOD

2.1. Methods of producing quantum dots

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 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 [15].

The crystal structure and optical properties of 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 [15].

2.2. The results of dose-finding study

According to our recent findings, there was no review of QDs in vivo toxicity. The 125, 250, and 500 mg/kg per kg of body weight doses were selected on the basis of the findings of in vitro studies particularly the study By Ming-Shu Hsieh. In the first stage, CdSe and CdSe:ZnS nanoparticles were prepared in normal saline solution and were intraperitoneally injected to 36 mice. All mice died in less than 24 hours after the injection. The remarkable finding was that in spite of in vitro results, including high control of CdSe QDs cytotoxicity by ZnS cover in embryonic culture environment, in vivo use of ZnS cover increased toxicity of CdSe and the rats showed greater responses to CdSe:ZnS compared to CdSe. So, the CdSe:ZnS -treated mice died within a shorter interval. Also some rats died almost immediately after the injection. In the next step DMSA-coated CdSe nanoparticles were injected to 16 mice intraperitoneally at 125 and 250 mg/kg doses. The results showed that 100% of the mice injected with DMSA-coated CdSe at 250 mg/kg dose and 75 to 80% of mice injected with DMSA-coated CdSe at 125 mg/kg dose died within one week. In the

third step, CdSe and CdSe:ZnS were intraperitoneally injected to 32 mice at 100 and 75 mg/kg doses. In CdSe:ZnS (at 100 and 75 mg/kg doses) groups, respectively 83% and 50% of mice and in CdSe (at 100 and 75 mg/kg doses) groups, respectively 66% and 50% of mice died within one week. Finally, were selected and injected to 24 mice. In CdSe groups 0% of mice died during 20 days at all three doses and in CdSe:ZnS groups 0, 12.5% and 20% of mice died at respectively 10, 20 and 40 mg/kg doses. 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.

2.3. Breeding and treatment of animals

Some male mice (about 60-70 days old) per 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, 32 adult male mice were divided randomly in 4 groups in each group of 8 sample: control, and treated with 10, 20 and 40 mg/kg doses of CdSe:ZnS QDs. CdSe:ZnS nanoparticles were prepared in normal saline solution and single-dose were injected intraperitoneally and Control group received only normal saline.

2.4. Tissue preparing

10 Days after CdSe:ZnS injection, 32 adult male mice following measurement of their bodies weight were anesthetized and liver and testis 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. Liver tissue histopathology, morphological structure of seminiferous tubes, and average number of spermatogonia, spermatocytes, spermatids, and matured sperms in testis and epithelial height, Connective tissue, Smooth muscle and sperm density were measured.

2.5. Statistical analysis

Data (testis, liver weight and the number of cells in seminiferous tubes of various groups) were analyzed using SPSS 16 program (by one way ANOVA). Statistical analysis Data were represented as means ± S.E. Differences was considered significant at *p<0.05, **p<0.01.

3. RESULTS AND DISCUSSION

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

The structure of the CdSe:ZnS QDs was investigated by XRD. The sample has a single phase and also a cubic crystal structure. The mean size of the particles was determined by Debye-Scherer formula. It was calculated as being of 2.4 nm for QDs. The size was determined around 3 nm from STM photograph (12).

3.2. Body, testis and liver weight changes

The body weight did not change significantly in any of the CdSe:ZnS treated groups (Figure 1). The testicular weight in the case of mice treated with 10, 20 mg/kg CdSe:ZnS QDs were similar to control group and no significant change was found in relative testis weights but the testis weight (TW) decreased significantly in mice that received 40 mg/kg CdSe:ZnS QDs (Figure 2) parallel with histological changes in mice testis in this group. Also weight liver in mice that received 10 mg/kg CdSe:ZnS QDs were similar in treated group, control and there was no statistically significant differences between control group and mice treated with 10 mg/kg CdSe:ZnS. But liver weight in the case of mice treated with 20, 40 mg/kg CdSe:ZnS QDs were

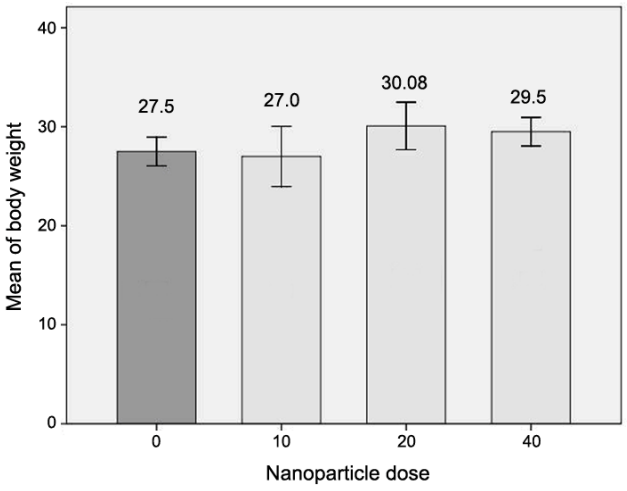

Figure 1: *Mean comparison of body weight in adult group 10 days after injection (**p<0.01).*

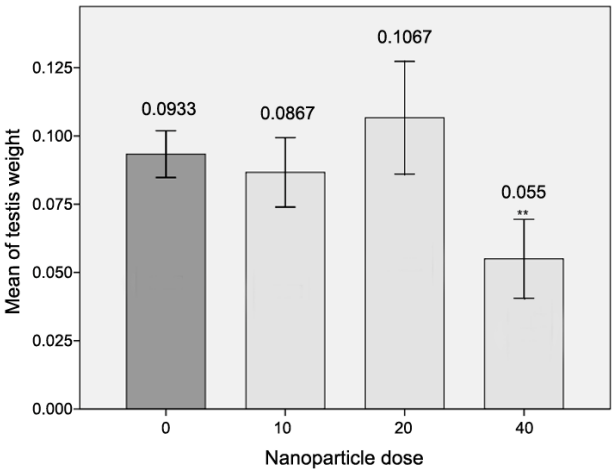

Figure 2: *Mean comparison of testis weight in adult group 10 days after injection (**p<0.01).*

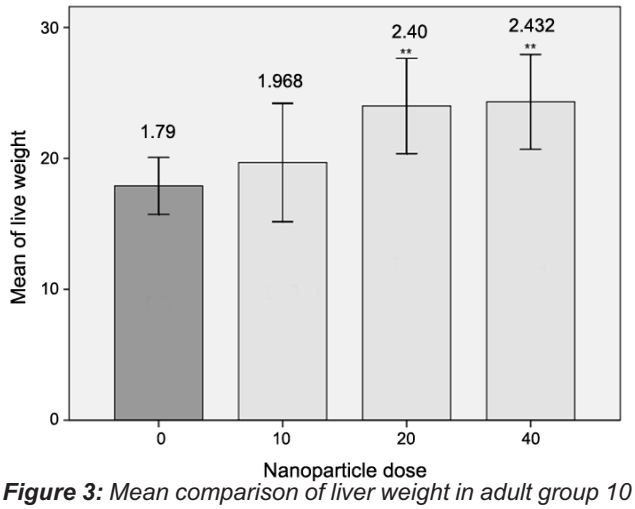

Figure 3: *Mean comparison of liver weight in adult group 10 days after injection (**p<0.01).*

increased the significantly (**p<0.01) parallel with sever histological changes in mice liver in the two groups (Figure 3).

3.3. Histological study of testis in adult

The seminiferous tubules are in different spermatogenic stages in control group, and in the mice treat-

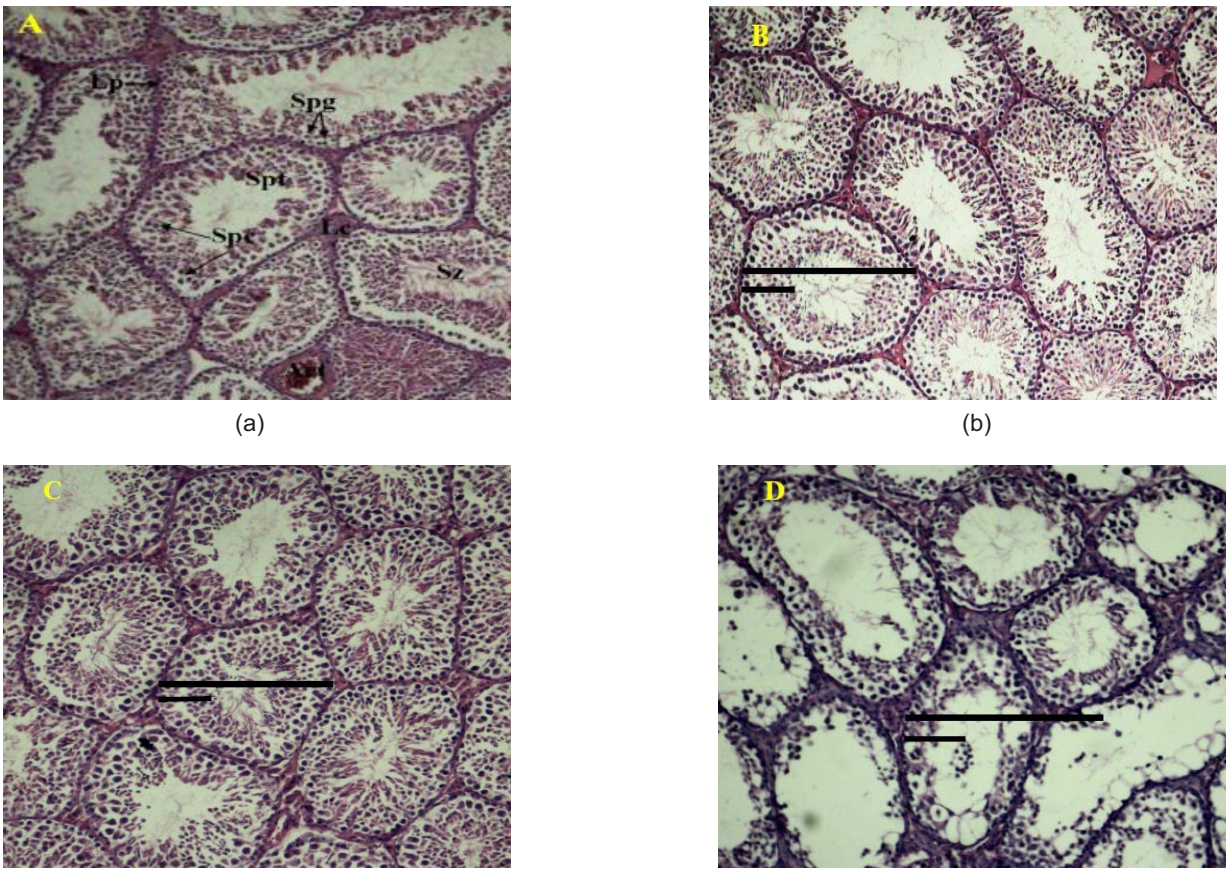

Figure 4: *Microscopic images of testis slides, 10 days after injection (H & E, 400×) (A) Control group and B, C, and D treated groups respectively with doses: 10, 20, 40 mg/kg CdSe:ZnS. (Sz: spermatozoa, Art: artery vessel, Lc: leydig cells, Lp: lamina propria, Spg: spermatogoni, Spc: spermatocyte, Spt: spermatid).*

Table 1: *Average and mean comparison of sperm stem cell numbers in one tubule in adult group after injection (*p < 0.05, **p < 0.01).*

Parameter	Groups (n =8 mice)			
	40 mg/kg	20 mg/kg	10 mg/kg	Control
18 ± 6.94	32 ± 6.67	33 ± 8.94	34 ± 6.39	Mean spermatogonia
29 ± 10.76**	43 ± 8.04	45 ± 8.45	44 ± 9.35	Mean spermatocyte I
83 ± 23.44**	109 ± 20.72	113 ± 23.29	111 ± 33.63	Mean spermatid

ed with all three doses of CdSe and CdSe:ZnS QDs, spermatozoids were observed in lumen tubules, 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, spermatids and an obvious decrease in matured sperms of lumen were noticed (Table 1). On the other hand, degeneration of the interstitial tissue and blood vessels and reduction in thickness of the lamina propria are illustrated in Figure 4.

3.4. Histological study of liver in adult

The results of histological changes in liver tissues examination in samples of liver in the group treated with 10, 20, 40 mg/kg QDs in dose-dependent manner revealed some abnormal morphology characteristics For acute toxicity study, liver tissues in group treated with 20, 40 mg/kg QDs showed presence of activated kupffer cells, sinusoidal dilatation and cytoplasmic vacuolation and nuclear destruction and nuclear matters, slight infiltrated inflammatory cell. The changes in the experimental histopathologic parameters for liver were shown in Figure 5.

With the increase in the quantum dots applications importance in biology and medicine fields, toxicity tests of quantum dots have been widely considered

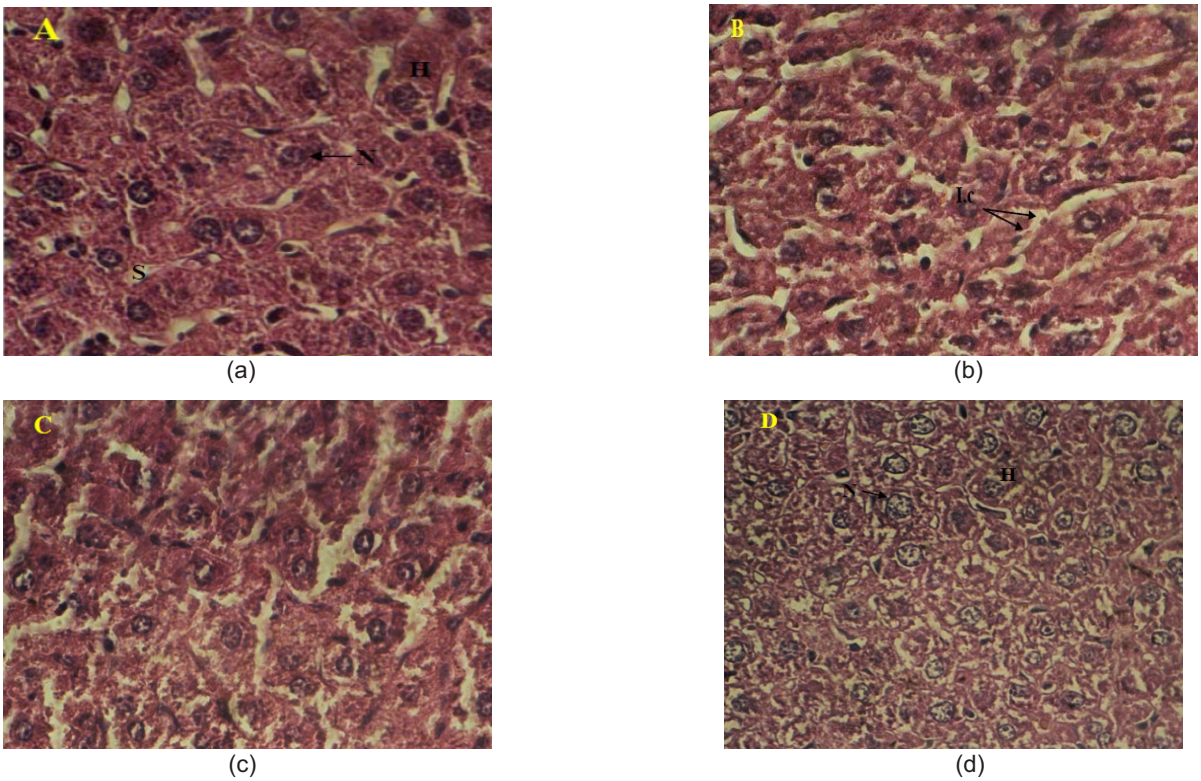

Figure 5: *Microscopic images of liver slides, 10 days after injection (H & E, 400×) (A) Control group and B, C and D treated groups with doses: 10, 20, 40 mg/kg CdSe (H: Hepahocyte, N: Nucleus, S:Sinusoid, Ic: Inflammatory cells).*

[2, 3, and 16] and Cytotoxicity of these particles is an important factor in their use in medicine and then has been considered highly in recent years [17, 18]. This study indicates that there are any previous study in toxicity of quantum fields on the testis and liver. However, the potential effects of nanoparticles on the reproductive system, placenta translocation, and fetus development are still far from even basic evaluations, although some researchers suggested the importance of reproductive toxicity of nanoparticles [2, 19, and 20]. For example, C60 nanoparticles intratracheally administered also induced adverse effects on the mouse male reproductive function [2, 19, and 21]. Also some researchers showed fetal CB exposure significantly reduced DSP in male offspring. When CB was administered to adult mice, DSP decreased significantly [21]. Therefore, CB reduces DSP through both fetal exposure and exposure during adulthood. Furthermore, it has been reported that fetal exposure to diesel exhaust (DE) lowers the DSP of male offspring [19, 22]. Diesel exhaust consists of various components, including DEP. The carbon nanoparticles used in the present study are made of carbon, which is the basic structure of DEP. Therefore, fetal DE exposure may lower DSP in male offspring due to particulate matters in DE, particularly CB. However, in the present study, it was not clear whether CB lowered DSP by altering the maternal environment or by directly affecting fetuses. In the future, it will be necessary to determine the effects of CB on both dams and offspring. In the testis of male offspring, intercellular adhesions of seminiferous epithelia and seminiferous tubules damage were observed. The low cellular adhesion of seminiferous epithelia may indicate reduced adhesion of Sertoli and spermatogenic cells. Because Sertoli cells supply nutrients and send signals for cellular differentiation to spermatogenic cells, weak adhesion of Sertoli and spermatogenic cells may inhibit spermatogenesis and thus fetal CB exposure decreases DSP in male pups [21, 22]. However, there is no linear relationship between ages and seminiferous tubule damage or DSP. The progression of sexual maturation might be related to such appearances, but not in a linear relationship. To further investigate these results, we should measure various factors, e.g., the adhesion molecule, testicular gene expression, and intratesticular regulators. When adult mice were exposed to CB, the incidence of seminiferous tubule damage was high; however, its severity was mild [7, 22]. In addition, fetal CB exposure leads to weak cellular adhesions in seminiferous tubules; however, CB administration to adult mice induced vacuolation of the seminiferous tubules [21]. As well as research results show treatments with TiO_2 and Gold nanoparticles for pregnant women is one of the previous studies incriminating cytotoxic effects on spermatogensis and histopathology changes of testis in their male children. In vitro studies showed also cyctotoxic effect of TiO_2 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 [9].

In this study, the cytotoxicity in vivo of CdSe:ZnS QDs with 2-3 nm size, synthesized by sedimentation method was studied. Histopathology studies of testis tissues did not show toxicity effect of these nanoparticles in the case of mice treated with dose 10 and 20 mg/kg of CdSe:ZnS. According to these studies, the number of spermatogonia, spermatocytes, spermatids, and matured sperms in seminiferous tubes were similar in treated with dose 10 and 20 mg/kg of CdSe:ZnS. groups and control. 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, spermatids and an obvious decrease in matured sperms of lumen were noticed Also, the study of body and testis weight showed a weight decreasing in the case of 40 mg/kg dose of CdSe:ZnS QDs. The toxicity effect of CdSe:ZnS quantum dot was a very significant increase in liver. So that in sections of liver in the group treated with 10, 20, 40 mg/kg QDs in dose-dependent manner revealed some abnormal morphology.

4. CONCLUSIONS

In this research cytotoxic effect of CdSe:ZnS QDs on liver and testis tissues of animals and testis , body, liver weight in adult mice indicated that the nanoparticles were passed through the membrane of different cells and even cross blood barrier-testicular and have affected of testis and liver. Although, CdSe:ZnS in vi-

tro condition shows high control of CdSe toxicity because of ZnS coverage in this study and higher in vivo toxicity of CdSe:ZnS even in nucleus and nuclear matters, with regard to the lack of review in this field, discussed in relation to the Reason for the increase body and testis weight need to further molecular research.

REFERENCES

1. Dabbousi B.O., Rodriguez-Viejo J., Mikulec F.V., Heine J.R., Mattoussi H., et al., *Phys. Chem. B*, **101** (1997), 9463.
2. Park E.J., Kim H., Kim Y and Park K., *Toxicol Environ Health Sci.*, **25** (2010), 279.
3. Oberdorster G., Maynard A., Donaldson K., Castranova V., Fitzpatrick J. et al., *Part. Fibre. Toxicol.*, **2** (2005), 8.
4. Alivisatos P., *Nat Biotechnol.*, **22** (2004), 47.
5. Aldana J., Wang Y.A., Peng X., *Am Chem. Soc.*, **123** (2001), 8844.
6. Fang B., Chaudhari N.K., Kim M.S., Kim J.H., Yu J.S., *Am. Chem. Soc.*, **131** (2009), 15330.
7. Yu W.W., Chang E., Drezek R., Colvin V.L., *Biochem. Biophys. Res. Commun.*, **348** (2006), 781.
8. Han M., Gao X., Su J.Z., Nie S., *Nat Biotechnol.*, **19** (2001), 631.
9. Eastman P.S., Ruan W., Doctolero M., Nuttall R., deFeo G., Park J.S. et al. *Nano Lett.*, **6** (2006), 1059.
10. Smith A.M., Duan H., Mohs A.M., Nie S., *Adv. Drug Deliv. Rev.*, **60** (2008), 1226.
11. Walling M.A., Novak J.A., Shepard J.R., *Mol. Sci.*, **10** (2009), 441.
12. Cano A.D., Sandoval S.J., Vorobiev Y. et al, *Nanotechnology*, **21** (2010), 4016.
13. Chang S.Q., Dai Y.D., Kang B. et al., *Toxicol. Lett.*, **188** (2009), 104.
14. Amiri G.R., Fatahian S., Mahmoudi S., *Mater Sci. Appl.*, **4** (2013), 134.
15. Oberdorster G., Overdorster E., Oberdorster J., *Environ. Health Perspect.*, **113** (2005), 823.
16. Yoshida S., Hiyoshi K., Oshio S., Takano H., Takeda K. et al., *Fertil. Steril.*, **93** (2010), 1695.
17. Bae P.K., Kim K.N., Lee S.J., Chang H.J., Lee C.K. et al. *Biomaterials.*, **30** (2009), 836.
18. Ahamed M., Posgai R., Gorey T.J. et al., *Toxicol Appl Pharmacol.*, **242** (2010), 263.
19. Roh J.Y., Sim S.J., Yi J., Park K., Chung KH. et al. *Environ. Sci. Technol.*, **43** (2009), 3933.
20. Yoshida S., Hiyoshi K., Ichinose T., Takano H., Oshio S. et al. *Androl.*, **32** (2009), 337.
21. Watanabe N., *Toxicol. Lett.*, **155** (2005), 51.
22. Guo W., Li J.J., Wang Y.A., Peng X., *Chem. Mater.*, **15** (2003), 3125.

AUTHOR (S) BIOSKETCHES

Akram Valipoor, *Assistant Professor, Physiology Department, Basic Sciences Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran*
Email: Valipoor.akram@gmail.com

Gholamreza Amiri, *Associate Professor, Department of Physic, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*

Jafar Taheri, *Instructor, Department of Chemistry, Islamic Azad University, Shahrekord, Iran*

Mehdi Abasi, *Instructor, Department of Statistics, Islamic Azad University, Shahrekord, Iran*

Amin Mirzakhani, *Instructor, Department of Mechanical Engineering, Payame Noor University, PO BOX 19395-3697 Tehran, Iran*