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Original Article

Changes in Reproductive Hormones Secretion Involved in Quantum Dots-induced Reproductive Toxicity in Adult Male Mice

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ABSTRACT

Background and Aims: Quantum dots (QDs), as colloidal nanocrystalline semiconductors, present QD wavelengths in terms of biomedical assays and imaging, though the high toxicity of their core demands to be taken into consideration. Investigating this subject is taken into account as an important concept concerning use of these nanoparticles in the medical applications.

Materials and Methods: 10, 20, and 40 mg/kg doses of mentioned QDs were injected into some male mice. 10 days after CdSe/ZnS, and the serum sample of mice were measured in regard with FSH, LH and testosterone assays. The testis and body weight of various groups were determined.

Results: Within 10 days after injection of 40 mg/kg CdSe:ZnS, the serum LH concentration increased from 0.64 to 0.79 ng/ml and the serum testosterone concentration declined from 1.33 to 0.58 mIu/ml. Mean concentration of LH and testosterone CdSe:ZnS in 40 mg/kg dose showed high toxicity of CdSe:ZnS in 40 mg/kg dose. The FSH concentration did not reveal any significant differences compared to the control group. The body weight in all groups and the testicular weight in the treated mice with 10, 20 mg/kg CdSe QDs were similar to the control group. No significant changes were observed in regard with relative testis weights, whereas the testis weight decreased significantly from 0.093 to 0.055 gr ($p< 0.01$) in the mice receiving 40 mg/kg CdSe:ZnS.

Conclusions: Quantum dots were demonstrated to be capable of inducing detrimental effects on the reproductive systems of male mice. Since no study has been conducted in this realm, the present study can serve as an introduction to more studies regarding the effects of quantum dots toxicity on the development of male sexual system.

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Introduction

Luminescent semiconductor quantum dots (QDs) involve a novel class of fluorescent probe which unique optical properties suggest their superiority to the conventional organic dyes for many biological applications. QD properties entail high quantum yields, broad absorption spectra, symmetric-narrow, size-tunable emission spectra, and exceptional resistance to photo and chemical degradation [1-3]. These attractive fluorescence properties are particularly appealing for the visualization of cellular processes, as they can potentially facilitate long-term and multicolour labeling of both fixed and living cells [1, 4, 5]. Successful use of QDs has been reported in various medical fields, though the high toxicity of core compounds of these nanoparticles should be taken into consideration which are composed of such heavy metals as cadmium and thallium [5-7]. Therefore, investigating the toxic effect of QDs is very important in regard with their biological use. Since it is regarded as a decisive factor in their wide use in medicine, it has absorbed much attention within recent years [8-10]. Combination of heavy metals is determined to have a minor role in the cytotoxicity of QDs; hence, they have a good chance for serving as contrast agents in the clinical use [7, 10].

Currently, relatively little work has been conducted on QDs toxicity specifically in vivo conditions; therefore, the present study intended to evaluate cyctoxic effect of CdSe:ZnS on testis, body, and testis weight of animals after puberty.

Materials and Methods

Producing CdSe:ZnS Quantum Dots

Nanoparticles were synthesized by the chemical precipitation method. For this purpose, three solutions of cadmium chloride (CdCl2.4H2O), mercaptoethanol and sodium selenite (Na2SeO3.5H2O) were prepared in the distilled deionized water under vigorous stirring (all from Merck Company). At first, CdCl2 solution was poured into a three-spout balloon container and in the meanwhile, mercaptoethanol solution was added to the same balloon. Ultimately, sodium selenite solution was added to the balloon by the same way under nitrogen 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 the room temperature. As a matter of fact, all processes were done at the room temperature [11]. The

crystal structure and optical properties of QDs were characterized by X-Ray Diffraction (XRD, Bruker D8 ADVANCE λ= 0.154 nm Cu Kα radiation) and Ultra Violet – Visible spectrophotometer (UV-Vis, UV-2600 Shimadzu, Japan). Scanning Tunneling Microscope (STM, NATSICO, Iran) was used in order to investigate the particle size distribution.

Breeding and Treatment of Animals

In this experimental study, 24 male Balb/c mice weighing 25-30g, aged 60-70 days were used. The mice were supplied from animal house of the histology department, faculty of medicine, Shahrekord University of Medical Sciences. Moreover, they were housed in plastic cages and kept under 12 h light/dark conditions under 20-22ºC, 50-60% humidity and free access to food and water. The male mice were divided randomly into 4 groups (N= 8): 1 control group and 3 treatment groups with 10, 20 and 40 mg/kg doses of CdSe:ZnS QDs. CdSe:ZnS nanoparticles were prepared in the single-dose saline injected intraperitoneally to the male mice.

Blood Sampling and Hormones Assay

Ten days after CdSe:ZnS injection, both control and treatment groups were anesthetized and killed by decapitation using a guillotine. Blood samples were collected in tubes containing anticoagulation heparin centrifuged at 1000g for 5 min at the room temperature. Then, the serum supernatant samples were collected. LH and FSH concentrations were measured by mouse immunoradiometric assay and the testosterone concentration was measured by radioimmunoassay method according to the manufacture's instructions (DRG Co, Germany). It should be noted that the study procedures were approved by the Ethics Committee of the Shahrekord University, Iran.

Statistical Analysis

The mean serum concentrations of hormones, the testis, as well as the body weight were analyzed in the various groups using SPSS software (Ver. 16, SPSS Inc, Chicago, IL, USA) via one-way ANOVA. The study data were represented as means±S.D. and, the differences were considered significant at $p<0.01$.

Results

The structure of the QDs was investigated by XRD. The study sample revealed a single phase as well as a cubic crystal structure. The particles mean size was determined by Debye-Scherer formula, which was calculated as 2.4 nm for QDs. Moreover, the size was determined around 3 nm via STM photograph [12]. The body weight did not change significantly in any of the CdSe:ZnS treated groups (Fig. 1).

The testicular weight were similar to that of the control group in the case of mice treated with 10, 20 mg/kg CdSe QDs and thus, no significant change was detected in the relative testis weights, whereas the testis weight decreased significantly from 0.093 to 0.055 gr ($p<0.01$) in mice that received 40 mg/kg CdSe:ZnS QDs parallel with histological changes in mice testis in this group (Fig.2). The mean plasma concentration of FSH was similar in the treated and control groups. The testosterone concentration in the mice treated with 10, 20 mg/kg CdSe:ZnS QDs were similar to the control group and no significant change was observed in the testosterone concentration. Plasma testosterone concentration decreased significantly from 1.33 to 0.58 mIu/ml in mice that received 40 mg/kg. Furthermore, LH concentration in mice receiving 10, 20 mg/kg CdSe:ZnS QDs demonstrated no significant differences in mean LH concentration. In contrast, LH was reported 0.79 ng/ml in the group treated with 40 mg/kg QDs, that it increased significantly from 0.648 to 0.798 ng/ml (Table 1).

Table 1. The mean plasma concentrations (±SE) of FSH, LH and testosterone

Nanoparticle dose	Groups (N =8)		
	Testosterone (mIu/ml)	**LH (ng/ml)**	**FSH (ng/ml)**
0 mg/kg	1.33	0.648	**1.17**
10 mg/kg	1.15	0.665	**0.998**
20 mg/kg	1.25	0.623	**1.04**
40 mg/kg	0. 58*	0.798*	**1.06**

*P<0.01

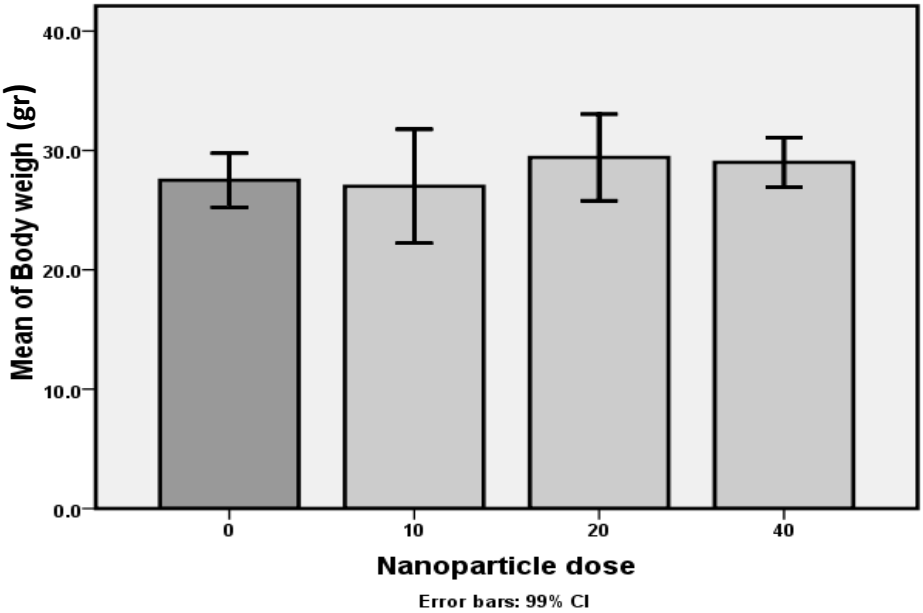

Fig. 1. Mean comparison of body weight in the adult group 10 days after injection. Data are presented as mean ± SD

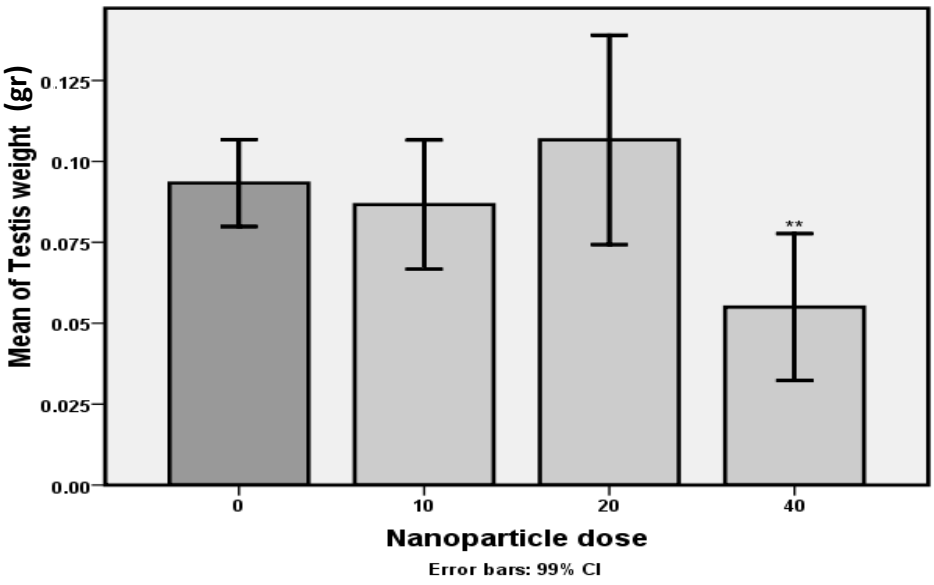

Fig. 2. Mean comparison of testis weight in adult group 10 days after injection
Data are presented as mean ± SD
$^{**}P<0.01$

Discussion

Nanomaterials are defined as materials having a physicochemical structure on a scale greater than typical atomic/molecular dimensions but less than 100 nm (nanostructure), which exhibit physical, chemical and/or biological characteristics associated with a nanostructure [2]. As a matter of fact, nanoparticles are defined as

particles with at least one dimension smaller than 100 nm including manufactured nanoparticles, ambient ultrafine particles and biological nanoparticles [2, 12]. Humans have been exposed to airborne nanoparticles throughout their evolution, but this exposure has dramatically increased due to such anthropogenic factors as combustion engines, power plants, and other sources of thermal degradation [12]. The distinctive and unique properties of nanomaterials offer the promise of broad advances in regard with a wide range of technologies. Nanomaterials are applied in a variety of areas including advanced materials, electronics, magnetics and optoelectronics, biomedicine, pharmaceuticals, cosmetics, energy, catalytic and environmental detection as well as monitoring [13-15]. Currently, there are relatively few environments where exposures are known to occur. However, if the commercialization of products using nanomaterials develops as it is anticipated, the potential for exposure is likely to increase notably over the coming decade [2, 14]. Despite the growing concern over the possible risk that nanomaterials pose, there is a lack of information on their potential toxicity. Currently, a knowledge gap can be observed between the increasing development, use of nanomaterials, and the prediction of possible health risks. Within recent years, reproductive and developmental toxicity has increasingly become recognized as an important part of the overall toxicology. In fact, adverse effects of environmental chemicals have been examined on the reproductive success of wildlife populations [14, 16]. To the best of our knowledge, no previous studies have been conducted concerning quantum dots toxicity fields on the reproductive system and a few studies have been carried out on quantum dots toxicity fields. There are some studies in another nanoparticle toxicity fields on the reproductive system. For example, Yoshida et al. revealed C60 (Carbon) nanoparticles intratracheally administered induced adverse effects on the mouse male reproductive function. Furthermore, fetal carbon black nanoparticles (CB-NPs) exposure has been significantly reduced daily sperm production (DSP) of male offspring [17]. In another study, some researchers showed that fetal exposure to diesel exhaust lowers the DSP of the male offspring [18].

In the present study, QDs of CdSe:ZnS with 2-3 nm size were investigated to be synthesized by the chemical sedimentation method and the cytotoxic effects of CdSe:ZnS QDs were evaluated on the abnormal secretion of male reproductive system hormones. Body weight was not changed significantly in the treatment groups, whereas testis weight was

decreased significantly by CdSe:ZnS at 40 mg/kg in the mature group. FSH was not changed by treatments, though testosterone was decreased significantly and LH increased significantly via CdSe:ZnS of 40 mg/kg. Regarding decrease of the testis weight with dose 40 mg/kg CdSe:ZnS QDs and nanometer-size of CdSe:ZnS crystallites (2-3 nm), it seems that nanoparticles are able to cross blood barrier-testicular. Furthermore, considering induction of CdSe:ZnS QDs toxicity in histopathology of the testis as well as the testis, it can be said that the adult testis have high sensitivity to CdSe:ZnS nanoparticles. LH results and testosterone hormones were in accordance to testis weight changes. Groups involved with reduction in testis weight demonstrated less testosterone and more LH. It seems that decrease testosterone hormone caused by testis and leydig cells often destroys. As a matter of fact, LH increase is a feedback reaction to testosterone reduction, and FSH was not changed in the various groups. However, more studies are demanded in this field in order to identify effective background mechanism of QDs cytotoxicity.

Conclusion

The study findings revealed that QDs are capable of inducing detrimental effects on the reproductive system of the male mice. The testis weight decreased significantly in mice that received 40 mg/kg CdSe:ZnS QDs. Plasma testosterone concentration decreased significantly in mice that received 40 mg/kg, though the LH concentration increased in the group treated with 40 mg/kg CdSe:ZnS QDs.

Conflicts of interest

The authors declare no conflict of interest.

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