Int. J. Bio-Inorg. Hybr. Nanomater., Vol. 3, No. 4 (2014), 197-202
ISSN Print: 2251-8533
ISSN Online: 2322-4142

International Journal of Bio-Inorganic Hybrid Nanomaterials

In vivo Effects of CdSe Injection on Embryonic Development of Reproductive System

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Received: 2 July 2014; Accepted: 6 September 2014

ABSTRACT

The use of quantum dots (QDots) as bright and photostable probes for long-term fluorescence imaging is gaining more interest. Thus far, (pre)clinical use of QDots remains limited, which is primarily caused by the potential toxicity of QDots. Most QDots consist of Cd^{2+} ions, which are known to cause high levels of toxicity. Therefore, the cytotoxic effects of CdSe quantum dots on embryonic development of male reproductive system are presented in this study.

Keyword: CdSe; Embryonic development; Reproductive; System; In vivo.

1. INTRODUCTION

Nanomaterials have found wide applications in biomedicine and biotechnology because of their novel physicochemical properties among them, quantum dots (QDs) have proven to be ideal optical probes for biological imaging [1, 2] and attracted great attention [3]. QDs are nanocrystals composed of a core of a semiconductor material, enclosed within a shell of another semiconductor that has a larger spectral band gap [1, 4]. They were initially prepared in 1982 for use as a probe for investigation of surface kinetics, where it was found that the quantum yield of the nanocrystals was sensitive to the concentration of surface-adsorbed species that can undergo reduction [1]. QD cores are usually composed of elements from groups II and VI, e.g., CdSe (most common) or groups III and V, e.g., InP, while the shell is typically a high band gap material such as ZnS. A typical QD has a diameter of about 2–10 nm which makes it of a size domain that allows one-

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on-one interaction with biomolecules such as proteins where the typical size ranges from 1 to 20 nm [5, 6].

Quantum dots are fluorescent nanoparticles with unique photophysical properties that allow them to be used as diagnostic, therapeutic, and theranostic agents, particularly in medical and surgical oncology [7, 8].

There for, Semiconductor Quantum dots (QDs) have raised great attention because of their superior optical properties and wide utilization in biological and biomedical studies. More recently, there have been intense concerns on cytotoxicity assessment of QDs. Most QDs are made of heavy metal ions (e.g., Cd^{2+}), which may result in potential in vitro toxicity that hampers their practical applications [9].

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 [10]. 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 [10].

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 and body, testis weight of animals Before and after puberty.

2. MATERIALS AND METHODS

2.1. Methods of producing CdSe 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 [11].

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.

2.2. Breeding and treatment of animals

In this experimental study used mice Balb C strain. Balb C is an inbred mice used mainly in immunology, monoclonal antibody production, pharmacology and toxicology. 24 female mice weighting 25-30 g with 60-70 days age were studied. The mice were supplied from animal house of the histology Department Faculty of Medicine, Shahrekord University of Medical Sciences. The mice were housed in plastic cages and kept under 12 h light/dark conditions under 20-22°C, 50-60% humidity and free access to food and water. The mice were mated and pregnancy was determined by detection of vaginal plug. The pregnant mice were divided randomly in 4 groups (n= 6): control and treated with 10, 20 and 40 mg/kg doses of CdSe` QDs. In this study, work with laboratory animals was approved by the ethics committee of the shahrekord University.

2.3. Study design

CdSe nanoparticles were prepared in saline and single dose with doses of 10, 20, 40 mg/kg injected intraperitoneally to the pregnant mice on gestation day 8, because the blood-placenta barrier and gonad development begin after 5 to 7 days after gestation. Gestation begins with the sign of a vaginal plug as evidence of copulation or gestation day 0.

2.4. Tissue preparing

Some 60 to 70 days male offsprings (injection in day 8 of pregnancy after CdSe) in both control and treatment following measurement of their bodies' weight were dissected under mild anesthesia and epididymis and testis organs were rapidly cut, weighted, and immersion-fixed in the fixative (formaldehyde). Sections

(5 micron) were prepared after dehydration and were embedded in paraffin. The sections were stained with hematoxilin and eosin and subsequently processed for histopathological examination under light microscope. The morphological structure of seminiferous tubules and mean number of spermatogonia, spermatocytes and spermatids were studied in testis and epithelial height, Connective tissue, Smooth muscle and sperm density were studied in epididymis.

2.5. Statistical analysis

Data (the mean testis and body weights 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.D. Differences was considered significant at $p<0.001$.

3. RESULTS AND DISCUSSION

3.1. The results of XRD and STM

The structure of the QDs was investigated by XRD. The sample had 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. Also the size was determined around 3 nm from STM photograph [12].

3.2. Body and testis weight changes

The testicular weight in the case of mice treated with 10, 20 mg/kg CdSe QDs were similar to control group

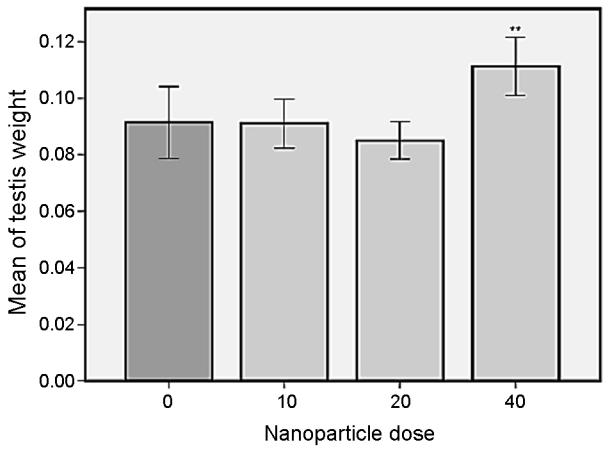

Figure 1: *Mean comparison of testis weight in embryonic groups of 60 to 70 days male offsprings ($p < 0.01$).*

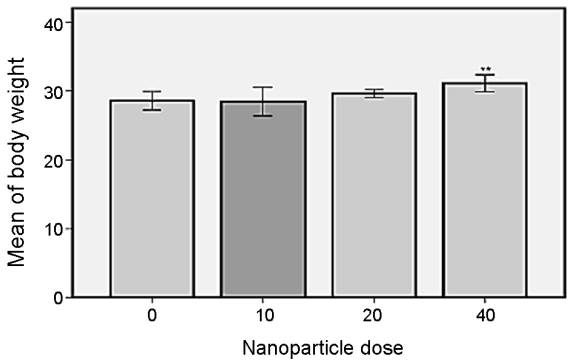

Figure 2: *Mean comparison of body weight in embryonic groups of 60 to 70 days male offsprings ($p < 0.01$).*

and no significant change was found in relative testis weights but the testis weight (TW) increased significantly in mice that received 40 mg/kg CdSe QDs parallel with histological changes in mice testis in this group (Figure 1). Also there were no significant differences between the groups treated with 10, 20 mg/kg CdSe QDs and control in body weight (Figure 2).

3.3. Histological study of testis tissue

All mice in the control Group (A) and in the case of mice treated with 10, 20 and 40 mg/kg CdSe QDs (B, C), showed normal testicular architecture with an orderly arrangement of germinal and The seminiferous tubules showed normal spermatogenesis pattern, In addition the normal structure of the interstitial tissue and blood vessels in testis tissue can also be seen (Figure 3). According to histophathology results of testis, Table 1 shows that the average number of spermatogonia, spermatocytes, spermatids was similar in treated groups and control (Table 1).

3.4. Histological study of body

Qualitative studies of epididymis tissues using optical microscope in all mice treated with a single injection of 10, 20 and 40 mg/kg CdSe showed that epithelia of epididymis, the interstitial tissue and spermatozoa volume in epididymis duct lumen were similar in treated groups and control (Figure 4).

Quantum dots (QDs), tiny light-emitting particles on the nanometer scale, are emerging as a new class of fluorescent probes for cancer cell imaging and molecular profiling [13]. In comparison with organic dyes and fluorescent proteins, QDs have unique optical and electronic properties, such as size-tunable

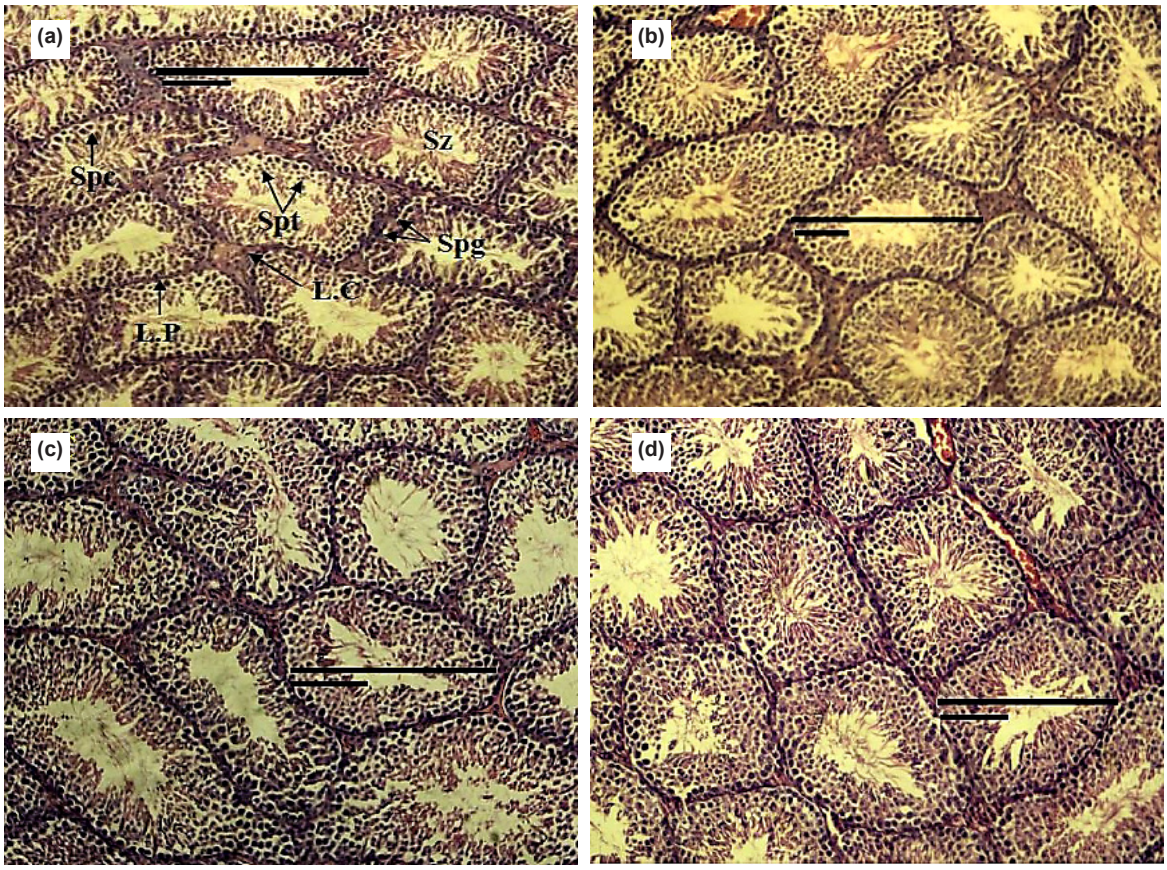

Figure 3: *Microscopic images of testis slides, in embryonic groups of 60 to 70 days male offsprings (H & E, 400×) (a) Control group and (b), (c) and (d) treated groups with doses: 10, 20, 40 mg/kg CdSe. (Sz: spermatozoa, Lc: Leydig cells, Lp: lamina propria, Spg: espermatogoni, Spc: espermatocyte, Spt: espermatid).*

light emission, improved signal brightness, resistance against photobleaching, and simultaneous excitation of multiple fluorescence colors [5, 8]. Thus far, (pre) clinical use of QDots remains limited, which is primarily caused by the potential toxicity of QDots. Most QDots consist of Cd^{2+} ions, which are known to cause high levels of toxicity [14]. As a result, toxicity tests of quantum dots have been widely considered [2] and Cytotoxicity of these particles is an important factor in their use in medicine and then has been considered highly in recent years [3]. Our study indicates that there are any previous studies in quantum dots toxicity fields on the reproductive system and they are a few studies in quantum dots toxicity fields. Including, But there are some studies in another nanoparticle toxicity fields on the reproductive system. For example, Yoshida and et al showed C60 (Carbon) nanoparticles intratracheally administered induced adverse effects

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

Parameter	Groups (n = 8 mice)			
	Control	10 mg/kg	20 mg/kg	40 mg/kg
Mean spermatogonia	34 ± 6.39	35 ± 7.55	35 ± 6.76	34 ± 7.55
Mean spermatocyte I	44 ± 9.3	45 ± 9.76	46 ± 8.58	45 ± 7. 01
Mean spermatid	111 ± 33.6	100 ± 18.64	100 ± 24.96	99 ± 24.68

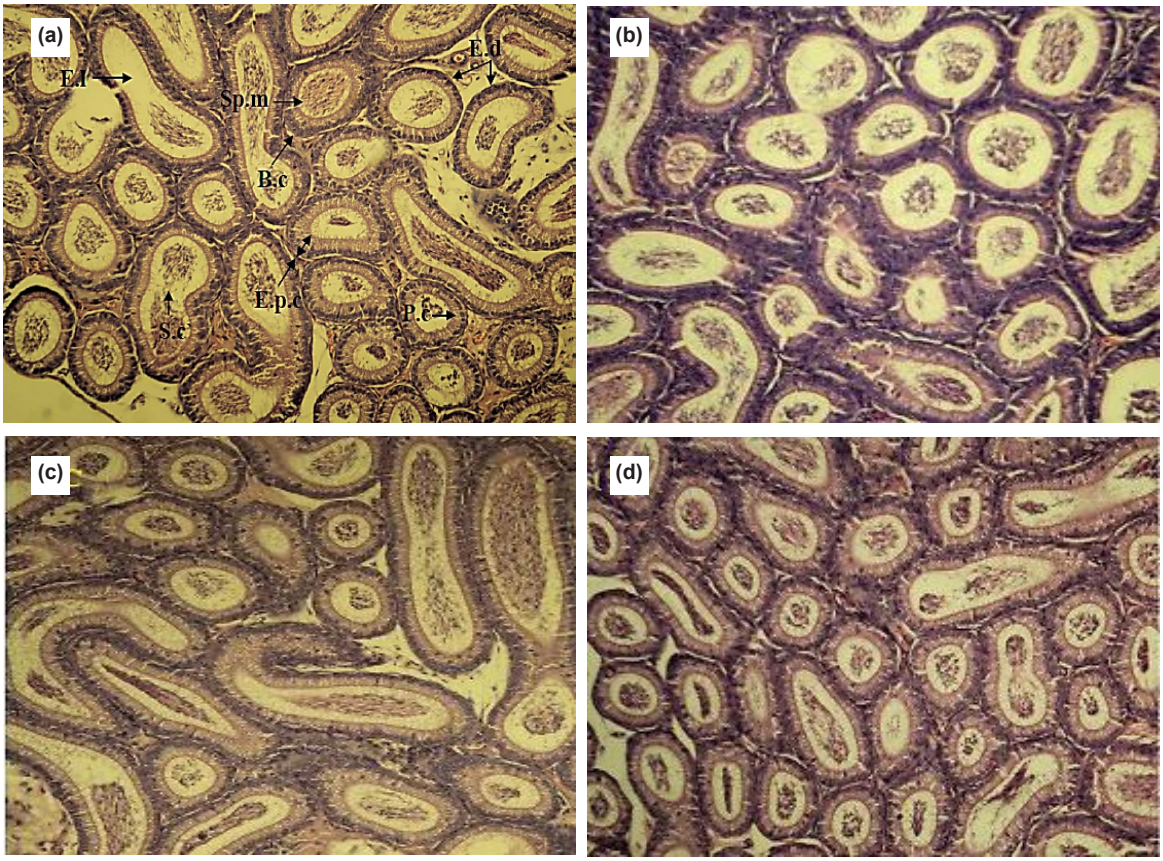

Figure 4: *Microscopic images of epididymis tissues, 60 to 70 days male offsprings (H & E, 400×) (a) Control group and (b), (c),and (d) treated groups with doses: 10, 20, 40 mg/kg CdSe. (L.c.t: Loose connective tissue., P.c: principal cells, S.c.e: pseudostratified stereociliated columnar epithelium, Stereocilia, Sm.m: Smooth muscle, Sp. m: Sperm mass, P.c: pseudostratified columnar, E.l: epididymis lumen, E.d: Epididymal duct).*

on the mouse male reproductive function [15]. Also In another study some researchers showed fetal CB-NPs (carbon black nanoparticles) exposure significantly reduced DSP (Daily sperm production) in male offspring [16]. Furthermore, it has been reported that fetal exposure to DE (Diesel Exhaust) lowers the DSP of male offspring [15]. In this study, Quantum Dots of CdSe with 2-3 nm size synthesized by chemical sedimentation method and the cytotoxic effects of CdSe quantum dots on embryonic development of male reproductive system was researched. Histopathology studies of testis tissues in the case of mice treated with doses 10, 20 and 40 mg/kg of CdSe no showed toxicity effect of these nanoparticles. According to these results, in this treated groups the number of spermatogonia, spermatocytes, spermatids and matured sperms in seminiferous tubes were similar in treated groups and control. But the study of testis weight showed a weight increasing in 40 mg/kg dose of CdSe QDs. In this research cytotoxic effect of CdSe QDs on epididymis tissue and testis of animals and testis, body weight in both adult and embryonic groups has been studied for the first time. More studies are necessary in this field in order to identify effective background mechanism of QDs cytotoxicity.

4. CONCLUSIONS

Considering increasing of the testis and the body weights in 40 mg/kg dose of CdSe QDs and nanometer-size CdSe crystallites (2-3 nm), It seems that nanoparticles are able to cross blood barrier-testicular and the blood-placenta barrier and Considering no induction CdSe quantum dots toxicity in histopathology of the testis and the epididymis it can be said that the testis and the epididymis of embryonic have Low sensitivity to CdSe nanoparticles. Therefore, these in-

teresting results are worrying and show complicated process of quantum dots in vivo toxicity and long way of biological use of them in spite of their obvious advantages in medicine.

ACKNOWLEDGMENT

This work was supported by Iran nanothecology initiative council and there is no conflict of interest.

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