Received: 19 March 2018 | Revised: 10 May 2018 | Accepted: 4 June 2018

DOI: 10.1002/jat.3660

REVIEW ARTICLE

WILEY Journal of Applied Toxicology

Review of toxicological effect of quantum dots on the liver

Jie Lu | Meng Tang | Ting Zhang

Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University and Jiangsu Key Laboratory for Biomaterials and Devices, Southeast University, Nanjing 210009, China

Correspondence
Ting Zhang, Key Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health & Collaborative Innovation Center of Suzhou Nano Science and Technology, Southeast University, Nanjing, Jiangsu 210009, China.
Email: zhangting@seu.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 81673218, 31671034, 81473003 and 81302461; Fundamental Research Funds for the Central Universities & Research, Grant/Award Number: 2242018K40021

Abstract

In recent years, quantum dots (QDs) have potential applications in technology, research and medicine. The small particle size is coupled to their unique chemical and physical properties and their excellent fluorescence characteristics. A growing number of studies have shown that QDs are distributed to secondary organs through multiple pathways, while the liver is the main reservoir of QDs. Here, we review current liver toxicity studies of QDs in vivo and in vitro. Mechanisms of hepatotoxicity are discussed and the problem of extrapolating knowledge gained from cell-based studies into animal studies is highlighted. In this context, there still exists significant discrepancies between in vitro and in vivo results, and the specific toxicity mechanism remains unclear. The hepatotoxicities of QDs are the need for a unifying protocol for reliable and realistic toxicity reports.

KEYWORDS

in vivo toxicity, in-vitro toxicity, liver toxicity, quantum dots, toxicity mechanism

1 | INTRODUCTION

Quantum dots (QDs) are semiconductor nanocrystals of size 1–10 nm and are usually composed of elements of groups IIB–VIA or IIIA–VA, which can consist of semiconductor materials such as CdS, CdSe, CdTe, ZnSe, InP and InAs, and can be comprised of two or more kinds of semiconductor materials (Lovric et al., 2005; Wu, Tian, Zhao, & Wu, 2013) (as shown in Figure 1). Compared with traditional organic dyes, the QDs have the following competitive features:

1 QDs have a broad excitation spectra and narrow emission spectra, greatly promoting the application of fluorescent markers. In addition, the emission spectrum of the QDs can be controlled by changing their size and chemical composition so that the emission spectra cover the entire visible area. In the case of CdTe QDs, the emission wavelengths can be shifted from 510 to 660 nm with its particle size increasing from 2.5 to 4.0 nm (Esteve-Turrillas & Abad-Fuentes, 2013).
2 QDs have the advantage in that they have long fluorescence lifetimes compared to the traditional organic dyes (Volkov, 2015).
3 Surface of QDs can be modified by a variety of chemical substances, which lately produced excellent biocompatibility and low cytotoxicity (Resch-Genger, Grabolle, Cavaliere-Jaricot, Nitschke, & Nann, 2008). QDs are currently being widely used in the biomedical field such as cell fluorescent labeling, virus detection, drug targeting transport and tumor imaging in vivo (Barroso, 2011; Esteve-Turrillas & Abad-Fuentes, 2013).

The liver is the major metabolic organ of animals, being responsible for detoxification, storage of glycogen, synthesis of secretory proteins, etc. In general, the liver has the following functions: (1) production and secretion of bile; (2) storage of vitamins and trace elements; (3) synthesis and decomposition of glycogen; (4) protein synthesis and secretion; and (5) phagocytosis of blood pathogens and the production of immune factors (Fischer, Liu, Pang, & Chan, 2006; Hoekstra et al., 2013). The liver is a solid organ, which mainly consists of substantial cells (hepatocytes) and non-substantial cells. The non-substantial cells range from macrophages (Kupffer cells), hepatic sinusoidal endothelial cells, hepatic stellate cells to some immune cells such as natural killer cells, dendritic cells and lymphocytes, etc. (Hoekstra et al., 2013) (as shown in Figure 2). Kupffer cells are the largest macrophages, comprising about 80% of the total macrophages of the body, as it is very important for the liver to clean up exogenous matter in the liver (Davies, Jenkins, Allen, & Taylor, 2013; Fischer, Hauck, Gomez-Aristizabal, & Chan, 2010; W. Zhang et al., 2016). The results of the existing studies also showed that the QDs inevitably enter the organism through a variety of ways and are mainly accumulated in the liver. Furthermore, by interacting with the cells of the liver,

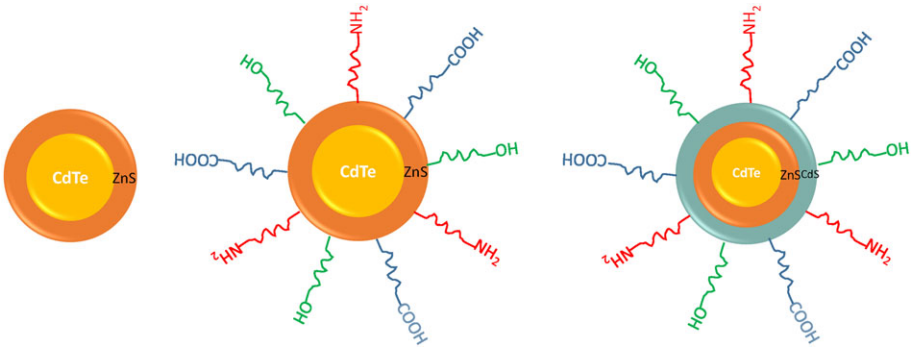

FIGURE 1 In the case of CdTe quantum dots, the left figure represents ZnS-coated QDs, the figure in the middle represents the QDs of ZnS-coated QDs modified by different functional groups, and the right figure represents the QDs that were coated with ZnS and CdS and modified with different functional groups. QDs, quantum dots [Colour figure can be viewed at wileyonlinelibrary.com]

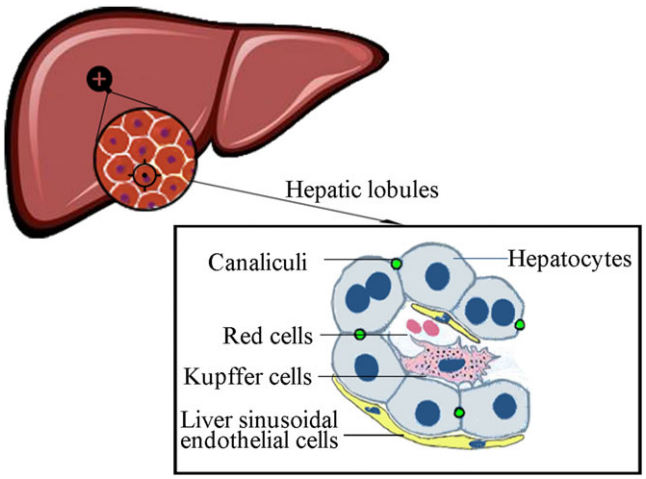

FIGURE 2 Two-dimensional structure diagram of hepatic lobule. Liver mainly consist of the substantial cells (hepatocytes), canaliculi and non-substantial cells, the non-substantial cells mainly include macrophages (Kupffer cells), hepatic sinusoidal endothelial cells [Colour figure can be viewed at wileyonlinelibrary.com]

the QDs would probably affect the liver's function and histopathology (Cho et al., 2007; Fischer et al., 2006; Zhang et al., 2016; Zhu et al., 2017). However, the results about hepatotoxicity of the QDs are inconsistent and determined from the diversities of QDs (including the composition and method of synthesis of the QDs), size, surface modification (surface charge and affinity), route and time of exposure, exposure dose, the types of cell and animal models. Therefore, the safety evaluation of the QDs still needs further study and serves as a scientific basis for the production of safer QDs. Recognizing these factors, the existing toxic effects of the QDs were reviewed, which provided reference for the follow-up study.

2 | TOXIC EFFECTS OF QUANTUM DOTS

A series of assays in vivo and vitro were used to evaluate the toxic effects of the QDs on organisms. In in vitro experiments, the liver cell lines were selected as evaluation models to explore the toxic effects and mechanisms of the QDs on cells and to provide a basis for the thorough animal experiment. However, the organisms are so sophisticated that the distribution and metabolism were difficult to determine using numerous cell tests after the QDs entered the body. Therefore, it is essential for in vivo experiments to focus on the toxic effects of the QDs. The liver, as the major metabolic organ of the mammal, is also one of the target organs for QD accumulation. There have been in vitro and in vivo studies about the toxic effects of QDs on the liver, as shown in Tables 1 and 2, respectively. In the in vitro study, the human hepatoma cells (HepG2 cells) and zebrafish liver cells were often used to evaluate the toxicity and mechanism of the QDs, but the normal liver cells and primary liver cells (usually containing a small amount of Kupffer cells) were selected as cell models. It has been recognized that the primary cells can better reflect the true situation of the body, but the acquisition and cultivation of primary cells are more difficult. As shown in Table 2, multiple experimental studies in vivo have been used to study the distribution, metabolism and exclusion processes of the QDs in organisms, as well as accumulation and toxicity in the liver by using rodents and zebrafish.

2.1 | Toxicity of quantum dots on liver cells

In the in vitro studies, cell viabilities are most often used to evaluate the toxicity of the QDs, which reflects the cytotoxicity of the QDs based on the quantitative changes of cells before and after exposure. Besides, the morphological changes in cells, cell membrane integrity and the release of certain intracellular substances into the medium such as lactate dehydrogenase and adenosine triphosphate are usually measured to reflect the toxic effects of the QDs. Many studies have shown that multiple types of QDs could diminish the survival rate of HepG2 cells in a time- and dose-dependent manner (Nguyen, Willmore, & Tayabali, 2013; Peng et al., 2013; Smith et al., 2012), which is mainly attributed to QDs causing a significant increase in the apoptosis and necrosis rates. Nguyena et al. confirms that CdTe QDs cause the HepG2 cell survival rate to decrease, the level of apoptosis-related proteins Fas, Bax, cytochrome *c* and caspase-8 are significantly increased, followed by a significant decrease in anti-apoptotic protein Bcl-2, and ultimately through endogenous and exogenous pathways, this sequence of transformation would induce apoptosis (Nguyen et al., 2013). In addition, some studies demonstrate that the QDs can cause cell autophagy and pytoptosis (a form of cell death that accompanied by inflammatory activation and inflammatory factors secretion) (Fan et al., 2016; Lu et al., 2016). Lu et al. have shown that CdSe QDs can cause NLRP3 inflammasome activation

TABLE 1 Summary of toxicity of QDs on liver cells

QDs type and mean size	Cell model	Exposure concentration	Hepatotoxicity	References
CdTe QDs: 13.98 ± 2.79 nm	HepG2 cells	0–100 μg ml^{-1} (containing 0.01–1 mg ml^{-1} of Cd)	ROS production; GSH, GSH/GSSG and SOD were changed; Calcium increased;QDs lead to apoptosis via both extrinsic and intrinsic pathways.Disruption of mitochondrial membrane potential; Impaired cellular respiration, the levels and activities of complexes II, III and IV were significantly decreased and increased the level of complex V, and reduced adenosine triphosphate synthesis;Peroxisome proliferator-activated receptor-c coactivator levels increased, the level of mitochondrial biogenesis was effected.	Nguyena et al., 2013.Nguyen et al., 2015
CdTe QDs: 5.06 ± 0.98 nm	HepG2 cells	0–400 μg ml^{-1}	Cells death, IC_{50} was 48.04 μg ml^{-1};Adenosine triphosphate-binding cassette transporters promotes the expulsion of intracellular QDs from HepG2 cells;	Chen et al., 2016
CdTe QDs: 2.2 nm	AML12 cells	0–40 μg ml^{-1}	AML 12 cells death in a dose- and time-dependent manner;ROS and apoptosis rate was increased;Upregulation of p53 and Bax, downregulation of Bcl-2;Both Nrf2 and HO-1 protein levels were significantly upregulated	Zhang et al., 2015
CdTe QDs: 3.4 nm	Zebrafish hepatocytes	19 μM	Cell viability significantly reduced;ROS production, DNA strand breaks;Upregulation of DNA repair gene expression; Nucleotide excision repair mechanism was inhibited with $CdSO_4$ but not with CdTe QDs.	S. Tang, Q. Cai et al., 2013
NAC-capped CdTe QDs: 2.8 nm	Mouse primary hepatocytes	0–200 nm	Cell death;Microenvironment of tyrosine residues, skeleton and secondary structure of Cu/Zn SOD were changed by QDs exposure, which caused activity inhibition of Cu/Zn SOD.	Sun et al., 2015
MPA-coated CdTe/CdS QDs: 12 nm	HL-7702 cells; HepG2 cells	20 nm	Viability of HL-7702, HepG2 cells was inhibited;Autophagy was induced after exposed to QDs, the expression level of LC3-II was significantly upregulated;Viability of the cells was rescued by blocking the autophagy;QDs preferentially enter the lysosomes after were swallowed by the HepG2 and HL-7702 cells, causing increased lysosome activity and then resulting in increased ROS and autophagy, which eventually leads to cell death.	Fan et al., 2016. Fan et al., 2018.
CdTe QDs coated with L-GSH (L-GSH-QDs): 3.2 and 4.2 nm; d-GSH (d-GSH-QDs): 3.2 and 4.2 nm	HepG2 cells.	0–160 nm	Both QDs increased the levels of autophagy in a size- and dose-dependent manner; Inhibition of autophagy increases cell viability;L-GSH-QDs being more effective to induce the activation of autophagy compared to D-GSH-QDs.	Li et al., 2011
CdTe QDs: 3.4 nm CdSe QDs: 4.4, 4.1 and 3.4 nm InP/ZnS QDs: 3.0 nm	Zebrafish liver cells	0–3000 nm	Unshelled CdTe QDs were more toxic to cells than any of the QDs possessing a ZnS shell, and smaller QDs showed greater toxicity than larger QDs;Concentration of Cd^{2+} was highest in zebrafish liver cells in CdTe QDs group, the CdSe/ZnS and InP/ZnS group was similar to the control;Gene expression levels of MT, MTF-1, DMT-1, ZIP-1 and ZnT-1 were significantly changed by all of QDs, but the CdTe QDs is the most efficient inducer.	S. Tang, V, Allagadda, et al., 2013
CdTe QDs: 3–5 nm; CdTe QD/PEG: 3–5 nm; CdTe QDs/BSA: 4–6 nm.	Liver cancer stem cells; Liver cancer cell	10 and 90 nm	Intracellular ROS levels of liver cancer cell increased by 2–5 times;However, intracellular ROS levels of liver cancer stem cells were almost reduced by 50%.	Li et al., 2017
CdSe/ZnS QDs: 7.1 nm	L02 cells	0–80 nm	QDs caused cytotoxicity;QDs induced a novel proinflammatory form of cell death named pyroptosis;QDs induce NLRP3 inflammasome and caspase 1 activation, and IL-1β maturation and secretion.	Lu et al., 2016
CdSe/ZnS QD-NH_2–525: 17.1 nm CdSe/ZnS QD-NH_2–585: 18.9 nm CdSe/ZnS QD-NH_2–625: 22.8 nm CdSe/ZnS QD-COOH-525: 16.7 nm	HepG2 cells;	0–100 nm	NH_2-QDs with smaller size were more easily taken up by HepG2 cells;4 CdSe/ZnS QDs did not caused obvious toxicity (10–100 nm); Increased of gene expression of MT1A and CYP1A1 at 100 nm, the NH_2–525 QD induced more upregulation than the remaining QDs, which was consistent with the tendency of their uptake.	Peng et al., 2013
Amphiphillic polymer-coated CdSe/ZnS QDs: 12.7 nm	HepG2 cells and PHL cells	0–40 nm	HepG2 cells avidly incorporated QDs, but PHL cells did not except for non-parenchymal cells;No toxicity in both cells at 0–40 nm;GCLC and GCLM were significantly downregulated, MT1A and HMOX1 were significantly upregulated in PHL cells, but only MT1A was upregulated in HepG2 cells.mRNA levels of TNF-β, CXCL8, CCL4 and CXCL10 were not changed in HepG2 cells, but the levels of CXCL8 and CCL4 were increased by	Smith et al., 2012

(Continues)

TABLE 1 (Continued)

QDs type and mean size	Cell model	Exposure concentration	Hepatotoxicity	References
			3.3- and 5.5-fold at 10 nm in PHL cells, respectively;Content of CXCL8, CCL4 and CXCL10 were increased in PHL cells, but no change in HepG2 cells.	
CdSe QDs: 4 nm	Hepa1–6 cells	5, 10 and 20 nm	Elevation of intracellular ROS and MDA along with cytotoxicity;Hepatotoxicity caused by QDs greater than Cd^{2+} ions at a similar dose.	Liu et al., 2011
CdS QDs: 5 nm	HepG2 cell	0–100 μg ml^{-1}	Mitochondrial functionality decreased in a dose-dependent manner;IC_{50} was 14 mg ml^{-1}; Bcl-2 were obviously downregulated, ATG3, ATG7, ATG13 and ATG14 were upregulated, AIFM2, APAF1 and BAD were upregulated.	Paesano et al., 2016
Realgar QDs: 5.48 nm	HepG2 cells	0–80 μg ml^{-1}	Cell death and MMP decreased in a dose-dependent manner, IC_{50} for HepG2 after 6 h was 23 μg ml^{-1};Bcl-2 showed dose-dependent decrease and Bax was increased at the level of gene and protein;ER stress gene GRP78 and CHOP increased by 30- and 10-fold, respectively.	Qin et al., 2015

GSH, reduced glutathione; GSSG, oxidised glutathione; MMP, matrix metalloproteinase; PHL, primary human liver (cells); QDs, quantum dots; ROS, reactive oxygen species; SOD, superoxide dismustase; TNF, tumor necrosis factor.

and interleukin (IL)-1beta secretion in L02 cells, thereby inducing the occurrence of cell pytoptosis (Lu et al., 2016). However, it is worth noting that the results will be different, as different liver cell lines are used to evaluate the toxicity of the QDs. For example, the liver cancer cells and normal liver cells exposed to the same QDs at the same concentration caused toxic effects that were significantly disparate, which may be ascribed to the biological characteristics of the cell and the inconsistent sensitivity to the QDs. For instance, Smith et al. studied amphiphilic polymer-coated CdSe/ZnS QDs simultaneously on HepG2 cells and primary human hepatocytes (including some non-solid liver cells), and they found that the HepG2 cells tend to incorporate more QDs, and the primary liver cells did not efficiently take up QDs (except Kupffer cells) (Smith et al., 2012). Consequently, this study showed that the QDs could not be seen to cause obvious cytotoxicity at the maximum exposure concentration (40 nM), but induced oxidative stress-related genes and proteins GCLC and GCLM were significantly downregulated, and MT1A along with HMOX1 was upregulated in primary liver cells, while HepG2 Cells were only elevated in MT1A. In normal liver cells, the proinflammatory cytokines tumor necrosis factor (TNF)-β, CXCL-8, CCL4 and CXCL10 were significantly upregulated, but HepG2 cells were not (Smith et al., 2012). This difference in results suggested that there are two notable questions, that more sensitive cellular models should be used to evaluate the toxicity of the QDs at the subtoxic dose level and more importantly, that primary cells may play a more vital role in the toxicity evaluation of QDs, which meant the obtained results were far more scientific. It was advantageous to provide a more reliable scientific basis for biosafety applications of the QDs.

2.2 | Mechanism of the cytotoxicity of the quantum dots on the liver

At present, the mechanism of cytotoxicity caused by QDs is still unclear. Excessive production of reactive oxygen species (ROS), elevation of intracellular Ca^{2+} levels, QDs and the release of Cd^{2+} ions from the Cd-containing QDs were considered to be the main source of cytotoxicity (as shown in Figure 3).

2.2.1 | Reactive oxygen species and oxidative stress

Excessive production of ROS was one of the common mechanisms for the toxic effects of nanomaterials; the ROS can react with macromolecules such as proteins, DNA, lipids etc., which affects the structure and function of macromolecules, and then causes cell oxidative damage (Cho et al., 2007; Juzenas, Generalov, Juzeniene, & Moan, 2008; Kermanizadeh, Gaiser, Johnston, Brown, & Stone, 2014; L. Liu et al., 2015; Migita et al., 2014; Skalickova et al., 2013; Tsay & Michalet, 2005; Wang & Tang, 2018; Wang, Gao, & Su, 2010; Yong & Swihart, 2012). The Tang et al. study outcomes showed that CdTe QDs and cadmium chloride caused ROS to increase significantly in zebrafish liver cells, which lead to DNA strand breaks, meanwhile, antioxidant genes and DNA repair gene expression was significantly upregulated (Tang, Allagadda, et al., 2013). Nguyen et al. had also verified that CdTe QDs caused an increase of ROS and changed antioxidant levels in treated cells, then induced oxidative stress and

TABLE 2 Summary of in vivo toxicity of QDs on the liver

QD type and size	Animal model	Exposure route, time and concentration	Liver toxicity	References
CdTe QDs: 2.2 nm	Male ICR mice	Intravenous injection 4.125, 8.25 and 16.5 mg kg^{-1} once a week for 4 weeks	Increased of the liver organ coefficient in the 16.5 mg kg^{-1} group; No obvious histopathological change in the liver;SOD and CAT activities were increased in the low-dose group, MDA level increased in the high-dose group.	Zhang et al., 2015
CdTe QDs: 3–4 nm	Male ICR mice	A single dose intravenous injection 1.5 μmol kg^{-1}, observed 1, 7, 14 and 28 days	ALT and AST increased slightly;MT levels reached their peak in the liver at 1 day, but did not change significantly from day 7 to 28;Histopathological changes were time-dependent with elevated Cd^{2+} and OH.	M. Wang et al., 2016
CdTe QDs: 3.8 nm CdTe/SiO_2: 65–70 nm	Female BALB/c mice	Intravenous injection 5 nmol per mouse, observed 30 days	Both QDs did not cause damage to the liver.	Sadaf et al., 2012
Cd/Se/Te-ZnS QDs: 12.3 ± 5.2 nm	Male ICR mice	Intravenous injection 40 and 160 pmol per mouse, observed 12 and 16 weeks	MT protein showed a distinct zonal distribution;No obvious change in histology, but QDs induced changes of essential trace metals (Cu, Zn, Mn, Se, Fe) about antioxidation enzymes in the liver;TNF-α and IL-6 were elevated.	Lin et al., 2011
CdSe/Te/ZnS-PEG: 23.83 nm, QD800-RGD: 16.67 nm,	Male BALB/C mice	Intravenous injection 200 pmol per mouse, observed 14 days	Liver histopathology and functional indicators showed both QDs not caused obvious damage to the liver	Wang et al., 2014
CdSeTe/-ZnS QD-PEG: 52.6 nm CdSeTe/-ZnS QD-COOH: 36.8 nm; CdSeTe/-ZnS QD-NH_2: 52.5 nm.	SD rats	Intravenous injection 200 pmol kg^{-1}, observed 10 min intervals for 5 h	NH_2-QDs and the other two types of QDs showed differences in biodistribution and blood clearance;QD-PEG half-life was 23.6 h, QD-COOH was 0.7 h, NH_2-QDs was 20.7 h in the blood.	Su et al., 2011
CdTe/CdS-MPA QDs: 12 nm	Male BALB/c mice	Intravenous injection 0.1–0.3 nmol per mouse, observed 1, 7 and 30 days	QDs were mainly distributed in liver, kidney and spleen;Autophagy induction by QDs in liver;QDs caused hepatocellular degeneration accompanied by increased relevant activity of AST and ALT.	Fan et al., 2016
CdTe/CdS-MSA: 2.1 nm	Male Wistar rats	Intravenous injection 97 nmol kg^{-1}, observed 3 h	QDs were mainly deposited in the liver sinusoids and selectively taken up by sinusoidal cells (Kupffer cells and liver sinusoidal endothelial cells) instead of by hepatocytes within 3 h.	Liang et al., 2015
CdSe QDs: 4 nm	Male Kunming mice	Intraperitoneal injection 20 and 200 nm for 48 h, 5 and 10 nm for 6 weeks, observed 48 h and 6 weeks	QDs were mainly deposited in the liver via both the acute and chronic exposure; QDs caused morphological alternation to the hepatic lobules and increased oxidative stress.	Liu et al., 2011
CdSe/CdS-MPA QDs: 5.5 nm	Female BALB/c mice	Intraperitoneal injection 0, 5, 10 and 25 mg kg^{-1} once every 3 days for 15 days	QDs were mainly distributed in the liver, spleen, lung and kidneys, but no histopathological damage was observed;Levels of IL-6, lactate dehydrogenase and NADP oxidase were increased in the liver.	Mamunul Haque et al., 2013
CdSe/ZnS QDs: 7.1 nm	Male C57BL/6 mice	Intravenous injection 10 mmol kg^{-1}, observed 2 weeks	Inflammation and dysfunction in the liver were induced by QDs;mtROS production and NLRP3 inflammasome activation in liver tissue; mtROS scavenger suppressed liver NLRP3 activation followed by eased QD-induced liver inflammation and dysfunction.	Lu et al., 2016
Phospholipid micelle capsulated CdSe/CdS/ZnS QDs: 7–8 nm	Male rhesus macaques	Intravenous drip 25 mg kg^{-1}, Observed 90 days	QDs were mainly accumulated in the liver, spleen and kidney, and more than 99% of the injected Cd remains in these organs after 90 days;QDs did not cause toxicity.	Ye et al., 2012
CdS QDs: 2.8 nm; ZnS QDs: 3.3 nm	Male BALB/c mice	Intravenous injection 0.4 and 1 μmol l^{-1} with 200 μl solution at days 1, 6 and 22, observed 30 days.	Both QDs were mainly accumulated in the liver, but did no significant toxicity.	Mansur et al., 2016
Si/SiO_2 QDs: 4.9 nm	Crucian carp	Intraperitoneal injection 2 mg kg^{-1}, observed 7, 14 and 21 days	QDs were detected in the hepatocytes at all time intervals;Increased antioxidant enzyme activity;Elevation activity of cyclooxygenase-2 and matrix metalloproteinases indicated that QDs induced an inflammatory response.	Serban et al., 2015
Si/SiO_2 QDs: 5 nm	Gibel carp			

(Continues)

TABLE 2 (Continued)

QD type and size	Animal model	Exposure route, time and concentration	Liver toxicity	References
		Intraperitoneal injection 2 mg kg^{-1}, observed 1, 3 and 7 days	QDs accumulated in the liver and cause histopathological damage;An increased number of macrophage clusters and fibrosis after 1 and 3 days, hepatocyte basophilia and isolated hepatolytic microlesions were observed in 7 days.Formation of MDA and advanced oxidation protein products, a decrease in protein thiol groups and glutathione levels.	Stanca et al., 2013
Si QD: 60 nm	Mice and monkeys	A single dose intravenous injection 200 mg kg^{-1}, observe for 3 months	In mice:QDs were mainly accumulated in the liver and kidneys;QDs did not result in abnormalities in the liver function, but pathological results showed proliferation of Kupffer cells, multifocal cholestasis and spotty necrosis of hepatic cells.In monkeys: No significant damage in the liver.	Liu et al., 2013
ZnO QDs: 5.4 nm ZnO QD-PEG: 94 nm	Male Kunming mice	Intravenous injection 1 and 5 mg kg^{-1} for ZnO QDs, 5 mg kg^{-1} for ZnO QD-PEG for 7 consecutive days, observed 24 h or 28 days	ZnO QDs cause oxidative stress at 5 mg kg^{-1} 24 h after injecting, but returned to control levels in 28 days;ZnO QD-PEG had less oxidative stress;Both QDs were located in the mitochondrion and induced nuclear malformation in 24 h;ZnO QDs were mainly trapped in the mitochondrion while ZnO QD-PEG mainly accumulated in the lysosomes in 28 days.	Yang, Zhao et al., 2014
ZnS QD-PEG: 4.2 nm ZnO QD-PEG: 5.4 nm	Male Kunming mice	Intravenous injection 2, 6 and 20 mg kg^{-1}, observed 1 h, 24 h and 7 days	Both QDs mainly accumulated in the lung and liver, but induced no harmful effects in lung and liver.	Yang et al., 2015
Mn-doped ZnS QDs: 4.79 nm; QD-PEG: 64.94 nm	Male Kunming mice	Intravenous injection 1 and 5 mg kg^{-1} for Mn-doped ZnS QDs, 5 mg kg^{-1} for QD-PEG for 7 days consecutively, observed 24 h and 28 days	Both QDs did not damage the liver.Mn-doped ZnS QDs were located in mitochondria at 24 h in hepatocytes, and detected in lysosomes and lipid droplets at 28 days; however, QD-PEG mainly distributed in the mitochondria at 24 h and 28 days.	Yang et al., 2015
GQDs: 3–5 nm	Female Balb/c mice	Intravenous and intraperitoneal injection 20 mg kg^{-1} every other day for 14 days observed 24 h and 1 month	No GQD accumulated in main organs of mice and fast clearance through kidneys; GQD showed no obvious harmful effects on mice.	Chong et al., 2014
GQDs: 3–6 nm,	SKH1 female nude mice and Rats	Intravenous injection 2.5 and 5 mg kg^{-1} for nude mice 5 and 10 mg kg^{-1} for rats, observed 1, 8 and 22 days	GQDs mainly accumulated in liver, spleen, lung, kidney;GQDs do not cause obvious toxicity and organ lesions in treated rats.	Nurunnabi et al., 2013
InP/ZnS QDs: 5–6 nm	BALB/c mice	Intravenous injection 25 mg kg^{-1}, observed 0–84 days	QDs were mainly distributed in liver and spleen; no harmful effects were observed over the 84-day period;	Lin et al., 2015
AgSe QD-MEA: 5.1 nm: AgSe QD-MPA: 4.9 nm; AgSe QD-PEG: 5.0 nm	Male ICR mice	A single intravenous injection 8 μmol kg^{-1}, observed 1, 7 and 26 days	3 types of QDs mainly accumulated in the liver and spleen and were significantly converted to Ag and Se within 1 week.QD-PEG and QD-MEA induced some edema and necrosis of the liver after 28 days of exposure.	Tang et al., 2016; Tang et al., 2017
Black phosphorus QDs: 2.7 nm	Male C57BL/6mice	Peritoneal cavity 0.34 mg per mouse, observed 1, 7 and 30 days	QDs caused oxidative stress in the liver after 1 day of exposure to QDs and returned to normal after 7 days;QDs caused a small amount of hepatocyte apoptosis after 1 day, and apoptotic cells were almost absent at 7 and 30 days as well as did not cause significant liver function impairment and pathological injury.	Mu et al., 2017

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GQDs, graphene quantum dots; QD-MEA, 2-aminoethanethiol-coated quantum dots; mtROS, mitochondrial reactive oxygen species; QD-MPA, mercaptopropionic acid-coated quantum dots; QD-PEG, polyethylene glycol-coated quantum dots; QDs, quantum dots.

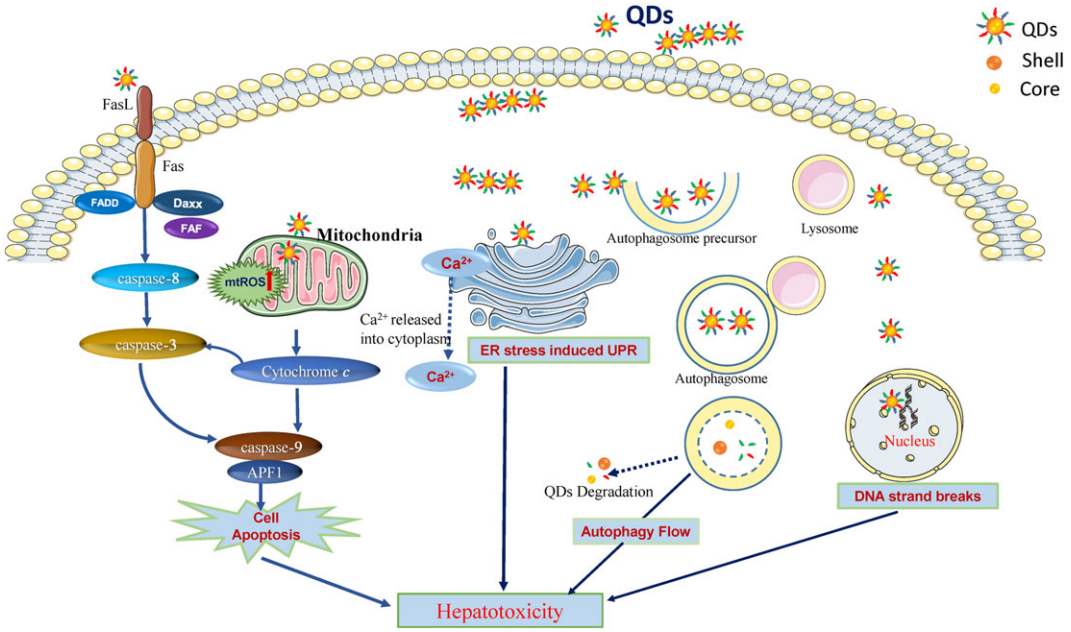

FIGURE 3 Schematic overview of cellular mechanisms in liver cell lines treated with QDs. It was summarized that the QD triggered impairments of the mitochondria, ROS formation, autophagy, DNA damage endoplasmic reticulum stress ultimately leading to multiple organelle damage and cell apoptosis. ER, endoplasmic reticulum; QDs, quantum dots; ROS, reactive oxygen species; UPR, unfolded protein response [Colour figure can be viewed at wileyonlinelibrary.com]

finally activated the apoptotic pathway (Nguyen et al., 2013). How was the excess active oxygen produced? At the subcellular level, it was mainly due to QD damage to mitochondria. Nguyen et al. studied the mitochondrial toxicity of CdTe QDs in mammalian hepatocytes, which indicated mitochondrial swelling and loss of cristae, and further led to the loss of mitochondrial membrane potential and mitochondrial permeability transition (MPT), which led to the release of cytochrome *c* into the cytosol, consequently triggered a series of downstream events in the apoptotic cascade. Apart from this, the CdTe QDs have a detrimental effect on the mitochondrial electron transfer chain (ETC), resulting in decreased levels and activity of the electron transport chain complex II, III and IV (Nguyen, Rippstein, Tayabali, & Willmore, 2015). Unfortunately, the mitochondria are the major organelles of ROS production in cells, and complexes I and III are the primary sites of ROS production (Li et al., 2002). The Li et al. study suggested that NAC-CdTe QDs showed an increased permeabilization of mitochondrial inner membrane to H^+ and K^+ in rat liver cells, which induced MPT. Simultaneously, CdTe QDs caused the increase in state 4 and decrease in state 3 on the mitochondrial respiration, prompting that CdTe QDs affected both electron transfer chain and transport of mitochondrial inner membrane, which caused excessive production of ROS and lipid peroxidation of mitochondrial membranes (Li et al., 2011).

In summary, QDs can damage the morphology and structure of mitochondria, induce mitochondrial membrane potential loss and MPT, and have a deleterious effect on the mitochondrial electron respiratory chain, disrupt the respiratory process, ultimately leading to ROS overproduction. These results are similar to other researchers and are observed in other cell lines. The Lai et al. study has shown that reduced glutathione (GSH)-CdTe QDs interacted with pore-forming proteins on the mitochondrial membrane in human embryonic kidney cells (HEK 293), resulting in an increased mobility of the mitochondrial membrane protein region and increased the probability of MPT formation, which eventually caused increased intracellular ROS levels (Lai et al., 2016).

2.2.2 | Release of cadmium from cadmium-containing quantum dots

In addition, the release of Cd^{2+} from QDs was thought to be one of the other mechanisms of Cd QD-induced cytotoxicity. The QDs can be degraded by acidic organelles such as lysosomes after uptake by cells, and Cd^{2+} can be combined with the thiol groups of the intracellular proteins, which destroyed the structure and dysfunction of the proteins (Mo et al., 2017). Some studies confirmed that QDs could lead to the upregulation of metallothionein (MT) gene expression in the HepG2 cells and zebrafish liver cells (Peng et al., 2013). Upregulation of MT gene expression was seen as a stress response to heavy metals (Klaassen, Liu, & Diwan, 2009). Tang, Cai, et al. research showed that only $CdSO_4$ and CdTe QDs could significantly increase MT and the bivalent metal transporter-1 gene in zebrafish liver cells; however, CdSe QDs and ZnP/ZnS QDs had no significant effect on the expression of both QDs (Tang, Allagadda, et al., 2013). The main reason for this difference was that ZnS limited the release of Cd. The Tang et al. study showed that both CdTe QDs and cadmium chloride caused DNA strands to break, in contrast to cadmium chloride; however, CdTe QDs did not inhibit DNA adduct removal and the mechanism of nucleic acid excision repair, which indicated that the Cd release process was part of the cytotoxicity mechanism but was not entirely the reason (Tang, Cai, et al., 2013). In addition to the reasons mentioned above, the QDs also affected the structure and function of macromolecular substances. For example, Sun et al. discovered that the QDs can interact with Cu/Zn superoxide dismutase (SOD) under the action of hydrophobic force, which caused tyrosine residue and

alteration in the secondary protein structure of the SOD, consequently inhibiting the activity of the SOD and causing oxidative damage (Sun, Cui, & Liu, 2015).

2.2.3 | Elevated intracellular Ca^{2+} levels

Apart from the two main reasons mentioned above, there are still others that deserve our attention and further research. At present, some study results showed that QDs can lead to increasing levels of intracellular Ca^{2+} ions, as well as autophagy and endoplasmic reticulum (ER) stress (Paesano et al., 2016; Richter et al., 2016). Ca^{2+} is one of the most significant and widespread of the second messengers in eukaryotic cells (Richter et al., 2016). Ca^{2+} can activate abundant, different, protein kinases involved in many biological processes such as cell proliferation, differentiation, and apoptosis. Elevated levels of Ca^{2+} impaired mitochondrial membrane permeability, leading to the release of cytochrome *c* and induced apoptosis (Paesano et al., 2016; Richter et al., 2016). Some researchers have described that QDs could cause elevated intracellular Ca^{2+} levels in HepG2 cells (Lu et al., 2016; Nguyen et al., 2015; Paesano et al., 2016). The Nguyen et al. study showed that CdTe QDs led to a 7.4-fold increase in Ca^{2+} levels and was mediated via ROS. Meanwhile high levels of Ca^{2+} concentrations further increased ROS and aggravated oxidative stress (Nguyen et al., 2015). Similarly, CdSe/ZnS QDs caused an increase in the Ca^{2+} concentration in L02 cells, which resulted in increasing mitochondrial ROS generation and activated NLRP3 inflammasomes (Lu et al., 2016). Current research suggests that the ER in organelles is mainly responsible for the dynamic balance of intracellular Ca^{2+}; harmful factors can trigger ER stress, leading to the breakdown of the Ca^{2+} balance (Richter et al., 2016).

2.2.4 | Other potential mechanisms

ER is an important organelle in protein synthesis, folding and secretion in eukaryotic cells and a reserve pool of intracellular Ca^{2+}. Many toxic factors can lead to the ER imbalance of homeostasis, triggering ER stress, which cause the accumulation of the unfolded proteins or misfolded proteins in the ER, giving rise to the risk of unfolded protein responses (Iurlaro & Munoz-Pinedo, 2016; Walter & Ron, 2011). In general, ER stress is performed mainly by three ER stress sensors: protein kinase R-like ER kinase, intron kinase 1 and transcription activator 6. Under physiological conditions, immunoglobulin-binding proteins (BiP) are bound to the inner ER of these three molecules, maintaining the inactive state of signal transduction factors (Iurlaro & Munoz-Pinedo, 2016). Appropriate ER stress is critical to cell survival, and disturbance of its function triggers cellular apoptosis (Iurlaro & Munoz-Pinedo, 2016). ER is abundant in hepatocytes and plays an important role in various liver diseases (Malhi & Kaufman, 2011). At present, some studies have elucidated that the nanomaterial-induced ER stress plays an important role in the cytotoxicity of hepatocytes (Brun, Christen, Furrer, & Fent, 2014; Cao et al., 2017; Huo et al., 2015; Yang et al., 2015; Zhang et al., 2012). Unfortunately, it is still unclear what role ER stress plays in the induction of hepatotoxicity by QDs and little research has been reported. The Qin et al. study reveals that Realgar QD-induced ER stress markers GRP78 and CHOP were significantly increased ($P < .05$) in HepG2 cells (Qin, Wang, Liu, Liu, & Wu, 2015). However, it does not get an in-depth exploration into the role of ER stress-induced apoptosis in the HepG2 cells by QDs. There are still many problems associated with the role of ER stress in hepatotoxicity by QDs that need to be addressed, including activation of the ER stress pathway and the interaction with mitochondria or lysosomes. Autophagy is a fundamental physiological phenomenon in eukaryotic cells and plays an important role in maintaining cell homeostasis. It clears damaged organelles and degrades long-term protein to promote the cell survival rate under the condition of nutritional deficiencies, hypoxia or other harmful factors, while excessive autophagy leads to cell death (Tanida, 2011). There are some studies confirming that hepatocyte toxicity caused by nanomaterials was mediated by autophagy. (Fan et al., 2016; Paesano et al., 2016; Zhang et al., 2017; Zhu et al., 2017). Studies have shown that low-dose (3 μg ml^{-1}) CdS QDs can lead to upregulation of autophagy related genes ATG3, ATG7, ATG13 and ATG14, whereas in high doses (14 μg/ml), there is no such inducement effect in HepG2 cells (Paesano et al., 2016), which suggest the significance of autophagy in preventing intracellular damage. In contrast to this, Fan et al. revealed that CdTe/CdS QDs only caused the autophagy of HL7702 cells and HepG2 cells, and without inducing apoptosis. Pretreatment with an autophagy inhibitor 3-methyladenine alleviated the cytotoxicity caused by QDs and increased cell viability (Fan et al., 2016). Importantly, in Fan et al.'s latest study they revealed that CdTe/CdS QDs preferentially enter the lysosome after being ingested by HepG2 and HL-7702 cells through the clathrin-mediated pathway, leading to a significant increase in lysosomal activity, which caused ROS production and autophagy. Inhibition of lysosome activity revealed that ROS levels and autophagy were significantly reduced, and cell viability was significantly increased. In contrast, the lysosomal activity did not change after ROS and autophagy were inhibited, and the cell survival rate increased significantly. It is suggested that QDs elicit hepatotoxicity through lysosome-dependent autophagy activation and ROS production (Fan et al., 2018). This inconsistency made the autophagy more inconceivable in QD-induced hepatotoxicity. Both autophagy and ER stress have dual roles in cells (Yorimitsu, Nair, Yang, & Klionsky, 2006; Zhang, Morris Jr., Dorsett-Martin, Drake, & Anderson, 2013). However, the interaction of autophagy and endoplasmic reticulum stress in quantum dots-induced liver toxicity in vivo and in vitro still is unclear.

2.3 | In vivo toxicity of quantum dots on the liver

In vivo toxicity tests were mainly used to evaluate the process of distribution, metabolism and excretion under certain circumstances such as QDs entering into organisms (such as mice) or some possible damage occurring in organ tissue. The QDs can be utilized as a drug carrier for the diagnosis and treatment of diseases. Therefore, the overall process starting from the entrance of QDs into the body must be studied in detail. Rodents have often been used to study the fate of QD in vivo toxicity, followed by zebrafish and *Caenorhabditis elegans*. There are very few researchers using primates (such as rhesus monkeys). So far, a considerable number of studies have confirmed the distribution and toxicity of QDs in vivo and have achieved some results. Unfortunately, many research results were not consistent, even

contradictory, indicating that researchers should make further joint efforts to complete the assessment of QD toxicity.

2.3.1 | Liver is the target organ of the largest accumulation of quantum dots

Most studies have shown that a very small number of dots are distributed in the brain and heart, and regardless of which routes of exposure, QDs are mainly stored in the reticular endothelial system such as the liver, spleen, lung and kidneys (Haque et al., 2013; Nurunnabi et al., 2013; Su et al., 2011; Yang, Lan, et al., 2014). Ye et al. studied phospholipid micell-coated CdSe/CdS/ZnS QDs (25 mg kg^{-1}) with intravenous infusion processed primate rhesus monkeys, which were observed for 90 days after exposure, and found that 99% QDs were stored in the liver, spleen and kidney, accounting for 58%, 6% and 35% of the total QD content respectively, but did not cause any toxic effects (Ye et al., 2012). Similarly, Haque et al. also confirmed that CdSe/CdS QDs mainly distributed in the liver and spleen by repeated intraperitoneal injection in mice (Haque et al., 2013). Liver is one of the main accumulation and metabolism organs of the QDs, the toxicity against the liver cannot be ignored. It is interesting that most of the current findings in vivo are quite different from in vitro toxicity results.

2.3.2 | Quantum dots have no deleterious effect on liver function

In analyzing the existing results from the perspective of QD liver toxicity it was found that QDs did not damaged to the liver or induced almost negligible toxicity after entry into the body', Sadaf et al. studied the effect of 5 nmol CdTe QDs and CdTe/SiO2 QDs on the BALB/C mice by intravenous injection and killed 30 days after exposure. Both QDs would not cause liver damage or any histopathological changes (Sadaf et al., 2012). Tang et al. studied the distribution, metabolism and elimination of three different surface-modified AgSe QDs of polyethylene glycol, 2-aminoethanethiol and mercaptopropionic acid coated Ag2Se QDs (denoted as QD-PEG, QD-MEA and QD-MPA, respectively) after intravenous injection into mice. The results showed that the three QDs accumulated mainly in the liver and spleen after exposure and were significantly converted to Ag and Se within 7 days. Importantly, all QDs did not cause damage to liver function and tissue, except for some edema and necrosis was found in the liver in the QD-MEA group at day 28 (Fan et al., 2016; Fan et al., 2018). Similar studies showed that black phosphorus QDs were mainly accumulated in the liver and spleen after intraperitoneal injection and caused a small amount of hepatocyte apoptosis on the first day after exposure, but apoptotic cells are almost absent after 7 and 30 days, suggesting that black phosphorus QDs have no long-term toxicity. It is emphasized that no apparent damages was detected in all organs, particularly in the liver and spleen during the entire time (Mu et al., 2017). This significant difference between in vivo and in vitro studies was mainly due to the complexity of the environment in the body. The QDs will be distributed to multiple organs and affected by substances in the blood after getting into the body, such as blood protein will be adsorbed by the QDs at their surface forming a "protein corona", which affects the redistribution and metabolism of the QDs because the protein coronal will affect the cell phagocytosis of QDs (Feliu et al., 2016). The immune system of the body will also play an important role in the phagocytosis and degradation of QDs, further affecting the QDs to reach the target organ concentration, ultimately reducing the potential toxic effects of the QDs (Fischer et al., 2010). For instance, Liang et al. found that the QDs were almost all incorporated by Kupffer cells and sinusoidal endothelial cells in the hepatic sinusoids, although the QDs distributed to the liver after injecting CdTe/CdS-MSA QDs into Wistar rats intravenously, the hepatocytes did not assimilate the QDs (Liang et al., 2015).

2.3.3 | QDs induced inflammation response

However, there are some studies that have shown that QDs have no significant effect on liver function or pathological damage, but can cause mild inflammation on liver tissue (Haque et al., 2013; Lin et al., 2011; Liu et al., 2013; Lu et al., 2016; Stanca et al., 2013). Lin et al. studied ICR mice injected intravenously with CdTe/ZnS (40 and 160 pmol per mouse). The results showed that the QDs did not cause any histopathological changes in the liver, but induced an elevated expression of some proinflammatory factors such as IL-6 and TNF-α, which suggested that it could create an immune response in the liver (Lin et al., 2011). IL-6 is a cytokine that can induce differentiation of B cells, promote the growth and differentiation of primitive bone marrow-derived cells with colony stimulating factor and enhance natural killer cell activity (Vallieres & Rivest, 1999). TNF-α is a proinflammatory cytokine secreted mainly by monocytes and macrophages, which can directly cause tumor cell death and promote neutrophil phagocytosis (Del Campo, Gallego, & Grande, 2018). Haque et al. studied the result of injecting CdSe/CdS-MPA QDs (25 mg kg^{-1}) into BALB/C mice by intravenous administration once every 3 days for 15 days. The results showed that the QDs were mainly accumulated in the liver, spleen, lung and kidney, but no pathological damage was observed at histological examination. However, the levels of IL-6, lactate dehydrogenase and NADPH oxidase were significantly increased in the liver after 7 days, which indicated that QDs could cause damage to liver cells and induced an inflammatory response, but did not clarify which cells were damaged (Haque et al., 2013). In addition, studies have shown that Si QDs can induce the cyclooxygenase 2 activity increased by 100%, 165% and 153% after exposed to Si QDs for one, two and three weeks compared to controls (Serban et al., 2015). Cyclooxygenase-2 is induced by proinflammatory cytokines, growth factors and tumor promoters, is always present at the site of inflammation and is considered as an indicator of immune response (El Sayed et al., 2018). Simultaneously, Si QDs can lead to a two- to threefold increase in the expression levels of matrix metalloproteinases (MMP) 2 and MMP-9 genes and a 141% increase in the activity of MMP-2 and MMP-9 (Serban et al., 2015). Changes in the levels of MMPs can be considered as an indicator of inflammation (Castillo-Briceno, Arizcun-Arizcun, Meseguer, Mulero, & Garcia-Ayala, 2010). Similarly, Lu et al. revealed that CdSe/ZnS QDs induce liver inflammation mediated by the significantly increased chemokine [C-X-C motif] ligand 1 (CXCL1) after QD administration. Meanwhile, QDs lead to the activation of NLRP3 inflammasomes and increase the secretion of IL-1β in the liver (Lu et al., 2016). IL-1β is one of the most potent proinflammatory cytokines that can induce rapid

recruitment of neutrophils to sites of inflammation, activate endothelial adhesion molecules, induce cytokines and chemokines, and stimulate specific types of adaptive immunity (Sahoo, Ceballos-Olvera, del Barrio, & Re, 2011). This results were consistent with other research (Lin et al., 2011). The inflammation response in the liver from QDs was closely related to the Kupffer cells, which are responsible for removing exogenous substances in the liver. Studies have shown that QDs were mainly ingested by Kupffer cells and blood sinus endothelial cells after entering the liver, and induced inflammation and promoted the proliferation of kupffer cells, while only a very small amount of liver parenchy cells phagocytize QDs (Liu et al., 2013; Manshian et al., 2015). These findings suggested that the QDs did not cause changes in liver function or significant pathological changes after entering into the organism, but still caused damage at subcellular levels. It is notable that QDs can be used for the diagnosis and treatment of disease, then QDs will reach the lesion of the organ that has already been damaged when entering into the body; however, it is possible that QDs can further cause damage to the compensatory capacity and current research is still limited to healthy animal models, and subsequent studies should be studied further with appropriate disease animal models.

2.3.4 | QDs caused oxidative stress

Studies have demonstrated that QDs can cause oxidative stress on various hepatocyte models in vitro. Studies have shown that QDs caused oxidative stress in liver tissues through changes in antioxidant enzyme activity and content, and trace element content related to antioxidant enzymes (Lin et al., 2011; Zhang et al., 2015). Lin et al. studied ICR mice injected intravenously with CdTe/ZnS (40 and 160 pmol per mouse). The results showed that the QDs caused the concentration of antioxidant system-related trace elements to increase in Zn, Se, Mn, Fe and Cu as well as corresponding to the amount of transporter (Lin et al., 2011). The Cu and Zn were indispensable for cytoplasmic SOD, the manganese was essential for SOD of mitochondria, the Fe and Se was irreplaceable for catalase (CAT) and GSH, respectively. Therefore, Lin et al. found that QDs caused an increase in glutathione peroxidase activity from 12 to 16 weeks after administration of QDs, while reducing Cu/Zn SOD activity. Meanwhile, the QDs caused an elevation in the ratio of GSH/oxidised glutathione at12 weeks, but significantly reduced at 16 weeks in QD705-treated animals, which reflected the oxidative stress (Lin et al., 2011). Other studies have also proven that QDs can lead to a significant increase in the levels of the ROS or ·OH, and changes in the concentration or activity of glutathione peroxidase, CAT and SOD (Serban et al., 2015; S. Wang et al., 2016; Y. Yang, T. Zhao et al., 2014; Yang et al., 2015; Zhang et al., 2015).

2.3.5 | Other toxic effects

A small number of studies have concluded that the QDs can cause significant liver damage, liver tissue disorders and even liver fibrosis (Fan et al., 2016; Stanca et al., 2013; M. Wang et al., 2016). Fan et al. have studied that MPA-CdTe/CdS QDs (0.1–0.3 nmol per mouse) by intravenous administration. Hepatocellular degeneration was observed at 30 days after exposure, accompanied by a significant increase in liver functional indicators of aspartate aminotransferase and alanine aminotransferase (Fan et al., 2016). Stanca et al. presented a study in which the Gibel carp were used as the test animals to observe the effect of Si/SiO_2 QDs (2 mg kg^{-1}) through intraperitoneal injection for 1–7 days. The results showed that the pathological changes in the liver were observed after 1–3 days, alkaline granulocyte aggregation, hepatic macrophage hyperplasia and hepatic fibrosis, and even after 7 days, liver cell lytic damage was observed and resulted in decreased levels of thiol-containing protein and GSH (Stanca et al., 2013). Clearly, it is justifiable to explore what caused such a wide gap in the results of all the in vivo studies. Unfortunately, the evidence that highlights this gap was not enough. The hepatotoxicity studies in vivo primarily focus on the accumulation of QDs in the liver and whether it caused liver function and pathological damage, and a few studies further illuminate how QDs interact with liver cells, which was helpful in revealing the mechanism involved in QDs disrupting the liver.

3 | PARAMETERS GOVERNING THE HEPATOTOXICITY OF QUANTUM DOTS

Until now, we have found that the current opinion about QD liver toxicity in vivo and in vitro was controversial. The discrepancy in the results of existing studies was caused by the different types, size, dose, exposure routes, exposure time of QDs and cell types or animal models. At present, the prevalent research interests of factors affecting the toxicity of QDs are converged on factors such as types, size and surface modification of QDs, but other influencing factors are rarely studied and need to be explored further.

3.1 | Size versus toxicity

The size of nanomaterials was the most important factor affecting its toxic effects (Chang, Kang, Liu, Dai, & Chen, 2012; Lai et al., 2016). Most studies have shown that the smaller QDs have the greater toxicity. Tang et al. studied the toxic effects of CdSe/ZnS QDs on zebrafish liver cells, and the smaller QDs resulted in greater cytotoxicity (Y. Tang et al., 2013). The results of the study showed that zebrafish liver cells were exposed to different sizes of CdSe/ZnS (3.4 and 4.4 nm) for 24 hours and the IC_{50} was 1327 and 3164 nm, respectively (Y. Tang et al., 2013). The CdSe/ZnS QDs increased by 1 nm in size and toxicity is reduced by almost 2.4-fold, which suggested that the smaller QDs resulted in greater cytotoxicity. The results of this study were likely compared to those of Peng et al. (2013). The results also showed that the smaller QDs were more likely to be ingested by HepG2 cells, which may be an important reason for the greater toxicity of smaller sized QDs. The size of QDs affects the distribution of QDs in cells and in vivo (Chang et al., 2012; Su & Sun, 2013; W. Zhang et al., 2016). Chang et al. have shown that smaller-sized QDs were more likely to enter the cells and are subsequently dispersed to the nucleus, yet the larger-sized QDs were distributed only in the cytoplasm after the MPA-CdTe QDs on pancreatic cancer cells (PANC-1). The DNA was more vulnerable to damage when QDs were transferred from the cytoplasm to the nucleus caused cytotoxicity. The difference in size of the QDs

influenced the phagocytosis and distribution in cells. However, how liver toxic effects are determined by size is still under discussion, and importantly, QDs are a class of nanomaterials with smaller size (2-10nm) than other nanomaterials (the size was generally more than tens of nanometers); so the results of other nanomaterials may not be applicable to QDs.

3.2 | Surface chemistry versus toxicity

The Cd-containing QDs have been proved toxic and the release of Cd^{2+} ions from Cd-containing QDs is one of the major aspects of toxicity, which limits applications in the biomedical field. To solve this problem, the molecules containing specific functional groups (as shown in Figure 1) were coated on the surface of the synthesized QDs to improve the biocompatibility of QDs, inhibiting the release of Cd^{2+} ions from QDs thus reducing its toxicity. The size of QDs will be significantly increased if the surface of the QD is coated and therefore conducive to reducing toxicity (Liang et al., 2015; Sadaf et al., 2012; Yang et al., 2014). Y. Yang et al. conducted a study in which the ZnO-PEG QDs (97 nm, dose: 5 mg kg^{-1}) were continuously exposed for 7 days. The results showed that the ZnO QDs could dramatically increase the antioxidant activity (CAT/GSH-PX, SOD and MDA), but the ZnO-PEG QDs had little effect on these indexes. Further observation revealed that both QDs were ingested by hepatocytes at 24 hours and distributed in mitochondria. However, after 28 days, the ZnO QDs were still evenly distributed in mitochondria, while the ZnO-PEG QDs were allocated in lysosomes (Yang et al., 2014). These intracellular differences in distribution clearly interpret the differences in toxicity between the two QDs. The QDs have different molecular groups that lead to the affinity surface charges of the QDs were diverse. The difference in surface modification also influences the distribution and metabolism of QDs in the body. Peynshaert et al. found that the CdSe/ZnS-MPA QDs could activate lysosomes, decrease the concentration of ROS, but that CdSe/ZnS-PEG QDs significantly elevated ROS concentration in HeLa cells, causing damage to lysosomes, and compared with the former one, CdSe/ZnS-PEG QDs have greater cytotoxicity, while the former one has better biocompatibility (Peynshaert et al., 2017). Q. Liu et al. have shown under certain circumstance that two human breast cancer cell lines (MDA-MB-231 cells and BT-20 cells) and murine peritoneal macrophages ((RAW264.7 cells) exposed to different surface-modified CdS/ZnS QDs with a positive, negative and neutral charge, the charged QDs were more likely to be phagocytosed by three cells compared to the neutral QDs, and the negative charge QDs were most likely to be incorporated. The positively charged QDs were mainly deposited on the cytoplasm, but the negatively charged QDs were mainly distributed in the endosomes and lysosomes after exposure. The neutral QDs were clustered around the cytoplasm and vesicular organelles (e.g., lysosomes and lipid droplets). The distribution of the three QDs in the body were also inconsistent, the positively charged QDs were mainly distributed in the kidney, while the other two QDs were more likely to deposit in the liver and spleen (Q. Liu et al., 2015). This result is similar to that of Y. Tang et al. (2013). The different charged QDs would accumulate in different organs. Compared with the liver, the positively charged QDs tend to accumulate in the lungs, while the negative and neutral QDs are mainly accumulated in the liver (Y Tang, S Han et al., 2013). From a toxicity perspective, the positively charged QDs had the highest hepatotoxicity, and the toxicity was minimal for the neutral QDs.

3.3 | Type and composition of quantum dots

With the rapid development of nanotechnology, a variety of QDs with better biocompatibility are synthesized; in particular, in the synthesis of Cd-free QDs, these QDs are synthesized by III AV A and IV group elements, such as InP/ZnS QDs, carbon QDs and silicon QDs (Iannazzo et al., 2017; Volkov, 2015; S. Wang et al., 2016; Wu et al., 2013). These QDs are more biocompatible and less toxicity compared with the Cd-containing QDs. In addition, proper surface modification of the Cd-containing contributes to enhance biocompatibility and reduce toxicity. S. Tang et al. have found that CdTe QDs at 50 nm has caused a significant reduction in the survival rate of zebrafish liver cells, and InP/ZnS QDs in the 500 nm cell survival rate began to reduce significantly ($P < .05$) (S. Tang, V. Allagadda et al., 2013). For the more biocompatible QDs, the effects of subtoxic dose levels on cells need to be attributed more attention, such as HepG2 and primary human normal hepatocytes treated with pro-amphoteric polymer-modified CdSe/ZnS QDs. Although the maximum exposure dose (40 nM) did not result in a significant decrease in both cell viability, QDs induced significant down-regulation of oxidative stress-related genes and protein GCLC and GCLM expression in both cells. At the same time, QDs caused up-regulation of CXCL-8, CXCL10 and CCL4 gene expression in the pro-inflammatory factors TNF-β and CXCL and primary normal human hepatocytes in both cells. Moreover, the levels of proinflammatory cytokines were significantly increased except for TNF-β, which indicated that an inflammatory response had been caused (Smith et al., 2012). In addition, more sensitive toxicity indicators should be tested, such as autophagy levels and ER stress. The concentration of QDs in the actual application process will be generally lower than the experimental concentration, taking into account the use of more sensitive and early indicators, which are more critical for bioapplications of QDs.

3.4 | Other factors

The complexity of the human body must be considered alongside the physicochemical properties of QDs. The blood supply of the liver is mainly derived from hepatic arteries and portal veins. The hepatic artery provides 30% blood and 70% oxygen, the portal vein provides many nutrients from the gastrointestinal tract. The blood from both the hepatic artery and portal veins converge together in hepatic sinusoids. The hepatic sinusoids is the gap between the adjacent liver plate, including a series of immune cells such as macrophages (Kupffer cells), NK cells and T cells, which plays an important role in eliminating harmful substances in the liver and protecting liver tissues. (Hoekstra et al., 2013; Y. N. Zhang, W, Poon et al., 2016). At present, the pathway of exposure in vivo was mainly by intravenous injection and a few studies were abdominal exposure refers to the exposure of exogenous chemicals by intraperitoneal injection. The QDs entered the liver through the venous pathway and then first came into

contact with macrophages and sinusoidal endothelial cells in the liver (Liang et al., 2015). Kupffer cells play a very important role in mediating the liver toxicity of QDs, and the intake of QDs in to Kupffer cells will greatly affect the toxic effects of QDs on other hepatocytes (Chen, Xue, & Sun, 2013; Fischer et al., 2010; Liang et al., 2015; Zhu et al., 2017). The Liang et al. study showed that CdTe/CdS-MSA QDs mainly accumulated in the liver sinusoids and were selectively taken up by Kupffer cells and liver sinusoidal endothelial cells instead of by hepatocytes within 3 h (Liang et al., 2015). This conclusion may explain why many studies have not observed liver function and pathological damage by QDs, but it did induce an inflammation response to a certain extent.

4 | CONCLUSION AND FUTURE PERSPECTIVES

In conclusion, we have summarized the recent research focus on the hepatotoxicity of QDs. In general, the majority of existing studies in vitro demonstrate that certain QDs can cause toxicity in certain cells via the generation of intracellular ROS or Cd^{2+} ion release, and the toxicity of Cd-containing QDs seems to be greater than that of Cd-free QDs (S. Tang, V, Allagadda et al., 2013). Thus, the Cd-free QDs with excellent biocompatibility and lower toxicity are promising for bioapplications such as bioimaging, bio-sensing and drug delivery. However, the hepatotoxicity profile of QDs varied in the reports with different test subjects and different QDs, which has brought great challenges for the study of the hepatotoxicity of QDs. Although hepatotoxicity in the toxicology studies of QDs was explicit, it does not apply to in vivo toxicity. There are many controversies in terms of liver toxicity in vivo, which are closely related to the type of QDs, animal models, exposure dose and time. More importantly, the biological environment of animals has an inevitable impact on distribution, metabolism and exclusion of QDs in the liver. In general, multiple QDs hold unclear toxicity or low toxicity to the liver, some QDs caused only mild inflammatory responses or oxidative stress at the level of the gene or protein and only a few studies have shown that QDs can cause liver dysfunction and pathological damage. It is a little astonishing about the outcomes of "highly toxic QDs" including both Cd-containing QDs and Cd-free QDs (and also both coated QDs and non-coated QDs). For example, CdSe QDs (size was 4 nm, 5–10 nmol per mouse, observed 48 hours and 6 weeks) caused morphological alternation to the hepatic lobules and increased oxidative stress (Liu et al., 2011). The CdTe/CdS-MPA QDs (size was 12 nm, dose was 0.1–0.3 nmol per mouse, observed 1–30 days) also caused hepatocellular degeneration accompanied by increasing the relevant activity of aspartate aminotransferase and alanine aminotransferase (Fan et al., 2016). The difference makes it more difficult to evaluate the hepatotoxicity of QDs.

Although the rise of the availability of hepatotoxicity studies of QDs has been trending over the past decade, there is still an urgent demand for more research effort to be made in this field so that a full spectrum of the hepatotoxicity of QDs could be explained clearly. Based on our evaluation of the current research situation, there are some possible future research perspectives:

1 There is more research in vitro than in vivo so that the systematic study of QDs in vivo liver toxicity is achieved.

2 Although there are more studies in vitro, system research is still not perfect. Many of the studies did not make a connection between the in vitro and in vivo studies so that they could not be properly summarized. Therefore, joint research must be carried out in vitro and in vivo, which will contribute to uncover the relationship between certain parameters and hepatotoxicity, such as QD size, surface chemistry or charge and chemical compositions.

3 At present the mechanisms of toxicity of QDs towards liver cells are still not clear enough, the liver cell uptake process of QDs, intracellular distribution, metabolism and elimination are still unclear. The effect of QDs on hepatocytes in subtoxic levels is superficial. It does not matter what role ER stress, mitochondrial dysfunction and autophagy play in QD-induced hepatotoxicity.

4 The study of in vivo hepatotoxicity in the future needs to elucidate the distribution of QDs in the liver, interaction with various cells in the liver and the role of non-parenchymal hepatocytes such as Kupffer cells and hepatic sinusoidal endothelial cells in the induction of hepatotoxicity by QDs.

Combined with practical applications, appropriate liver disease model animals should be established to assess the toxic effects of QDs to elucidate the deleterious effects that may be caused by the use of QDs in the presence of liver cells.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (no. 81673218, 31671034, 81473003, 81302461) and Fundamental Research Funds for the Central Universities & Research (no.2242018K40021). Meanwhile, we appreciate the reviewers' comments, which improved our manuscript. We thank Dr. Ting Zhang (School of Public Health of Southeast University) and Dr. Meng Tang (School of Public Health of Southeast University) for their suggestions.

CONFLICT OF INTEREST

The authors did not report any conflict of interest.

ORCID

Jie Lu http://orcid.org/0000-0003-4195-8015

REFERENCES

Barroso, M. M. (2011). Quantum dots in cell biology. *The Journal of Histochemistry and Cytochemistry*, *59*, 237–251. https://doi.org/10.1369/0022155411398487

Brun, N. R., Christen, V., Furrer, G., & Fent, K. (2014). Indium and indium tin oxide induce endoplasmic reticulum stress and oxidative stress in zebrafish (*Danio rerio*). *Environmental Science & Technology*, *48*, 11679–11687. https://doi.org/10.1021/es5034876

Cao, Y., Long, J., Liu, L., He, T., Jiang, L., Zhao, C., & Li, Z. (2017). A review of endoplasmic reticulum (ER) stress and nanoparticle (NP) exposure. *Life Sciences*, *186*, 33–42. https://doi.org/10.1016/j.lfs.2017.08.003

Castillo-Briceno, P., Arizcun-Arizcun, M., Meseguer, J., Mulero, V., & Garcia-Ayala, A. (2010). Correlated expression profile of extracellular matrix-related molecules during the inflammatory response of the teleost fish gilthead seabream. *Developmental and Comparative*

Immunology, *34*, 1051–1058. https://doi.org/10.1016/j.dci.2010.05.007

Chang, S., Kang, B., Liu, X., Dai, Y., & Chen, D. (2012). The combined influence of surface modification, size distribution, and interaction time on the cytotoxicity of CdTe quantum dots in PANC-1 cells. *Acta Biochimica et Biophysica Sinica*, *44*, 241–248. https://doi.org/10.1093/abbs/gmr126

Chen, M. L., Yin, H. C., Bai, P. L., Miao, P., Deng, X. D., Xu, Y., … Yin, J. (2016). ABC transporters affect the elimination and toxicity of CdTe quantum dots in liver and kidney cells. *Toxicology of Applied Pharmacology*, *303*, 11–20. http://doi.org/10.1016/j.taap.2016.04.017

Chen, Q., Xue, Y., & Sun, J. (2013). Kupffer cell-mediated hepatic injury induced by silica nanoparticles in vitro and in vivo. *International Journal of Nanomedicine*, *8*, 1129–1140. https://doi.org/10.2147/IJN.S42242

Cho, S. J., Maysinger, D., Jain, M., Roder, B., Hackbarth, S., & Winnik, F. M. (2007). Long-term exposure to CdTe quantum dots causes functional impairments in live cells. *Langmuir*, *23*, 1974–1980.

Davies, L. C., Jenkins, S. J., Allen, J. E., & Taylor, P. R. (2013). Tissue-resident macrophages. *Nature Immunology*, *14*, 986–995. https://doi.org/10.1038/ni.2705

Del Campo, J. A., Gallego, P., & Grande, L. (2018). Role of inflammatory response in liver diseases: Therapeutic strategies. *World Journal of Hepatology*, *10*, 1–7. https://doi.org/10.4254/wjh.v10.i1.1

El Sayed, M. T., El-Sharief, M. A. M. S., Zarie, E. S., Morsy, N. M., Elsheakh, A. R., … Abdel-Aziz, H. A. (2018). Design, synthesis, anti-inflammatory antitumor activities, molecular modeling and molecular dynamics simulations of potential naprosyn (R) analogs as COX-1 and/or COX-2 inhibitors. *Bioorganic Chemistry*, *76*, 188–201. https://doi.org/10.1016/j.bioorg.2017.11.002

Esteve-Turrillas, F. A., & Abad-Fuentes, A. (2013). Applications of quantum dots as probes in immunosensing of small-sized analytes. *Biosensors & Bioelectronics*, *41*, 12–29. https://doi.org/10.1016/j.bios.2012.09.025

Fan, J., Sun, Y., Wang, S., Li, Y., Zeng, X., Cao, Z., … Ju, D. (2016). Inhibition of autophagy overcomes the nanotoxicity elicited by cadmium-based quantum dots. *Biomaterials*, *78*, 102–114. https://doi.org/10.1016/j.biomaterials.2015.11.029

Fan, J., Wang, S., Zhang, X., Chen, W., Li, Y., Yang, P., … Ju, D. (2018). Quantum dots elicit hepatotoxicity through lysosome-dependent autophagy activation and reactive oxygen species production. *ACS Biomaterials Science & Engineering*, *4*, 1418–1427. https://doi.org/10.1021/acsbiomaterials.7b00824

Feliu, N., Docter, D., Heine, M., Del Pino, P., Ashraf, S., Kolosnjaj-Tabi, J., … Parak, W. J. (2016). In vivo degeneration and the fate of inorganic nanoparticles. *Chemical Society Reviews*, *45*, 2440–2457. https://doi.org/10.1039/c5cs00699f

Fischer, H. C., Hauck, T. S., Gomez-Aristizabal, A., & Chan, W. C. (2010). Exploring primary liver macrophages for studying quantum dot interactions with biological systems. *Advanced Materials*, *22*, 2520–2524. https://doi.org/10.1002/adma.200904231

Fischer, H. C., Liu, L., Pang, K. S., & Chan, W. C. W. (2006). Pharmacokinetics of nanoscale quantum dots: in vivo distribution, sequestration, and clearance in the rat. *Advanced Functional Materials*, *16*, 1299–1305. https://doi.org/10.1002/adfm.200500529

Haque, M. M., Im, H. Y., Seo, J. E., Hasan, M., Woo, K., & Kwon, O. S. (2013). Acute toxicity and tissue distribution of CdSe/CdS-MPA quantum dots after repeated intraperitoneal injection to mice. *Journal of Applied Toxicology*, *33*, 940–950. https://doi.org/10.1002/jat.2775

Hoekstra, L. T., de Graaf, W., Nibourg, G. A., Heger, M., Bennink, R. J., Stieger, B., & van Gulik, T. M. (2013). Physiological and biochemical basis of clinical liver function tests: a review. *Annals of Surgery*, *257*, 27–36. https://doi.org/10.1097/SLA.0b013e31825d5d47

Huo, L., Chen, R., Zhao, L., Shi, X., Bai, R., Long, D., … Chen, C. (2015). Silver nanoparticles activate endoplasmic reticulum stress signaling pathway in cell and mouse models: The role in toxicity evaluation. *Biomaterials*, *61*, 307–315. https://doi.org/10.1016/j.biomaterials.2015.05.029

Iannazzo, D., Pistone, A., Salamo, M., Galvagno, S., Romeo, R., Giofre, S. V., … Di Pietro, A. (2017). Graphene quantum dots for cancer targeted drug delivery. *International Journal of Pharmaceutics*, *518*, 185–192. https://doi.org/10.1016/j.ijpharm.2016.12.060

Iurlaro, R., & Munoz-Pinedo, C. (2016). Cell death induced by endoplasmic reticulum stress. *The FEBS Journal*, *283*, 2640–2652. https://doi.org/10.1111/febs.13598

Juzenas, P., Generalov, R., Juzeniene, A., & Moan, J. (2008). Generation of nitrogen oxide and oxygen radicals by quantum dots. *Journal of Biomedical Nanotechnology*, *4*, 450–456.

Kermanizadeh, A., Gaiser, B. K., Johnston, H., Brown, D. M., & Stone, V. (2014). Toxicological effect of engineered nanomaterials on the liver. *British Journal of Pharmacology*, *171*, 3980–3987. https://doi.org/10.1111/bph.12421

Klaassen, C. D., Liu, J., & Diwan, B. A. (2009). Metallothionein protection of cadmium toxicity. *Toxicology and Applied Pharmacology*, *238*, 215–220. https://doi.org/10.1016/j.taap.2009.03.026

Lai, L., Li, Y. P., Mei, P., Chen, W., Jiang, F. L., & Liu, Y. (2016). Size effects on the interaction of QDs with the mitochondrial membrane in vitro. *The Journal of Membrane Biology*, *249*, 757–767. https://doi.org/10.1007/s00232-016-9920-3

Li, J., Zhang, Y., Xiao, Q., Tian, F., Liu, X., Li, R., … Liu, Y. (2011). Mitochondria as target of quantum dots toxicity. *Journal of Hazardous Materials*, *194*, 440–444. https://doi.org/10.1016/j.jhazmat.2011.07.113

Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., … Nel, A. (2002). Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environmental Health Perspectives*, *111*, 455–460. https://doi.org/10.1289/ehp.6000

Li, K. M., Xia, C. H., Wang, B. Q., Chen, H., Wang, T., He, Q., … Wang, Y. (2017). Effects of quantum dots on the ROS amount of liver cancer stem cells. *Colloids and Surfaces B Biointerfaces*, *155*, 193–199. http://doi.org/10.1016/j.colsurfb.2017.04.019

Liang, X., Grice, J. E., Zhu, Y., Liu, D., Sanchez, W. Y., Li, Z., … Roberts, M. S. (2015). Intravital multiphoton imaging of the selective uptake of water-dispersible quantum dots into sinusoidal liver cells. *Small*, *11*, 1711–1720. https://doi.org/10.1002/smll.201402698

Lin, C. H., Yang, M. H., Chang, L. W., Yang, C. S., Chang, H., Chang, W. H., … Lin, P. (2011). Cd/Se/Te-based quantum dot 705 modulated redox homeostasis with hepatotoxicity in mice. *Nanotoxicology*, *5*, 650–663. https://doi.org/10.3109/17435390.2010.539712

Lin, G. M., Ouyang, Q. L., Hu, R., Ding, Z. C., Tian, J. L., Yin, … Yong, K. T. (2015). In vivo toxicity assessment of non-cadmium quantum dots in BALB/C mice. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *11*, 341–350. http://doi.org/10.1016/j.nano.2014.10.002

Liu, J., Erogbogbo, F., Yong, K. T., Ye, L., Liu, J., Hu, R., … Prasad, P. N. (2013). Assessing clinical prospects of silicon quantum dots: studies in mice and monkeys. *ACS Nano*, *7*, 7303–7310. https://doi.org/10.1021/nn4029234

Liu, L., Tao, R., Huang, J., He, X., Qu, L., Jin, Y., … Fu, Z. (2015). Hepatic oxidative stress and inflammatory responses with cadmium exposure in male mice. *Environmental Toxicology and Pharmacology*, *39*, 229–236. https://doi.org/10.1016/j.etap.2014.11.029

Liu, Q., Li, H., Xia, Q., Liu, Y., & Xiao, K. (2015). Role of surface charge in determining the biological effects of CdSe/ZnS quantum dots. *International Journal of Nanomedicine*, *10*, 7073–7088. https://doi.org/10.2147/IJN.S94543

Liu, W., Zhang, S., Wang, L., Qu, C., Zhang, C., Hong, L., … Jiang, G. (2011). CdSe quantum dot (QD)-induced morphological and functional impairments to liver in mice. *PLoS One*, *6*, e24406. https://doi.org/10.1371/journal.pone.0024406

Lovric, J., Bazzi, H. S., Cuie, Y., Fortin, G. R., Winnik, F. M., & Maysinger, D. (2005). Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. *Journal of Molecular Medicine*, *83*, 377–385. https://doi.org/10.1007/s00109-004-0629-x

Lu, Y., Xu, S., Chen, H., He, M., Deng, Y., Cao, Z., … Zhou, Z. (2016). CdSe/ZnS quantum dots induce hepatocyte pyroptosis and liver

inflammation via NLRP3 inflammasome activation. *Biomaterials*, *90*, 27–39. https://doi.org/10.1016/j.biomaterials.2016.03.003

Malhi, H., & Kaufman, R. J. (2011). Endoplasmic reticulum stress in liver disease. *Journal of Hepatology*, *54*, 795–809. https://doi.org/10.1016/j.jhep.2010.11.005

Manshian, B. B., Soenen, S. J., Al-Ali, A., Brown, A., Hondow, N., Wills, J., … Doak, S. H. (2015). Cell type-dependent changes in CdSe/ZnS quantum dot uptake and toxic endpoints. *Toxicological Sciences*, *144*, 246–258. https://doi.org/10.1093/toxsci/kfv002

Migita, S., Moquin, A., Fujishiro, H., Himeno, S., Maysinger, D., Winnik, F. M., & Taniguchi, A. (2014). Quantum dots induce heat shock-related cytotoxicity at intracellular environment. *In Vitro Cellular & Developmental Biology. Animal*, *50*, 367–372. https://doi.org/10.1007/s11626-013-9693-2

Mo, D., Hu, L., Zeng, G., Chen, G., Wan, J., Yu, Z., … Cheng, M. (2017). Cadmium-containing quantum dots: properties, applications, and toxicity. *Applied Microbiology and Biotechnology*, *101*, 2713–2733. https://doi.org/10.1007/s00253-017-8140-9

Mu, X., Wang, J. Y., Bai, X., Xu, F., Liu, H., Yang, J., … Zhang, X. D. (2017). Black phosphorus quantum dot induced oxidative stress and toxicity in living cells and mice. *ACS Applied Materials & Interfaces*, *9*, 20399–20409. https://doi.org/10.1021/acsami.7b02900

Nguyen, K. C., Rippstein, P., Tayabali, A. F., & Willmore, W. G. (2015). Mitochondrial toxicity of cadmium telluride quantum dot nanoparticles in mammalian hepatocytes. *Toxicological Sciences*, *146*, 31–42. https://doi.org/10.1093/toxsci/kfv068

Nguyen, K. C., Willmore, W. G., & Tayabali, A. F. (2013). Cadmium telluride quantum dots cause oxidative stress leading to extrinsic and intrinsic apoptosis in hepatocellular carcinoma HepG2 cells. *Toxicology*, *306*, 114–123. https://doi.org/10.1016/j.tox.2013.02.010

Nurunnabi, M., Khatun, Z., Huh, K. M., Park, S. Y., Lee, D. Y., Cho, K. J., & Lee, Y. K. (2013). In vivo biodistribution and toxicology of carboxylated graphene quantum dots. *ACS Nano*, *7*, 6858–6867. https://doi.org/10.1021/nn402043c

Paesano, L., Perotti, A., Buschini, A., Carubbi, C., Marmiroli, M., Maestri, E., … Marmiroli, N. (2016). Markers for toxicity to HepG2 exposed to cadmium sulphide quantum dots; damage to mitochondria. *Toxicology*, *374*, 18–28. https://doi.org/10.1016/j.tox.2016.11.012

Peng, L., He, M., Chen, B., Wu, Q., Zhang, Z., Pang, D., … Hu, B. (2013). Cellular uptake, elimination and toxicity of CdSe/ZnS quantum dots in HepG2 cells. *Biomaterials*, *34*, 9545–9558. https://doi.org/10.1016/j.biomaterials.2013.08.038

Peynshaert, K., Soenen, S. J., Manshian, B. B., Doak, S. H., Braeckmans, K., De Smedt, S. C., & Remaut, K. (2017). Coating of quantum dots strongly defines their effect on lysosomal health and autophagy. *Acta Biomaterialia*, *48*, 195–205. https://doi.org/10.1016/j.actbio.2016.10.022

Qin, Y. U., Wang, H., Liu, Z. Y., Liu, J., & Wu, J. Z. (2015). Realgar quantum dots induce apoptosis and necrosis in HepG2 cells through endoplasmic reticulum stress. *Biomedical Reports*, *3*, 657–662. https://doi.org/10.3892/br.2015.489

Resch-Genger, U., Grabolle, M., Cavaliere-Jaricot, S., Nitschke, R., & Nann, T. (2008). Quantum dots versus organic dyes as fluorescent labels. *Nature Methods*, *5*, 763–775.

Richter, M., Vidovic, N., Honrath, B., Mahavadi, P., Dodel, R., Dolga, A. M., & Culmsee, C. (2016). Activation of SK2 channels preserves ER Ca^{2+} homeostasis and protects against ER stress-induced cell death. *Cell Death and Differentiation*, *23*, 814–827. https://doi.org/10.1038/cdd.2015.146

Sadaf, A., Zeshan, B., Wang, Z., Zhang, R., Xu, S., Wang, C., & Cui, Y. (2012). Toxicity evaluation of hydrophilic CdTe quantum dots and CdTe@SiO_2 nanoparticles in mice. *Journal of Nanoscience and Nanotechnology*, *12*, 8287–8292. https://doi.org/10.1166/jnn.2012.6667

Sahoo, M., Ceballos-Olvera, I., del Barrio, L., & Re, F. (2011). Role of the inflammasome, IL-1 beta, and IL-18 in bacterial infections. *TheScientificWorldJOURNAL*, *11*, 2037–2050. https://doi.org/10.1100/2011/212680

Serban, A. I., Stanca, L., Sima, C., Staicu, A. C., Zarnescu, O., & Dinischiotu, A. (2015). Complex responses to Si quantum dots accumulation in carp liver tissue: Beyond oxidative stress. *Chemico-Biological Interactions*, *239*, 56–66. https://doi.org/10.1016/j.cbi.2015.06.015

Skalickova, S., Zitka, O., Nejdl, L., Krizkova, S., Sochor, J., Janu, L., … Kizek, R. (2013). Study of interaction between metallothionein and CdTe quantum dots. *Chromatographia*, *76*, 345–353. https://doi.org/10.1007/s10337-013-2418-6

Smith, W. E., Brownell, J., White, C. C., Afsharinejad, Z., Tsai, J., Hu, X., … Eaton, D. L. (2012). In vitro toxicity assessment of amphiphillic polymer-coated CdSe/ZnS quantum dots in two human liver cell models. *ACS Nano*, *6*, 9475–9484. https://doi.org/10.1021/nn302288r

Stanca, L., Petrache, S. N., Serban, A. I., Staicu, A. C., Sima, C., Munteanu, M. C., … Dinischiotu, A. (2013). Interaction of silicon-based quantum dots with gibel carp liver: oxidative and structural modifications. *Nanoscale Research Letters*, *8*, 254. https://doi.org/10.1186/1556-276x-8-254

Su, C. K., & Sun, Y. C. (2013). In vivo monitoring of distributional transport kinetics and extravasation of quantum dots in living rat liver. *Nanotechnology*, *24*, 165101. https://doi.org/10.1088/0957-4484/24/16/165101

Su, Y., Peng, F., Jiang, Z., Zhong, Y., Lu, Y., Jiang, X., … He, Y. (2011). In vivo distribution, pharmacokinetics, and toxicity of aqueous synthesized cadmium-containing quantum dots. *Biomaterials*, *32*, 5855–5862. https://doi.org/10.1016/j.biomaterials.2011.04.063

Sun, H., Cui, E., & Liu, R. (2015). Molecular mechanism of copper-zinc superoxide dismutase activity change exposed to N-acetyl-L-cysteine-capped CdTe quantum dots-induced oxidative damage in mouse primary hepatocytes and nephrocytes. *Environmental Science and Pollution Research International*, *22*, 18267–18277. https://doi.org/10.1007/s11356-015-5035-0

Tang, S., Allagadda, V., Chibli, H., Nadeau, J. L., & Mayer, G. D. (2013). Comparison of cytotoxicity and expression of metal regulatory genes in zebrafish (*Danio rerio*) liver cells exposed to cadmium sulfate, zinc sulfate and quantum dots. *Metallomics: Integrated Biometal Science*, *5*, 1411–1422. https://doi.org/10.1039/c3mt20234h

Tang, S., Cai, Q., Chibli, H., Allagadda, V., Nadeau, J. L., & Mayer, G. D. (2013). Cadmium sulfate and CdTe-quantum dots alter DNA repair in zebrafish (*Danio rerio*) liver cells. *Toxicology and Applied Pharmacology*, *272*, 443–452. https://doi.org/10.1016/j.taap.2013.06.004

Tang, Y., Han, S., Liu, H., Chen, X., Huang, L., Li, X., & Zhang, J. (2013). The role of surface chemistry in determining in vivo biodistribution and toxicity of CdSe/ZnS core-shell quantum dots. *Biomaterials*, *34*, 8741–8755. https://doi.org/10.1016/j.biomaterials.2013.07.087

Tanida, I. (2011). Autophagy basics. *Microbiology and Immunology*, *55*, 1–11. https://doi.org/10.1111/j.1348-0421.2010.00271.x

Tsay, J. M., & Michalet, X. (2005). New light on quantum dot cytotoxicity. *Chemistry & Biology*, *12*, 1159–1161. https://doi.org/10.1016/j.chembiol.2005.11.002

Vallieres, L., & Rivest, S. (1999). Interleukin-6 is a needed proinflammatory cytokine in the prolonged neural activity and transcriptional activation of corticotropin-releasing factor during endotoxemia. *Endocrinology*, *140*, 3890–3903. https://doi.org/10.1210/en.140.9.3890

Volkov, Y. (2015). Quantum dots in nanomedicine: recent trends, advances and unresolved issues. *Biochemical and Biophysical Research Communications*, *468*, 419–427. https://doi.org/10.1016/j.bbrc.2015.07.039

Walter, P., & Ron, D. (2011). The unfolded protein response: from stress pathway to homeostatic regulation. *Science (New York, N.Y.)*, *334*, 1081–1086. https://doi.org/10.1126/science.1209038

Wang, C., Gao, X., & Su, X. (2010). Study the damage of DNA molecules induced by three kinds of aqueous nanoparticles. *Talanta*, *80*, 1228–1233. https://doi.org/10.1016/j.talanta.2009.09.014

Wang, M., Wang, J., Sun, H., Han, S., Feng, S., Shi, L., … Sun, Z. (2016). Time-dependent toxicity of cadmium telluride quantum dots on liver

and kidneys in mice: histopathological changes with elevated free cadmium ions and hydroxyl radicals. *International Journal of Nanomedicine*, *11*, 2319–2328. https://doi.org/10.2147/IJN.S103489

Wang, S., Cole, I. S., & Li, Q. (2016). The toxicity of graphene quantum dots. *RSC Advances*, *6*, 89867–89878. https://doi.org/10.1039/c6ra16516h

Wang, Y., & Tang, M. (2018). Review of in vitro toxicological research of quantum dot and potentially involved mechanisms. *Science of the Total Environment*, *625*, 940–962. https://doi.org/10.1016/j.scitotenv.2017.12.334

Wu, X., Tian, F., Zhao, J. X., & Wu, M. (2013). Evaluating pharmacokinetics and toxicity of luminescent quantum dots. *Expert Opinion on Drug Metabolism & Toxicology*, *9*, 1265–1277. https://doi.org/10.1517/17425255.2013.807797

Yang, X., Shao, H., Liu, W., Gu, W., Shu, X., Mo, Y., … Jiang, M. (2015). Endoplasmic reticulum stress and oxidative stress are involved in ZnO nanoparticle-induced hepatotoxicity. *Toxicology Letters*, *234*, 40–49. https://doi.org/10.1016/j.toxlet.2015.02.004

Yang, Y., Lan, J., Xu, Z., Chen, T., Zhao, T., Cheng, T., … Zhang, H. (2014). Toxicity and biodistribution of aqueous synthesized ZnS and ZnO quantum dots in mice. *Nanotoxicology*, *8*, 107–116. https://doi.org/10.3109/17435390.2012.760014

Yang, Y., Zhao, T., Cheng, T., Shen, J., Liu, X., Yu, B., … Zhang, H. (2014). Hepatotoxicity induced by ZnO quantum dots in mice. *RSC Advances*, *4*, 5642. https://doi.org/10.1039/c3ra46583g

Ye, L., Yong, K. T., Liu, L., Roy, I., Hu, R., Zhu, J., … Prasad, P. N. (2012). A pilot study in non-human primates shows no adverse response to intravenous injection of quantum dots. *Nature Nanotechnology*, *7*, 453–458. https://doi.org/10.1038/nnano.2012.74

Yong, K. T., & Swihart, M. T. (2012). In vivo toxicity of quantum dots: no cause for concern? *Nanomedicine (London, England)*, *7*, 1641–1643. https://doi.org/10.2217/nnm.12.152

Yorimitsu, T., Nair, U., Yang, Z., & Klionsky, D. J. (2006). Endoplasmic reticulum stress triggers autophagy. *The Journal of Biological Chemistry*, *281*, 30299–30304. https://doi.org/10.1074/jbc.M607007200

Zhang, J., Morris, M. W. Jr., Dorsett-Martin, W. A., Drake, L. C., & Anderson, C. D. (2013). Autophagy is involved in endoplasmic reticulum stress-induced cell death of rat hepatocytes. *The Journal of Surgical Research*, *183*, 929–935. https://doi.org/10.1016/j.jss.2013.02.043

Zhang, J. Q., Zhou, W., Zhu, S. S., Lin, J., Wei, P. F., Li, F. F., … Wen, L. P. (2017). Persistency of enlarged autolysosomes underscores nanoparticle-induced autophagy in hepatocytes. *Small*, *13*. https://doi.org/10.1002/smll.201602876

Zhang, R., Piao, M. J., Kim, K. C., Kim, A. D., Choi, J. Y., Choi, J., & Hyun, J. W. (2012). Endoplasmic reticulum stress signaling is involved in silver nanoparticles-induced apoptosis. *The International Journal of Biochemistry & Cell Biology*, *44*, 224–232. https://doi.org/10.1016/j.biocel.2011.10.019

Zhang, T., Hu, Y., Tang, M., Kong, L., Ying, J., Wu, T., … Pu, Y. (2015). Liver toxicity of cadmium telluride quantum dots (CdTe QDs) due to oxidative stress in vitro and in vivo. *International Journal of Molecular Sciences*, *16*, 23279–23299. https://doi.org/10.3390/ijms161023279

Zhang, W., Yang, L., Kuang, H., Yang, P., Aguilar, Z. P., Wang, A., … Xu, H. (2016). Acute toxicity of quantum dots on late pregnancy mice: Effects of nanoscale size and surface coating. *Journal of Hazardous Materials*, *318*, 61–69. https://doi.org/10.1016/j.jhazmat.2016.06.048

Zhang, Y. N., Poon, W., Tavares, A. J., McGilvray, I. D., & Chan, W. C. (2016). Nanoparticle-liver interactions: Cellular uptake and hepatobiliary elimination. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, *240*, 332–348. https://doi.org/10.1016/j.jconrel.2016.01.020

Zhu, S., Zhang, J., Zhang, L., Ma, W., Man, N., Liu, Y., … Wen, L. (2017). Inhibition of Kupffer cell autophagy abrogates nanoparticle-induced liver injury. *Advanced Healthcare Materials*, *6*. https://doi.org/10.1002/adhm.201601252

How to cite this article: Lu J, Tang M, Zhang T. Review of toxicological effect of quantum dots on the liver. *J Appl Toxicol*. 2018;1–15. https://doi.org/10.1002/jat.3660