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Cellular responses of normal (HL-7702) and cancerous (HepG2) hepatic cells to dust extract exposure

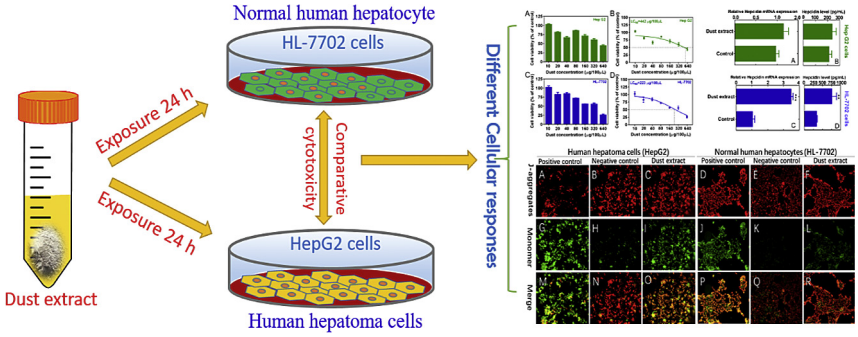
Ping Xiang [a, b], Rui-Wen He [b, c], Rong-Yan Liu [b], Kan Li [b], Peng Gao [d], Xin-Yi Cui [b], Hongbo Li [b], Yungen Liu [a, **], Lena Q. Ma [a, d, *]

[a] Research Institute of Rural Sewage Treatment, Southwest Forestry University, Kunming, 650224, People's Republic of China
[b] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210046, People's Republic of China
[c] Institute for Risk Assessment Sciences, Utrecht University, 3508 TD Utrecht, The Netherlands
[d] Soil and Water Science Department, University of Florida, Gainesville, FL 32611, United States

HIGHLIGHTS

- Investigated cellular responses of normal and cancerous hepatic cells to dust exposure.
- Observed cytotoxicity, oxidative damage, inflammatory response and MMP loss.
- More cell viability decrease in HL-7702 with higher oxidative damage to HepG2.
- Fe regulatory hormone expression was perturbed only in HL-7702 cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Cancerous human liver cell line has been used to test the hepatic toxicity of indoor dust, showing its organic extract decreases cell viability. However, little is known about its impact on normal human liver cell line. In the present study, we compared the cellular responses between carcinoma cell line (HepG2) and normal cell line (HL-7702) after exposing to 10–640 μg/100 μL organic dust extract for 24 h. The dust extract caused cytotoxicity, oxidative damage, inflammatory response and loss of mitochondrial transmembrane potential (MMP) in both cells. The inhibition of cell viability in HL-7702 cells was stronger than that in HepG2 cells, with HL-7702 cells having lower LC_{50}. Higher production of oxidative stress, more loss of MMP and stronger suppression of antioxidant enzymes mRNA level occurred in HepG2 cells, while mRNA expression and hepcidin secretion were enhanced in HL-7702 cells at 40/100 μL, indicating the dust extract probably perturbed their liver Fe homeostasis. Our data showed considerable differences in cellular responses between normal and cancerous cell lines. To obtain accurate data, normal hepatocytes should be employed as they better match with the *in vivo* tissue than cancerous cell lines.
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1. Introduction

Epidemiological and experimental studies have associated

* Corresponding author. Research Institute of Rural Sewage Treatment, Southwest Forestry University, Kunming, 650224, People's Republic of China.
** Corresponding author.
E-mail addresses: yungenliu@swfu.edu.cn (Y. Liu), lqma@ufl.edu (L.Q. Ma).

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human health problems with indoor dust exposure (Geens et al., 2009; Mendell, 2007). Indoor dust is a complex heterogeneous mixture of particles, containing different contaminants and human pathogens (Mitro et al., 2016). Research showed that organic contaminants including polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) in indoor dust negatively impact human health as they tend to accumulate in mammalian liver (El-Shahawi et al., 2010). Besides, one study found decreased activities of serum aspartate aminotransferase and alkaline phosphatase in workers occupationally exposed to dust, suggesting dust exposure may perturb liver function (Mojiminiyi et al., 2008). Human liver is one of the most important organs responsible for detoxification, metabolism and hormone regulation. As such, dust-induced liver damage and its mechanisms are of concern and warrant further investigations.

To understand the underlying mechanisms of dust-induced adverse effects on humans, various human cell lines have been employed (Zhang et al., 2016). Human liver carcinoma cell line (HepG2) has a number of characteristic enzyme pathways of human hepatocytes, thereby being widely used in hepatic toxicology test. In addition to direct dust exposure, indoor dust has been extracted using organic solvent to separate organic contaminants (Poole and Romberger, 2012). Organic extract of indoor dust effectively decreased cell viability in HepG2 cells, with greater PAHs concentration in organic dust extract leading to lower LC_{50} of HepG2 cells (Kang et al., 2010). However, compared to normal human liver cell line (HL-7702), HepG2 cells show different metabolism and CYP450 activities (Gerets et al., 2012; Wilkening et al., 2003). As such, the impact of the dust extract on normal human hepatocytes is unclear. Therefore, it is important to compare normal hepatic cells to cancerous cells as they better match with the *in vivo* liver tissue (Cree and Andreotti, 1997).

Oxidative stress and inflammation are the main molecular mechanism of dust-induced toxicity in human cells. Under oxidative stress, increase in ROS (reactive oxygen species) suppresses antioxidant enzymes and decreases mitochondrial membrane potential in HepG2 and HL-7702 cells (Xiong et al., 2015). Moreover, altered biomarkers of DNA damage, lipid peroxidation, and inflammatory responses are also detected (Cederbaum et al., 2001). Additionally, recent studies demonstrated that organic contaminants, frequently detected in dust, altered the expression of hepcidin, thereby disrupting Fe homeostasis in HepG2 cells (Qian et al., 2015). The hepcidin, a key Fe regulatory hormone, is generated in the hepatocytes to negatively regulate intestinal Fe absorption (Rossi, 2005). Hepcidin overproduction results in hypoferremia and the anemia of inflammation, whereas downregulated hepcidin tissue leads to Fe overload (Nemeth et al., 2004). Several studies showed that IL-1β and IL-6 strongly stimulated hepcidin transcription in hepatocytes (Lee et al., 2005). In addition, dust extract significantly induced IL-1β and IL-6 expressions in human corneal epithelial cells (Xiang et al., 2016b). Whether dust extract affects hepcidin expression in human hepatocytes is still unknown.

In this study, we compared the cellular responses and molecular mechanisms between human hepatocellular carcinoma cell line (HepG2) and human normal liver cell line (HL7702) after exposing to organic dust extract. Notably, the change in hepcidin level as a new endpoint was observed for dust-induced hepatotoxicity in this study.

2. Materials and methods

2.1. Chemicals and indoor dust sample

Cell culture medium, plates, and dishes were from Thermo Fisher Scientific (Invitrogen, USA) and Corning Inc. (NY, USA). The CCK-8 cell viability assay kit and SYBR green qPCR master mix were from Yi Fei Xue Biotech. Co., Ltd. (Nanjing, China). JC-1 probe and ROS assay kit (DCFH-DA) were from Beyotime Institute of Biotechnology (Haimen, China). Malondialdehyde assay kits, enhanced BCA protein assay kit, and cell lysis buffer were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The TaKaRa MiniBEST universal RNA extraction kit and cDNA synthesis kit were from TaKaRa Biotech. Co., Japan. Human interleukin 1β (IL-1β), human interleukin 6 (IL-6), and human hepcidin ELISA Kits were from R&D Systems (MN, USA). Chemical standards for 16 organochlorine pesticides (OCPs) were from J&K Scientific (Shanghai, China) and Aladdin Industrial Corporation (Shanghai, China) with purity > 98%. Other chemicals were from Sigma-Aldrich, Inc. (MO, USA).

Fifteen indoor dust samples were collected from air conditioner filter of various offices in Nanjing, China by a vacuum cleaner equipped with a paper bag. Prior to dust extraction, all samples were sieved through a nylon sieve (<100 μm) after being freeze-dried, with 50 samples being made to one composite sample. The sample (1.6 g) was extracted with 160 mL n-hexane under sonication for 30 min twice, which was concentrated by a rotatory evaporator (IKA®RV10, Germany) under N_2 stream to reconstitute in 2 mL n-hexane. One mL was solvent-exchanged to 0.5 mL dimethyl sulfoxide (DMSO) under N_2 stream, which was used for cell exposure and one mL was filtered through 0.45 mm nylon filter for chemical analysis. The analysis of OCPs and PAHs as conducted on gas chromatography (Thermo Scientific, Trace 1310) coupled with mass spectrometry (Thermo Scientific, ISQ Single Quadrupole) (GC-MS) in selective ion-monitoring mode. Detailed information can be found in our previous studies (He et al., 2016; Xiang et al., 2016a).

2.2. Cell culture and cell viability assay

Human hepatoma cell line (HepG2) and normal human hepatocyte cell line (HL-7702) were from the American Type Tissue Culture Collection (VA, USA) and Chinese Academy of Sciences (Shanghai, China). The cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% antibiotic-antimycotic solution at 37 °C in a humidified incubator with 5% CO_2. Cell viability was evaluated using a commercial kit. Briefly, both HepG2 and HL-7702 cells were reseeded into a 96-well plate at a density of 5×10^3 cells/100 μL/well. After overnight culture, the cells were treated with 100 μL fresh medium containing 7 different concentrations of dust extract (10, 20, 40, 80, 160, 320 or 640 μg/100 μL). After 24 h incubation, 10 μL CCK-8 solution was added to the cells in each well and cultured for 2 h in a CO_2 incubator. Absorption was measured at 450 nm using Multiskan™ FC microplate photometer (Thermo, MA, USA). In addition, Curve fitting was conducted to calculate LC_{50} using nonlinear regression analysis [log(agonist) vs response] by GraphPad Prism Version 6 (USA).

2.3. Mitochondrial membrane potential and oxidative stress biomarkers: ROS, MDA, and 8-OHdG

To test whether dust-induced oxidative stress affected mitochondrial function, mitochondrial transmembrane potential was measured with JC-1 probe following (Xiang et al., 2017a). Briefly, cells were grown in a 24-well plate at 4×10^5 cells/well, and then exposed to dust extract at 40 μg/100 μL for 24 h. After exposure, 250 μL of JC-1 staining solution diluted in 500 μL fresh medium was added and incubated in a CO_2 incubator for 20 min followed by washing twice with HBSS. Results were observed and recorded by Eclipse Ti-U fluorescent microscopy (Nikon, Japan). In addition, the

alternations in color were quantified and analyzed by using Image J software (NIH, USA). The abundance of green fluorescence intensity indicates mitochondrial depolarization. Carbonyl cyanide 3-chlorophenylhydrazone at 10 μmol/L and DMSO (0.1%) were employed to treat cells as a positive and negative control.

The ROS level was detected using an oxidation-sensitive fluorescent probe 2′,7′-dichlorofluorescin diacetate (DCFH-DA). In brief, cells were plated into 96-well black plates at a density of 3×10^3 cells/well. After treatment with dust extract at 40 μg/100 μL for 24 h, 10 μM DCFH-DA dissolved in basic DMEM medium was added and incubated for 30 min at 37 °C. The fluorescence intensity in the cells was detected by fluorescence microplate reader (TECAN, USA) at excitation 488 nm and emission 525 nm wavelength. The ROS production was presented as the fold of control.

The level of dust extract induced lipid peroxidation was assessed by measuring malondialdehyde (MDA) production using a commercial kit. Briefly, 3×10^5 cells were plated into 60 mm dishes overnight to allow attachment. The culture medium was replenished by dust extract diluted in fresh medium at 40 μg/100 μL and incubated for 24 h. The contents of MDA were monitored spectrophotometrically after reaction with thiobarbituric acid at a wavelength of 532 nm. Total protein concentrations were determined by enhanced BCA protein assay kit according to manufacturer's instruction. The MDA level was expressed as micromoles per milligram protein.

Oxidative DNA damage was estimated by 8-hydroxydeoxyguanosine generation using an enzyme linked immunosorbent assay (ELISA) kit. In brief, cells at 5×10^4 cells/well were pre-cultured in 6-well plate overnight, and treated with dust extract (40 μg/100 μL) for 24 h. Levels of 8-OHdG were determined based on the manufacturer's guideline and presented as fold of control.

2.4. RNA extraction and quantitative PCR analysis

Both HepG2 and HL-7702 cells were detached and seeded into 60 mm dishes at the initial density of 1×10^6 cells/well overnight, followed by exposing to dust extract at 40 μg/100 μL for 24 h. Subsequently, total RNA was extracted from control and dust-exposed cells using RNAiso Reagent (TaKaRa Biotech. Co., Japan). RNase-Free DNase I was used to remove contaminated DNA. The quality of RNA was measured using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific Inc., USA). One microgram total RNA was reverse transcribed into the first-strand cDNA via PrimeScript RT reagent kit (TaKaRa Biotech. Co., Japan). The mRNA expression of *interleukin (IL)-1β*, *IL-6*, and *Hepcidin* were analyzed with respect to housekeeping gene *β-Actin* in a CFX Connect™ Real-Time PCR detection system (Bio-Rad, CA, USA) at the following cycling conditions: 95 °C for 2 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Besides, the melting curve was also done from 65 to 95 °C with 0.5 °C s^{-1} increments to exclude unspecific amplification after the last reaction. The relative expression levels in terms of fold changes of target genes were calculated by $2^{-\Delta\Delta CT}$ method. The specific primers were obtained from Harvard PrimerBank (Table 1).

2.5. Pro-inflammatory cytokines and iron hormone using ELISA

Both HepG2 and HL-7702 cells were seeded into the 24 well plates at an initial density of 3×10^4 cells/well overnight to allow attachment. Cells were exposed to dust extract at 40 μg/100 μL for 24 h. After exposure, the supernatants were collected and centrifuged at to remove cell debris. The release of interleukin (IL)-1β, IL-6 and hepcidin in the cell-free medium were detected by the enzyme-linked immunosorbent assay (ELISA) using commercial ELISA kits according to the manufacturer instructions. DMSO (0.1%) treated cultures were served as control. Color intensity was read by the Multiskan™ FC microplate photometer at 450 nm (Thermo, MA, USA). The values were presented as pg/mL.

2.6. Statistical analysis

All experiments were carried out in triplicate. Data are expressed as the mean ± standard error of the mean. Statistical analyses were conducted by GraphPad Prism Version 6 (USA). Differences between control cells and dust extract treated cells were examined using unpaired t-tests. JC-1 staining fluorescence intensity analysis was conducted using one-way ANOVA. Significant differences were set at $\alpha \leq 0.05$.

3. Results and discussion

3.1. Contaminant concentrations in dust extract

Concentration-dependent relationships between organic pollutants such as PAHs in indoor dust and human health issues have been established (Kolarik et al., 2008; Meeker et al., 2013). OCPs have been widely used worldwide before the late 1970s. However, elevated levels of OCPs are still frequently detected in human serum, adipose tissue samples, and mammalian liver (Dirtu et al., 2006; Erdogrul et al., 2004; Pan et al., 2014; Pulkrabová et al., 2009). It is suggested that indoor dust could be a main route for human exposure to OCPs (Ali et al., 2013). Moreover, OCPs can disturb cellular metabolism of lipids, proteins, and carbohydrates and induce liver lesion (Benarbia Mel et al., 2013; Karami-Mohajeri and Abdollahi, 2011). Thus, concentrations of 16 OCPs and 16 PAHs in dust extract were quantified by GC-MS (Table 2). The total concentration of 16 PAHs in organic dust extract was 25.3 μg/g (Xiang et al., 2016b), which was higher than that reported in Hong Kong (6.18 μg/g), and Pearl River Delta (7.30 μg/g) (Kang et al., 2010), but lower than in Texas, USA (73.5 μg/g) (Mahler et al., 2010). OCPs levels in organic dust extract at 3462 ng/g was significantly higher than that in the dust extract from Singapore at 171 ng/g (Tan et al., 2007) and from Romania at 1200 ng/g (Dirtu and Covaci, 2010). This was because OCPs are not volatile with low solubility, thereby accumulating in dust (Bräuner et al., 2011). Among OCPs, endrin was the most abundant compound at 1270 ng/g, followed by heptachlor at 400 ng/kg and endosulfan at 390 ng/kg, while other compounds including three common OCPS (pp'DDE, pp'DDD, and pp'DDT) were all low at < 300 ng/g (Table 2).

3.2. Dust extract decreased cell viability and perturbed cellular redox status

Cell viability assay is a vital criterion to screen cellular responses to contaminants, which is widely used to quantify metabolic activities, cell proliferation, and cell survival after exposure (Xiang et al., 2011, 2017b). The effects of dust extract on the viability of HepG2 and HL-7702 cells were determined using CCK-8 assay. After 24 h exposure, organic dust extract increased cell mortality with increasing concentration of dust extract in both cells (Fig. 1). At 40 μg/100 μL, dust extract showed higher cytotoxicity on HepG2 cells than HL-7702 cells, which was consistent with previous study where a curcumin derivative induced stronger inhibition in HepG2 than in HL7702 (Li et al., 2017), which may be due to lower CYP450 activities in HepG2 cells (Gerets et al., 2012). Moreover, ≥ 80 μg/100 μL, dust extract strongly suppressed cell viability of HL-7702, which may be due to altered metabolic pathways of cancerous HepG2 cells at higher exposure (Vander Heiden et al., 2009). Based on the fitted curve, the LC_{50} of dust extract on cells was calculated.

Table 1
Primers for RT-qPCR of anti-oxidative enzymes, pro-inflammatory mediator and hepcidin genes.

Gene	Forward primer (5′-3′)	Reserve primer (5′-3′)	Accession no.	Production size (bp)
CAT	TGGAGCTGGTAACCCAGTAGG	CCTTTGCCTTGGAGTATTTGGTA	NM_001752	209
GSTM	GCAGGAAACAAGGGCTTGGA	CCTACTTGTTGCCCCAGACA	NM_146421.2	103
SOD1	GGTGGGCCAAAGGATGAAGAG	CCACAAGCCAAACGACTTCC	NM_000454	227
TRXR1	ATATGGCAAGAAGGTGATGGTCC	GGGCTTGTCCTAACAAAGCTG	NM_001261446.1	140
HO-1	AAGACTGCGTTCCTGCTCAAC	AAAGCCCTACAGCAACTGTCG	NM_002133	247
IL-1β	ACAGATGAAGTGCTCCTTCCA	GTCGGAGATTCGTAGCTGGAT	NM_000576.2	73
IL-6	CAATCTGGATTCAATGAGGAGAC	CTCTGGCTTGTTCCTCACTACTC	NM_000600.3	118
HEPC	CTGACCAGTGGCTCTGTTTTC	GAAGTGGGTGTCTCGCCTC	NM_021175	129
β-Actin	GTACCACTGGCATCGTGATGGACT	CCGCTCATTGCCAATGGTGAT	NM_001101.3	323

Table 2
Total concentrations of OCPs and PAHs in the organic extract of composite dust sample from commercial offices in Nanjing (ng/g).

OCPs		PAHs (Xiang et al., 2016b)	
Name of OCPs	Concentration (ng/g)	Name of PAHs	Concentration (ng/g)
α-BHC	60 ± 3.0	Naphthalene	0.14 ± 0.03
γ-BHC	230 ± 10	Acenaphthylene	0.18 ± 0.02
β-BHC	110 ± 4.0	Acenaphthene	0.04 ± 0.007
Heptachlor	400 ± 100	Fluorene	0.08 ± 0.014
δ-BHC	60 ± 0.4	Phenanthrene	0.84 ± 0.06
Aldrin	180 ± 6.0	Anthracene	0.20 ± 0.01
Heptachlor epoxide	290 ± 10	Fluoranthene	1.37 ± 0.07
Endosulfan	390 ± 80	Pyrene	1.01 ± 0.04
pp'DDE	30 ± 10	Benz(a)anthracene	1.1 ± 0.08
Dieldrin	120 ± 50	Chrysene	2.5 ± 0.11
Endrin	1270 ± 300	Benzo(b)fluoranthene	4.99 ± 0.24
pp'DDD	32 ± 1	Benzo(k)fluoranthene	1.20 ± 0.11
Endosulfan II	ND	Benzo(a)pyrene	5.01 ± 0.32
pp'DDT	ND	Indeno(1,2,3-cd)pyrene	1.13 ± 0.03
Endosulfan sulfate	ND	Dibenzo(a,h)anthracene	5.50 ± 0.26
Methoxychlor	290 ± 20	Benzo(ghi)perylene	0.14 ± 0.03
pp'DDE	30 ± 10	Naphthalene	0.18 ± 0.02

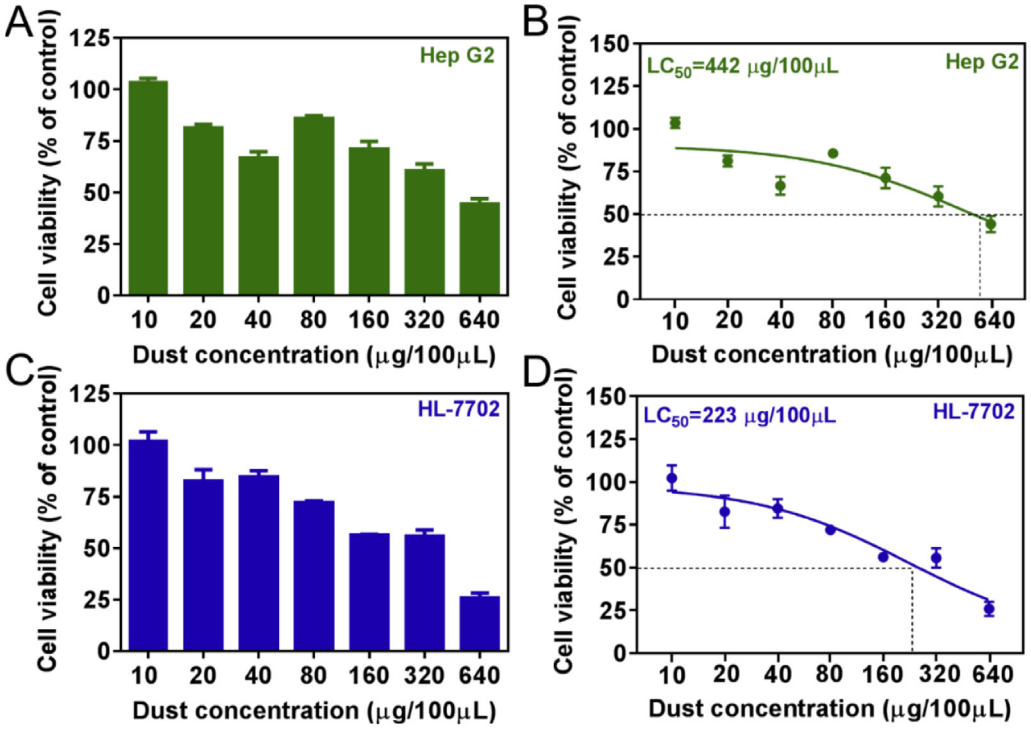

Fig. 1. The cytotoxicity of dust extract to HepG2 cells (A) and HL-7702 cells (C). Cells were exposed to different concentrations of dust extract for 24 h. Logarithmic transformation of dust extract concentrations and cell viability data was fit to a nonlinear regression curve (log(agonist) vs. response - Variable slope) to determine LC_{50} of HepG2 (B) and HL-7702 (D). Error bar means standard error of three replicates.

The LC_{50} of dust extract of HepG2 cells (442 μg/100 μL) was higher than that of Kang et al. (2010) at 230 μg/100 μL, which was attributed to different contaminants in dust extract. Besides, in the present study, LC_{50} of HepG2 (442 μg/100 μL) was 2-fold higher than HL-7702 cells (223 μg/100 μL) (Fig. 1 BD), indicating HL-7702 cells were more sensitive to the dust extract than HepG2 cells. To better understand different cellular responses between HepG2 and HL-7702 cells to dust extract, a lower concentration at 40 μg/100 μL was selected for subsequent studies.

Guerrero-Castilla and Olivero-Verbel (2014) reported that organic extract of coal-dust induced oxidative stress and DNA damage in HepG2 cells. However, it was unclear how dust extract affects normal hepatocytes. In this study, the oxidative stress caused by exposing to organic dust extract at 40 μg/100 mL for 24 h in both cells was examined by determining intracellular formation of ROS (Fig. 2 AD). The ROS levels in both cells were increased after exposure. Dust extract caused more ROS production in HepG2 (Fig. 2A) than HL-7702 cells (Fig. 2D). Excessive generation of ROS leads to oxidative injury to cellular components including DNA and lipids. The contents of 8-OHdG as an inductor of oxidative DNA damage were measured. The 8-OHdG level in HepG2 cells was up to 2.1-fold of control (Fig. 2B) while little difference in HL-7702 cells was observed (Fig. 2E), indicating that dust extract at 40 μg/100 mL may not induce oxidative DNA damage in HL-7702 cells. Lipid peroxidation was also evaluated based on MDA, with MDA production in HepG2 and HL-7702 being increased to 1.8- and 1.3-fold after dust exposure (Fig. 2 CF). In short, at 40 μg/100 mL, greater generation of ROS, MDA, and 8-OHdG appeared in HepG2 cells (Fig. 2), suggesting higher oxidative damage to HepG2 cells including lipids and DNA, which was consistent with Xiong et al. (2015).

3.3. Gene expression of antioxidant enzymes and mitochondrial transmembrane potential

In a biological system, antioxidant enzymes detoxify reactive intermediates and repair the resulting damage to maintain a balance in an oxidant-antioxidant system (Mates, 2000). Potential mechanisms of cellular responses to dust-induced oxidative stress may include changes in the expression of certain genes in antioxidant enzymes (Schins and Borm, 1999). To better understand why dust extract induced higher oxidative damage to HepG2 cells than that of HL-7702 cells, we compared their mRNA expression of antioxidant enzymes (Fig. 3). Superoxide dismutase (SOD) and catalase (CAT) are responsible for catalyzing the dismutation of O_2^- to H_2O_2, thereby protecting cells by removing O_2^-. In this study, the mRNA level of *SOD* and *CAT* was decreased in HepG2 cells, whereas mRNA expression of *SOD1* in HL-7702 cells was elevated after dust extract exposure. This may be because SOD1 and CAT enzymes were more sensitive in HepG2 cells than that in HL-7702 cells. Glutathione S-transferase mu 1 (GSTM1), a member of the GST family of proteins, serves as an important antioxidant enzyme to protect cellular DNA from oxidative damage (Lin et al., 2009). *GSTM1* mRNA expression was decreased by 8.3-fold in HepG2 cells , while increased by 7.2-fold in HL-7702 cells (Fig. 3C), which may be responsible for a higher 8-OHdG level in HepG2 (Fig. 2B). Heme oxygenase-1 (HO-1) and thioredoxin reductase (TRXR1) are a rate-limiting enzyme catalyzing the degradation of pro-oxidant heme and an antioxidant of the thioredoxin system catalyzing the NADPH-dependent reduction of thioredoxin, respectively. They also play an important role to balance cellular redox status (Wu et al., 2012). The mRNA expression of *HO-1* in both HepG2 and HL-7702 cells was elevated, consistent with literature that exposure to air particles or indoor dust increased *HO-1* gene expression in human alveolar macrophages, airway epithelial cells, and human corneal epithelial cells (Becker et al., 2005; Xiang et al., 2016a). However, *TRXR-1* mRNA level in HL-7702 was declined by 10-fold while not affected in HepG2. Taken together, mRNA expression of more antioxidant enzymes was inhibited in HepG2 than HL-7702. Different gene expression patterns of antioxidant enzymes implied that human normal liver cells and cancer liver cells showed a different sensitivity to organic dust extract.

Mitochondria is one of the most important cytoplasmic organelles to supply the necessary energy for cellular activities and

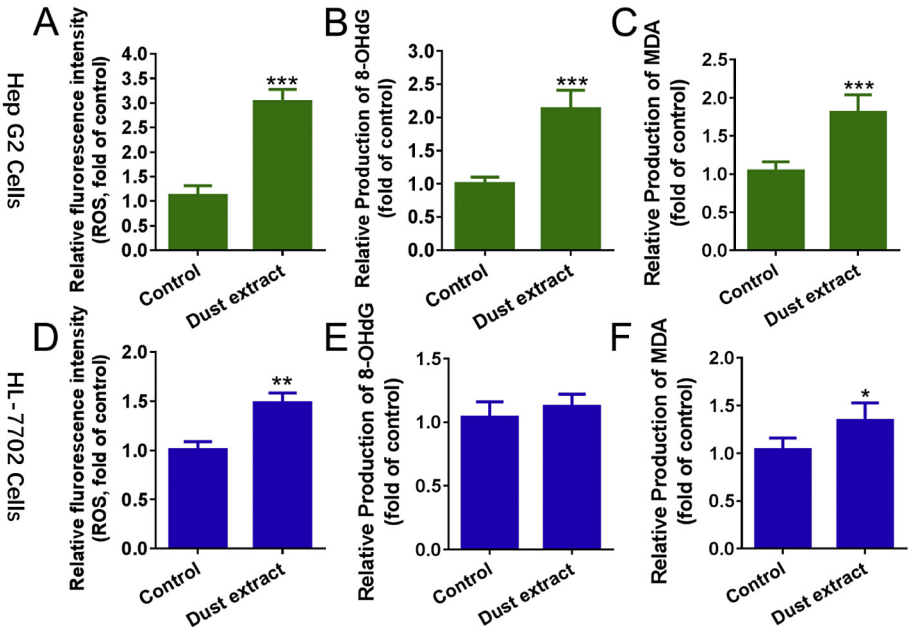

Fig. 2. Organic dust extract induced oxidative stress in HepG2 cells (A–C) and HL-7702 cells (D–F) after 24 h exposure at 40 μg/100 μL. The levels of ROS, MDA and 8-OHdG (H) were significantly increased after dust extract exposure in HepG2 cells (ABC), suggesting aggravation of oxidative stress induced DNA damage. The production of ROS and MDA, but no 8-OHdG was also elevated in HL-7702 cells. Each bar represents the mean ± SEM of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

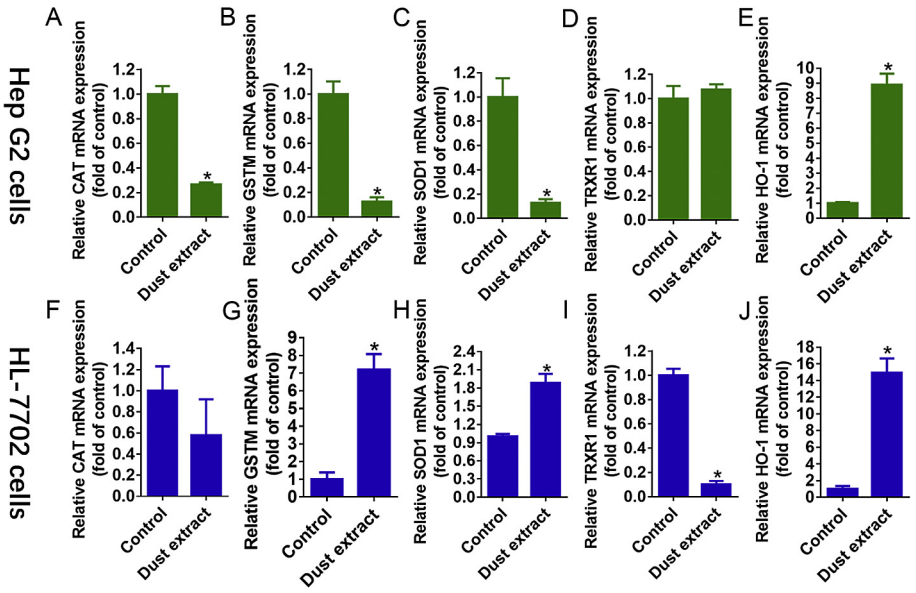

Fig. 3. Effects on the expression of antioxidant enzyme genes in HepG2 cells (A–E) and HL-7702 (F–J) after exposure to 40 μg/100 μL organic dust extract for 24 h. Dust exposuredecreased the mRNA expression of *CAT* (A), *GSTM* (B), and *SOD1* (C) but elevated HO-1 (E) mRNA expression in HepG2 cells .Up-regulation of *GSTM* (G), *SOD1* (H), and *HO-1* (J) mRNA expressions were observed, but *CAT* (F) and *TRXR1* (I) were decreased in HL-7702 cells. Each bar represents mean ± SEM of three replicates. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

functions. Several studies found a significant association between low antioxidant capacity and mitochondrial dysfunction in human cells, which frequently induces events upstream of mitochondrial pathways of apoptosis (Ott et al., 2007). To test whether oxidative stress affected mitochondrial functions in cells, we further examined mitochondrial transmembrane potential (MMP) after exposing to organic dust extract for 24 h. Dust exposure resulted in dissipation of MMP, which was evidenced by loss of red fluorescence and abundance of green fluorescence (Fig. 4 CIO, FLR), demonstrating its toxicity to mitochondria. However, the ratio of green fluorescence in HepG2 cells was 52% (Fig. 4S), higher than that in HL-7702 cells (32%) (Fig. 4T), indicating more severe damage of mitochondria in HepG2 cells. Impaired mitochondrial usually release more ROS (Xiang et al., 2016a), which also explained why higher ROS was observed in HepG2 cells in this study, with lower expression of antioxidant enzymes in HepG2 cells.

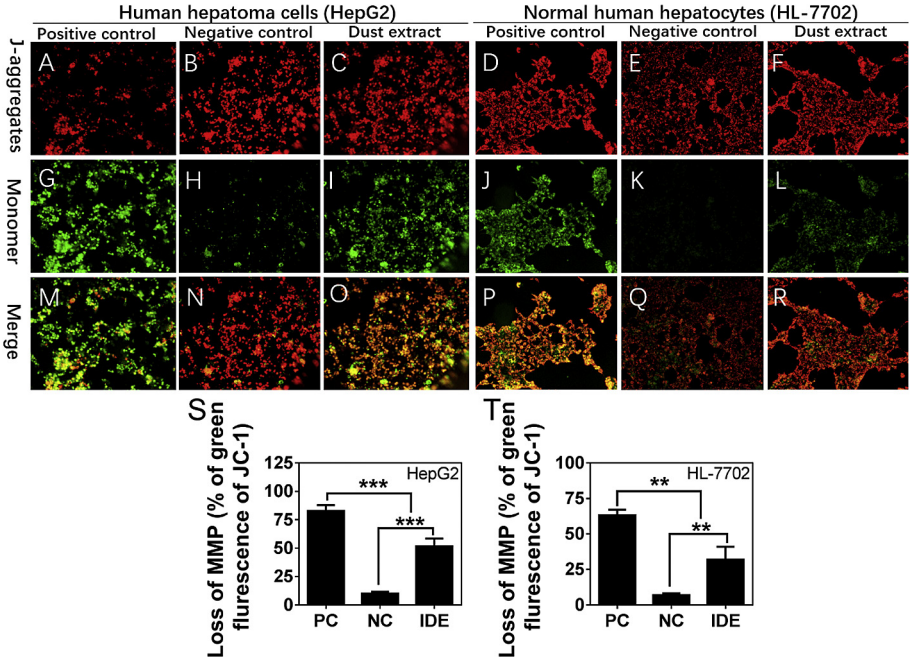

Fig. 4. Loss of mitochondrial membrane potential (ΔΨm) in HepG2 cells (A–O) and HL-7702 cells (D–R) after exposing to 40 μg/100 μL organic dust extract for 24 h at 200 × magnification. After exposure, both HepG2 and HL-7702 cells exhibited an increased green fluorescence intensity (IL) and an attenuated red fluorescence signal (CF) compared to negative control (BH, EK). The bar diagrams represent the loss of mitochondrial potential (% green fluorescence of JC-1) as analyzed by Image J software (S for HepG2 cells, T for HL-7702 cells). Data are presented as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$. NC, PC, and IDE mean negative control, positive control and organic dust extract respectively.

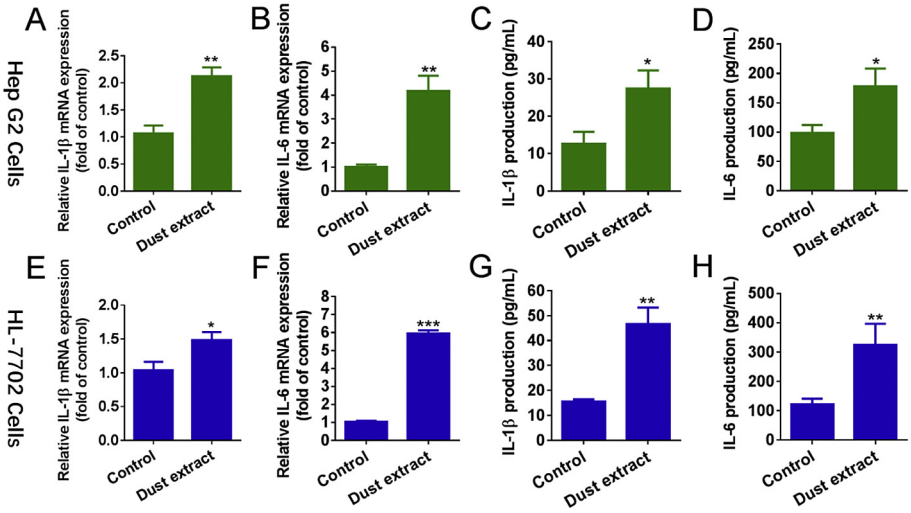

Fig. 5. Organic dust extract induced gene expressions and secretion of pro-inflammatory cytokines in HepG2 cells (A–D) and HL-7702 cells (E–H) after 24 h exposure at 40 μg/100 μL. Dust exposure elevated *IL-1β* and *IL-6* mRNA expression and release in both HepG2 cells and HL-7702 cells. Each bar represents mean ± SEM of three replicates. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.4. Pro-inflammatory cytokines and hepcidin mRNA

Oxidative stress also activates the transcription of pro-inflammatory mediators (e.g., IL-1β and IL-6). The increase of pro-inflammatory mediators has a pivotal role in controlling various liver diseases including liver damage, steatosis, inflammation, and fibrosis (Szabo and Csak, 2012; Tsutsui et al., 2015). Tilg et al. (1992) demonstrated that the level of IL-1β concentration was highly correlated with liver cirrhosis. Naugler et al. (2007) found an increase of IL-6 concentration in hepatocarcinogenesis. In the present study, compared with control, *IL-1β* and *IL-6* mRNA expression were 2.1- and 4.2-fold higher in HepG2 cells (Fig. 5 AB), while they were 1.5 and 5.9-fold higher in HL-7702 cells (Fig. 5 EF). To further evaluate IL-1β and IL-6 expression at the protein level, the cell culture supernatant was collected and measured by an ELISA assay. Consistent with their mRNA expression, the dust extract enhanced releases of IL-1β and IL-6 in both HepG2 (Fig. 5CD) and HL-7702 cells (Fig. 5GH), indicating dust-induced oxidative stress also elicited inflammatory responses, which may be attributed to OPCs, PAHs and other contaminants in the dust extract (Xiang et al.,

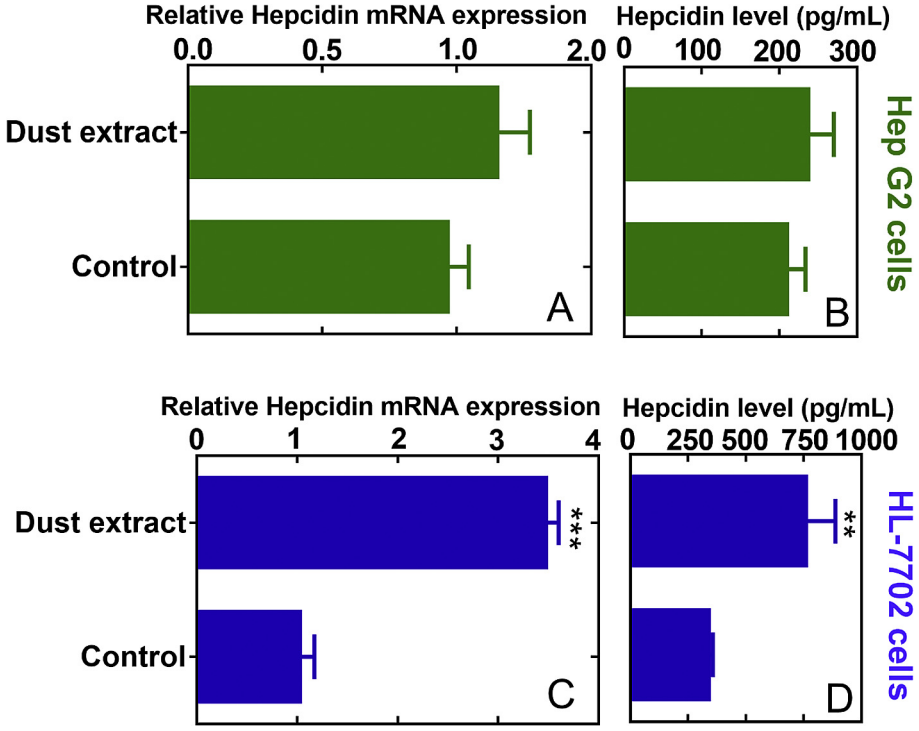

Fig. 6. Expression and secretion of hepcidin in HepG2 cells (AB) and HL-7702 cells (CD) after exposure to organic dust extract at 40 μg/100 mL for 24 h. Dust exposure elevated *hepcidin* mRNA expression and secretion in HL-7702 cells, whereas no significant change was observed in HepG2 cells. Each bar represents mean ± SEM of three replicates. $^{**}p < 0.01$, $^{***}p < 0.001$.

2016b). The release of both pro-inflammatory mediators in HL-7702 cells was higher than that in HepG2 cells, suggesting normal human hepatic cells are more susceptive to dust-induced inflammation than cancerous liver cells.

Hepcidin, a well-known Fe regulatory hormone, is generated in the liver hepatocytes to negatively regulate intestinal Fe absorption and inhibit its release from macrophages (Rossi, 2005). Several studies showed that its production can be induced under inflammation (Schmidt, 2015). It has been demonstrated that IL-1β and IL-6 stimulate hepcidin transcription in hepatocytes (Lee et al., 2005). Thereof, we compared hepcidin expression at mRNA and protein level in both cells after exposing to organic dust extract. The dust extract increased hepcidin expression at both mRNA and protein level in HL-7702 (Fig. 6 CD), which may attribute to their higher level of IL-1β and IL-6 production after dust extract exposure. However, the hepcidin level in HepG2 cells (Fig. 6 AB) did not alter even at elevated IL-1β and IL-6 condition, which may be due to the fact that HepG2 cells are not responsive to the bone morphogenetic protein pathway, a well-known molecular signal pathway to stimulate hepcidin expression in hepatic cells (Kanamori et al., 2014). In short, regulation of hepcidin in HL-7702 cells at mRNA and protein level by the dust extract indicated its adverse effects on Fe metabolism. Given that, further studies using human normal hepatic cells and enterocytes co-culture models are warranted to elucidate the regulatory mechanisms of possible Fe metabolism perturbation in enterocyte after dust extract exposure.

4. Conclusion

Our data showed that organic dust extract caused cytotoxicity, oxidative damage, inflammatory response and loss of MMP in both human cancerous liver cells (HepG2) and human normal hepatic cells HL-7702. The inhibition of cell viability by the dust extract in HL-7702 cells was stronger than that in HepG2 cells. However, higher production of oxidative stress biomarkers, loss of MMP and stronger suppression of antioxidant enzymes mRNA expression appeared in HepG2 cells at 40 μg/100 μL. In addition, our study demonstrated organic dust extract altered the hepcidin expression in HL-7702 cells but not HepG2 cells, indicating that the dust extract exposure may have perturbed Fe homeostasis in HL-7702 cells. These results indicated there were differences in cellular responses between human normal and cancerous hepatic cells to dust exposure. Further investigations are warranted to compare different cellular models and determine the best suited for hepatotoxicity of contaminants. Furthermore, the differences observed between cancerous and normal cell lines suggest that it is important to use normal cultures to assess contaminant toxicity to humans.

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