



In vitro and in vivo toxicity study of diesel exhaust particles using BEAS-2B cell line and the nematode *Caenorhabditis elegans* as biological models

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Abstract

It is well accepted that diesel exhaust particles (DEPs) are highly associated with improper function of organ systems. In this study, DEP toxicity was performed by using in vitro human BEAS-2B cell line and in vivo animal model, namely, *Caenorhabditis elegans* (*C. elegans*). The potential toxicity of DEP was assessed by the apical endpoints of BEAS-2B cell line and reflections of *C. elegans* under exposure scenarios of 0–50 $\mu\text{g mL}^{-1}$ DEP. With the increase of DEP exposure concentration, microscopic accumulations in the cytoplasm of cell line and intestine of *C. elegans* were observed. Such invasion of DEP impaired the behaviors of *C. elegans* as well as its un-exposed offspring and caused significant impeded locomotion. Moreover, the disorders of dopaminergic function were observed simultaneously under DEP exposure, specifically manifested by the decreased transcriptional expression of *dat-1*. The stress responses instructed by the expression of *hsp-16.2* were also increased sharply in TJ375 strain of *C. elegans* at DEP concentrations of 1 and 10 $\mu\text{g mL}^{-1}$. In the case of cellular reactions to DEP exposure, the injuries of membrane integrity and the decreased viability of cell line were simultaneously identified, and reactive oxygen species (ROS), damaged DNA fragment, and upregulated apoptosis were monotonically elevated in cell lines with the increase of DEP concentrations. This study provided a systematic insight into toxicity of DEP both in vivo and vitro, demonstrating that DEP exposure could disturb the stability of cell system and further threat the stability of organism.

Keywords Diesel exhaust particles · *Caenorhabditis elegans* · BEAS-2B cell · Accumulation · Oxidative stress

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Introduction

Particulate matter (PM) pollution has become one of the leading contributors to the global burden of diseases (Lim et al. 2012). It has been reported that PM exposure impairs multiple aspects of organism, including respiratory disorders, neurotoxicity, and cardiovascular disease. As one of the major components of PM, diesel exhaust particles (DEPs) are believed as core of various contaminants, which absorb other toxic substances due to its nanometer size and large specific surface (Mazzarella et al. 2007; Reis et al. 2018). Although the usage of diesel fuel has been limited and further substitutable by certain clean powers, it is enormously consumed amount globally which inevitably causes a series of healthy problems (Steiner et al. 2016). Generally, nano-scale size allows DEP to enter into the tissues, which is considered as one of its sources of toxicity to organism (Donaldson et al. 2005). In addition, complex mixture of organic compounds absorbed on the particle surface, such as particle-bound polyaromatic hydrocarbons (PAHs), can permeate into the organism tissues

accompanied by the DEP, which is also regarded as a main contributor for the adverse effects of DEP (Cordier et al. 2004; Levesque et al. 2013). Moreover, natural factors, such as sunlight, can trigger the action of free radicals on DEP surface, causing a higher risk for public health.

Previous toxicological studies have emphasized that the public health risk of DEP exposure for cardiopulmonary system, such as lung cancer, could be considered as the original health concern (Chu et al. 2018; Pope et al. 2002), while accumulating evidences show that central nervous system (CNS) is further affected by the DEP exposure (Danysh et al. 2015; Morales et al. 2009). Most studies focused on the biological responses of lung epithelial cells to DEP that of concentrations ranging from 10 to 100 $\mu\text{g mL}^{-1}$ (Cao et al. 2010; Totlandsdal et al. 2015; Zheng et al. 2019). In addition, there has been extensive literature reported on model animals that exposure to DEP can disrupt the respiratory, nervous and cardiovascular systems of mice (Block et al. 2004; Block and Calderon-Garciduenas 2009; Hartz et al. 2008). Moreover, the abnormal alterations of locomotor and cognitive activities are tightly associated with DEP exposure. The existed several hypotheses regarding how the PM affects the normal nervous function. Specifically, monoamine system, such as dopaminergic function, is verified as an important candidate for DEP-related neurobehavioral disorders (Yokota et al. 2009). Simultaneously, negative effects of parental generation caused by the DEP invasion even potentially influence the neurodevelopmental processes of next generation (Reis et al. 2018; Wang et al. 2019).

Generally, the main approaches to qualitatively evaluate DEP toxicities include models *in vitro* and *in vivo*. Cell culture studies are normally applied as the primary step to test how an agent reacts *in vitro* due to their low-cost and convenience. However, systems *in vitro* can only provide a small part information of the whole organism, without the information related systemic reactions and the buffering capacity (Steiner et al. 2016). In contrast, the consequences from model animal can help us comprehend the connection of entire organism with the presence of pollutions, ignoring detail changes appeared at the level of single cell. To date, most of current researches unilaterally focus on the single cell or individual level for DEP exposure. Apparently, the integrative toxicity in both levels of cell line and individual can provide systematical messages from those two aspects (Ma et al. 2018; Zhang et al. 2019). However, toxicological analyses of the effects associated with DEP *in vitro* and *in vivo* have rarely been reported.

Herein, the toxicological evaluation *in vivo* and *in vitro* for DEP exposure was conducted under environmental concentrations (10~5570 $\mu\text{g m}^{-3}$) to address issues of DEP toxicological effects in this study (Ghio et al. 2012). *C. elegans* was selected as animal model *in vivo* to evaluate the toxicity of DEP. The basic locomotive activities of worms were performed to determine the systematic stability. The biological

effects, such as the morphological alterations of dopaminergic neurons and stress response following exposure to DEP, were also evaluated by the usage of transgene strains. In addition, human BEAS-2B cell line, which has widely applied in the assessment of air pollution, was also selected due to its identity as first target for DEP invasion. Assays using cell line were further measured by disruption of multiple aspects of cellular processes (Fiorito et al. 2006; Ma et al. 2018), including the damage cell membranes (LDH, lactate dehydrogenase), cellular metabolic activity (MTT), oxidative stress, apoptosis, and DNA damage. This study would offer a preeminent understanding for the DEP toxicity.

Materials and methods

Chemicals and concentration determination

SRM2975 was a standard reference material for diesel exhaust particles industrial diesel-powered forklift provided by the National Institute of Standards and Technology (NIST), which was collected from a filtration system designed for diesel-powered forklifts. The specific components of DEP were listed in the Supporting Information (Table S1). In addition, the SEM test was used to characterize its surface and dimension for exposure in this study (Fig. S1). The samples of DEP for different exposure scenarios were dissolved in 100% DMSO as storage solutions, and then diluted by specific cell's mediums and worm's mediums with the final concentration of DMSO 0.1%.

C. elegans assays

Preparation of *C. elegans* strains and cultures

The cultivation of *C. elegans* was based on previous works (Stiernagle 2006). Briefly, the strains of worm, including wild type, BZ555 (egIs1 [*dat-1p::GFP*]), TJ375 (gpIs1 [*hsp-16.2p::GFP*]), and CF1553 (mul84 [*sod-3p::GFP + rol-6(su1006)*]) were cultivated on nematode growth medium (NGM, 51 mM NaCl, 25 mM KH_2PO_4 , 1.7% agar, 0.25% peptone, 1 mM CaCl_2 , 1 mM MgSO_4 , 5 mg L^{-1} cholesterol) seeded with *E. coli* OP50 to form adequate pregnant adult worms at 20°C. Furthermore, the collected worms were dissolved in alkaline bleach solution (1.6 M NaOH, 3.3% NaClO) and thoroughly washed by sterile K solution (Yang et al. 2019). Then, the killed worms were hatched on the NGM plates with food at 20°C for 24 h to obtain age-synchronized populations of the L1 stage. The worm's exposure experiments were performed with DEP suspensions (0, 0.01, 0.1, 1, 10 $\mu\text{g mL}^{-1}$) for 72 h, and *E. coli* OP50 was provided as a food source.

Locomotion behavioral assay

The wild-type worms in all exposure groups were washed three times with K medium to remove the *E. coli* OP 50. The conditions of DEP accumulation in *C. elegans* were recorded by microscope (Olympus, Japan). Specific neurobehaviors, including head thrashing, body bending, were chosen as neurobehavioral indicators to evaluate the toxicity of DEP based on previous studies (Hart et al. 1999; Tsalik and Hobert 2003). Briefly, Head thrashing of worms was defined as a change in the direction of bending at the mid body per 1 min (Liu et al. 2020). Body bending of worms refers to the movement of *C. elegans* by a wavelength in the direction relative to the long axis of the body per 20 s (Qu et al. 2019). The exposed nematodes were transferred into NGM plates without food sources to maintain a stable state for 1 min. Then, the rate of head thrashing and body bending were respectively calculated. 30 worms were used for each indicator.

For the assessment of offspring, 12 adult worms in reproductive period of F0 generation that exposed to different dose of DEP were randomly chosen to transfer to new NGM plates without DEP. After spawning, the adult worms were transferred from plates and worms' eggs were kept. Eggs were cultivated in incubator for 48 h into L4 stage for counting the number of offspring (F1). The method of culturing F2 generations' worms was the same as the F1 generation (Zhou et al. 2016). In addition, the adult worms from all generations were collected to test worms' head thrashing.

DAT-1, HSP-16.2, and SOD-3 expression determination

The protein expression of strain BZ555, *dat-1* promoter coupled with the gene encoding green fluorescent protein (GFP), was recorded after exposure to DEP on base of our previous study (Cao et al. 2020). In addition, TJ375 and CF1553 stains were both used to estimate the extent of stress response to DEP exposure. In brief, 100 mM NaN₃ was applied to anaesthetize worms, and inactive worms were visualized using a laser scanning confocal microscope (Nikon Corporation, Japan), with an excitation wavelength of 485 nm and an emission wavelength of 535 nm. At least 20 worms for each exposure group were counted, and the experiments were repeated three times. The percentage of morphologically intact neurons was calculated as previous study (Sammi et al. 2018), and plotted against its respective neuronal type in the control. Fluorescence intensity of GFP was quantified through Image Pro Plus software (American). The ratio of integrated Density/Area was chosen as the quantitative index (Cordeiro et al. 2020).

BEAS-2B cell line assays

Cell line culture

The human bronchial epithelial cell line BEAS-2B, provided by Icell company (Shanghai, China), was cultured in 5% CO₂ atmosphere at 37°C in DMEM containing 10% FCS, 1% penicillin-streptomycin, and 1% glutamine referenced as previous methods (Künzi et al. 2013). For all exposure groups, bronchial epithelial cells (1×10^4) were cultured in ninety-six well plates in 200 μ L complete medium (DMEM + 10% FBS) with the different DEP concentrations. Cells maintained in complete medium without DEP were designed as controls. The BEAS-2B cell line in different concentrations of DEP suspension (0, 0.1, 1, 10, and 50 μ g mL⁻¹) were cultivated in cell culture incubator for 24 h and further to proceed the experiments. All test endpoints were in triplicates for each experiment. The DEP accumulation in BEAS-2B cells was recorded by microscope phase-contrast microscopy.

MTT assay

MTT assay was carried out to assess the viability of cell based on reported methods (Mosmann 1983) and the instruction of reagent kit. Briefly, 200 μ L PBS was added to each well to remove the remaining diesel particles as much as possible after 24 h DEP exposure. And then, 200 μ L complete culture medium was added to each well with 20 μ L MTT solution (5 mg mL⁻¹). The configured mixtures were cultured for 4 h in incubator at 37°C. After cultivation, the culture medium was aspirated from 96-well plate and 150 μ L dissolving solution of formazan (Icell company, China) was added to each well, which was further sharked 10–30 min at low speed to fully dissolve the crystals inside cells. Finally, microplate reader (TECAN, Switzerland) was applied to measure the absorbance of each well at OD 570 nm.

LDH assay of cell line

The release of lactate dehydrogenase (LDH) was applied to detect cytotoxicity as previously described (Künzi et al. 2013). Lactate dehydrogenase cytotoxicity detection kit (Beyotime, China) was used to determine LDH release. In brief, 60 μ L LDH solution was added into each well contained with BEAS-2B cells and then the mixtures were mixed completely. The prepared solutions were incubated at room temperature (approximately 25°C) in the dark for 30 min. The absorbance was then measured by microplate reader at 490 nm based on formula: LDH release rate (%) = (absorbance of treated sample - absorbance of control sample) / (absorbance of maximum enzyme activity - absorbance of control sample) \times 100.

ROS assay

The intracellular levels of reactive oxygen species (ROS) in BEAS-2B cell line were measured to reflect the extent of oxidative stress triggered by xenobiotic substances as previously described (Jakubowski and Bartosz 2000). The non-fluorescent molecular probe, DCFH-DA, diluted by serum-free medium with 1:1000 to realize final treatment solution ($10 \mu\text{mol L}^{-1}$). The cells collected from 96-well were suspended in DCFH-DA solutions and incubated in cell incubator at 37°C for 20 min. During the period of cultivation, it was necessary to blend the solutions every 3–5 min to fully realize the contact of the probe with cells. And then, the cells were washed three times with serum-free culture medium to sufficiently remove DCFH-DA for fluorescence detection.

Apoptosis assay

Apoptosis was detected by an annexin V-FITC/PI double-staining detection Kit (Solarbio, USA) based on method's instruction and previously reported (Zhao et al. 2018). Simply, the tested cells were harvested after 24 h DEP exposure and were washed by cold PBS solution (1×10^6 cells/time). The cells were suspended with 1 mL binding buffer and then centrifugalized with $300 \times g$ for 10 min. Successively, cells were re-suspended to realize the density of cells up to 1×10^6 /mL and prepared for staining (1×10^5 cells to each tested tube). Next, 5 μL annexin V-FITC and PI were in turn added into each tube being incubated for 10 and 15 min at room temperature in the dark, respectively. Finally, 500 μL PBS was added to each tube with suspension, and the stained cells were determined by flow cytometry to analyze the apoptotic rate within 1 h.

Comet assay

For comet assay, the preparation of reagents, including lysis, electrophoresis, and neutralization solutions, and gelatin were prepared based on previously reported (Azqueta and Collins 2013). Briefly, the pre-chilled lysate was used to lysed cells on slides at 4°C in the dark for 2 h. The slides contained cells were subsequently immersed completely in freshly prepared pre-cooled electrophoresis solution in 4°C and unscrewed for 30 to 60 min. The voltage of electrophoresis was set to 25 V and this process lasted for 20–30 min. And then, neutralization was proceeded with pre-cooled 0.4 mol/L Tris-HCl buffer for 5 min and repeated 3 times. After dehydration using absolute ethanol (gradient dehydration) for 15 min, the slide glass carried with cells was taken out and dripped ethidium bromide on the surface with staining for 20 min. Finally, fluorescence microscope was used to inspect the DNA integrity under the fluorescence of 510–560 nm wavelength. The DNA migration

length was calculated by measuring head length and full length of the tailing cells using an eyepiece micrometer.

Statistical analysis

For the statistical analysis, all data were displayed as means \pm standard deviation (SD) and one-way ANOVA with Dennett's post-test was used to compare significance between the exposure and control groups (SPSS 24.0). p values lower than 0.05 were considered as statistical significance.

Results and discussion

DEP accumulation in *C. elegans*

Once encountered DEP, the first line of defence was the intestinal system for *C. elegans*. The results from observation showed that DEP distributed throughout the intestine of the nematode from the pharynx to the tail, especially on the pharynx of the worm after exposure DEP for 72 h (Fig. 1A and B). Virtually, the accumulation of particles appeared at whole intestinal lumen under $10 \mu\text{g mL}^{-1}$ DEP, while the enrichment was not significant at $1 \mu\text{g mL}^{-1}$ of DEP, without detectable DEP at lower concentrations by optical microscope (Fig. S2). This could be probably explained by the limited affordability of excretion ability of intestine, which was unbearable on such higher dose of DEP intake. It is remarkable that *C. elegans* periodically expels undigested luminal contents to keep the balance and stability of intestinal lumen (Chauhan et al. 2013). The changes of intestinal conditions, such as pH and the activity of hydrolases, could be affected inevitably by the accumulation of DEP. However, the results accessed by spontaneous fluorescence in intestine from this research indicated that DEP exposure could not affect the formation processes of lipofuscin (Fig. S3, SM), implying that the existence of DEP did not accelerate the aging processes of the intestine. It was once reported that besides intestine, nano-particles can also additionally appear in other organs, such as vulva and brain (Riediker et al. 2019; Scharf et al. 2013). However, on the basis of above results, we could not elucidate the DEP's distribution in other tissues because of the invisible or indistinguishable nano-size particles under such magnification.

DEP-impaired neurobehaviors and DA neurons on *C. elegans*

As rapid and reliable indicators, neurobehaviors of *C. elegans* were proceed to identify whether the accumulation of DEP could further disturb the normal behaviors controlled by the complex neurons. In the presence of $0.1 \mu\text{g mL}^{-1}$ DEP, the frequency of head thrashing was significantly influenced ($p < 0.05$), and the maximum dose used in this study ($10 \mu\text{g mL}^{-1}$)

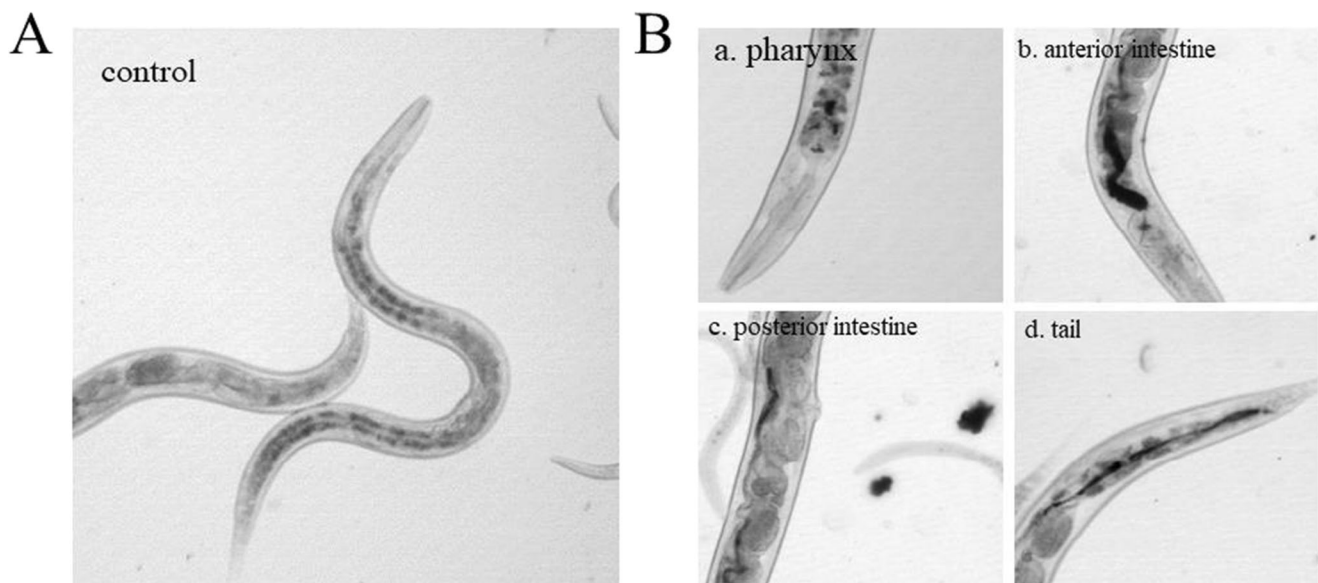


Fig. 1 The accumulation condition of DEP in *C. elegans* as well as cell. (A) The morphology of *C. elegans* in the control group (1:100); (B) Representative pictures of DEP accumulation in *C. elegans* under the dose of $10 \mu\text{g mL}^{-1}$

mostly aggravated this locomotive deflection ($p < 0.01$, Fig. 2A). Consistent with head thrashing, the frequency of body bending under $10 \mu\text{g mL}^{-1}$ DEP exposure was also severely affected compared with control, with the mean times of sinusoidal motion decreased from 13.5 to 9.8 per 20 s (Fig. 2B). Similar with the results from *C. elegans*, behavior disorders, such as spontaneous locomotor activities, also occurred on mice after DEP exposure (Suzuki et al. 2010; Yokota et al. 2009). More seriously, workers who have been exposed to DEP for a long time are affected by neurobehavioral disorders, indicating that DEP have effects on the central nervous system (Kilburn 2000). Head thrashing, as the most sensitive indicator for DEP exposure, was also induced to verify the effect of DEP on offspring of worms. Negative defections were also detected in F1 generation ($p < 0.05$), while no significant alteration in F2 generation, indicating that DEP exposure potentially interfered the nervous developmental processes of the next offspring. Similar results have been confirmed by previous studies that the negative effects of DEP exposure to individual can inherit to the next generation (Palkova et al. 2015; Pencikova et al. 2019).

In the case of *C. elegans*, the protein expression controlled by genes, which was coupled with the signal of green/yellow fluorescent protein (GFP/YFP), can provide a direct information of physiological conditions of worms. Specifically, the application of transgenic strain helps to reveal the underlying mechanisms of certain behavioural or pathological changes. It was once reported that DEP neurotoxicity is sensitive for DA neurons by comparing the ability to uptake $[^3\text{H}]\text{DA}$ and $[^3\text{H}]\text{GABA}$ of neuron-glia cultures (Block et al. 2004). Hence, the strain of BZ555, in which fluorescent protein represented transcription level of dopaminergic transporter,

namely, *dat-1*, was applied to further detected the DEP neurotoxicity in vivo (Au-Kudumala et al. 2019). DA neurons in *C. elegans* shows 4 CEP, 2 ADE in head region along with 2 PDE neurons in tail region (Sammi et al. 2018). Considering the fact that neurobehaviors of worms were disturbed under 1 and $10 \mu\text{g mL}^{-1}$ DEP, the neurobiology of dopamine neurons was examined at the same dosages. As seen in Fig. 3, the signals of *dat-1* decreased significantly at the concentration of 1 and $10 \mu\text{g mL}^{-1}$ DEP in comparison with the control group. Simultaneously, worms showed neuropathological alterations in the presence of $1 \mu\text{g mL}^{-1}$ DEP, especially for the loss of CEP (Fig. 3B). Such morphological changes of neuron were also observed at posterior deirid (PDE), which exhibited considerable decrease compared with the control group (Fig. 3C). Neuronal loss of CEP showed the most sensitive change under the DEP exposure, which could be attributed to the DEP's diffusion or passive transportation into the CEP or ADE neurons that has been inferenced by other neurotoxins (Nass et al. 2002). The abnormal expression of *dat-1* is incapable of re-accumulating DA into the neurons, which ultimately results in the inhibited locomotion (Sawin et al. 2000). Levesque et al. (2013) demonstrated that the function of dopaminergic neuron was damaged with the decreasing DA uptake in the presence of DEP (Levesque et al. 2013). Notably, nanometer-size DEP were proposed to reach into the brain through blood circulation in mammals (Valavanidis et al. 2008). Sugamata et al. (2006) have also documented that DEP exposure would cause accumulation in various brain regions, indicating the potential negative effect of DEP on the DA neurons (Sugamata et al. 2006). Moreover, the adverse impact to offspring could also be attributed to the maternal exposure to DEP, which is believed to be highly

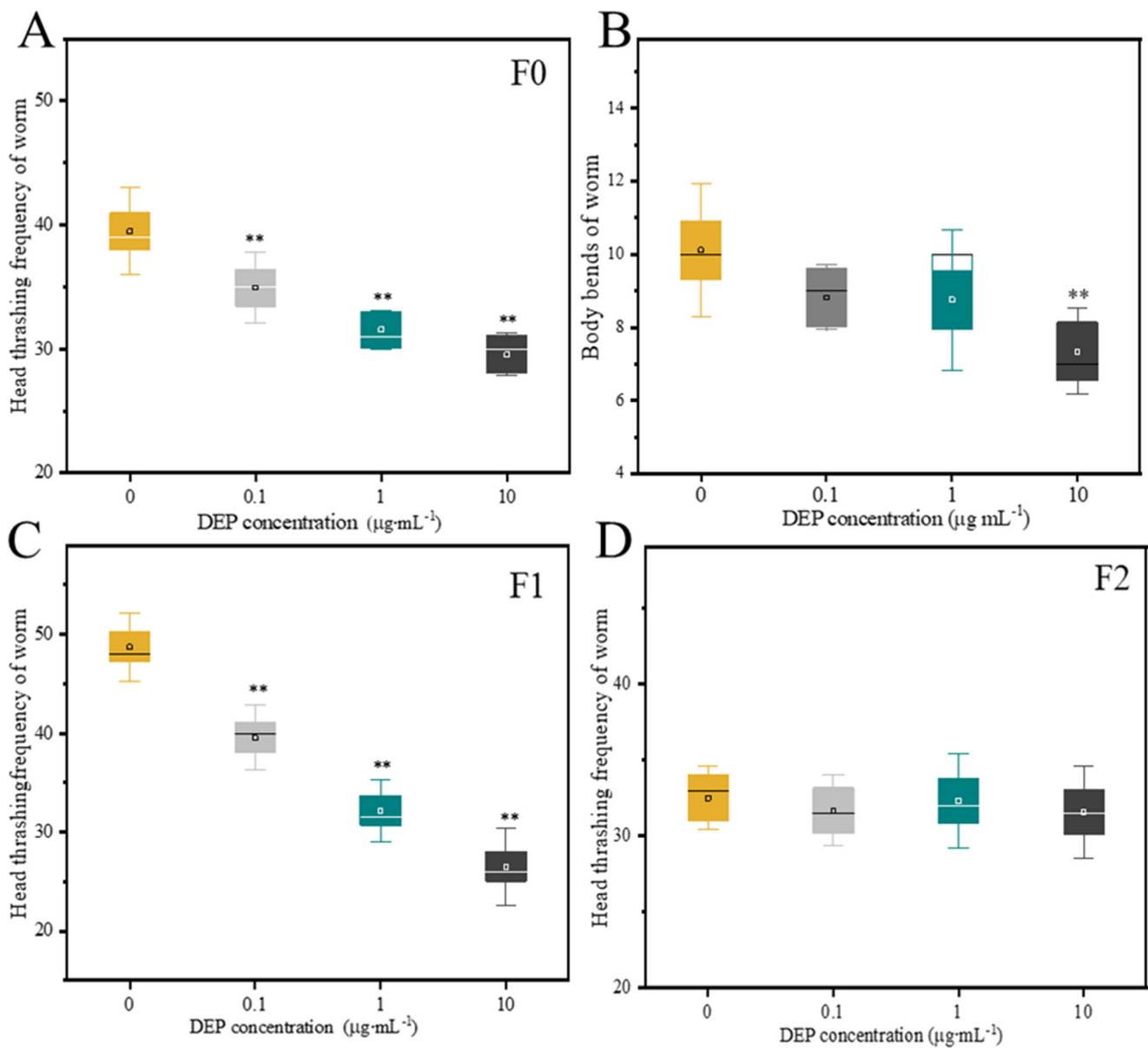


Fig. 2 Toxicity evaluation of neurobehavioral indicators after exposure to DEP. (A) Head thrashing of F0; (B) Body bends of F0; (C) Head thrashing of F1; (D) Head thrashing of F2; Data (mean ± SD) were expressed as the percentage value compared to the control group. * $p < 0.05$; ** $p < 0.01$

associated with development of central dopaminergic system (Yokota et al. 2009). To sum up, the obstructed activities of dopamine could be mainly or partially responsible for the changes of neurobehaviors observed in this study. It was also hypothesised that hydrophobic molecules that absorbed on the surface of particles are more likely to directly cause influences to sensitive neurons (Collier et al. 2013).

Transgenic stains related to stress response were also used to detect the abnormal regulation factors by DEP exposure in *C. elegans*, including TJ375 and CF1553 stains. TJ375 can provide an accurate assessment of the total amount of native HSP-16.2 expression (Rea et al. 2005), which has been

accepted as a direct visualization reporter of stress response (Link et al. 1999). Obviously, the appearance of DEP evoked the expression of HSP in worm (Fig. 4A). On exposure condition of the animals to 1 µg mL⁻¹ DEP, significantly increased GFP signal was observed, and a stronger expression appeared at a higher concentration of 10 µg mL⁻¹ DEP. In addition, the distribution of HSP was more widespread throughout the worm’s body compared with the control group (Fig. 4A and B). In contrast, the influence of DEP exposure on CF1553, which carried the expression marker of *sod-3*, was quite limited in the presence of DEP (Fig. S4). It was also reported that the intracellular catalase (CAT) and superoxide

Fig. 3 Toxicity evaluation of DA neurons exposure to DEP. **(A)** The morphology of *P. dat-1::GFP* animal; **(B)** The morphology of CEP neurons; **(C)** The morphology of PED neurons; **(D)** The intensity of fluorescence; **(E)** The percentage of intact neurons; Data (mean \pm SD) were expressed as the percentage value compared to the control group. * $p < 0.05$; ** $p < 0.01$

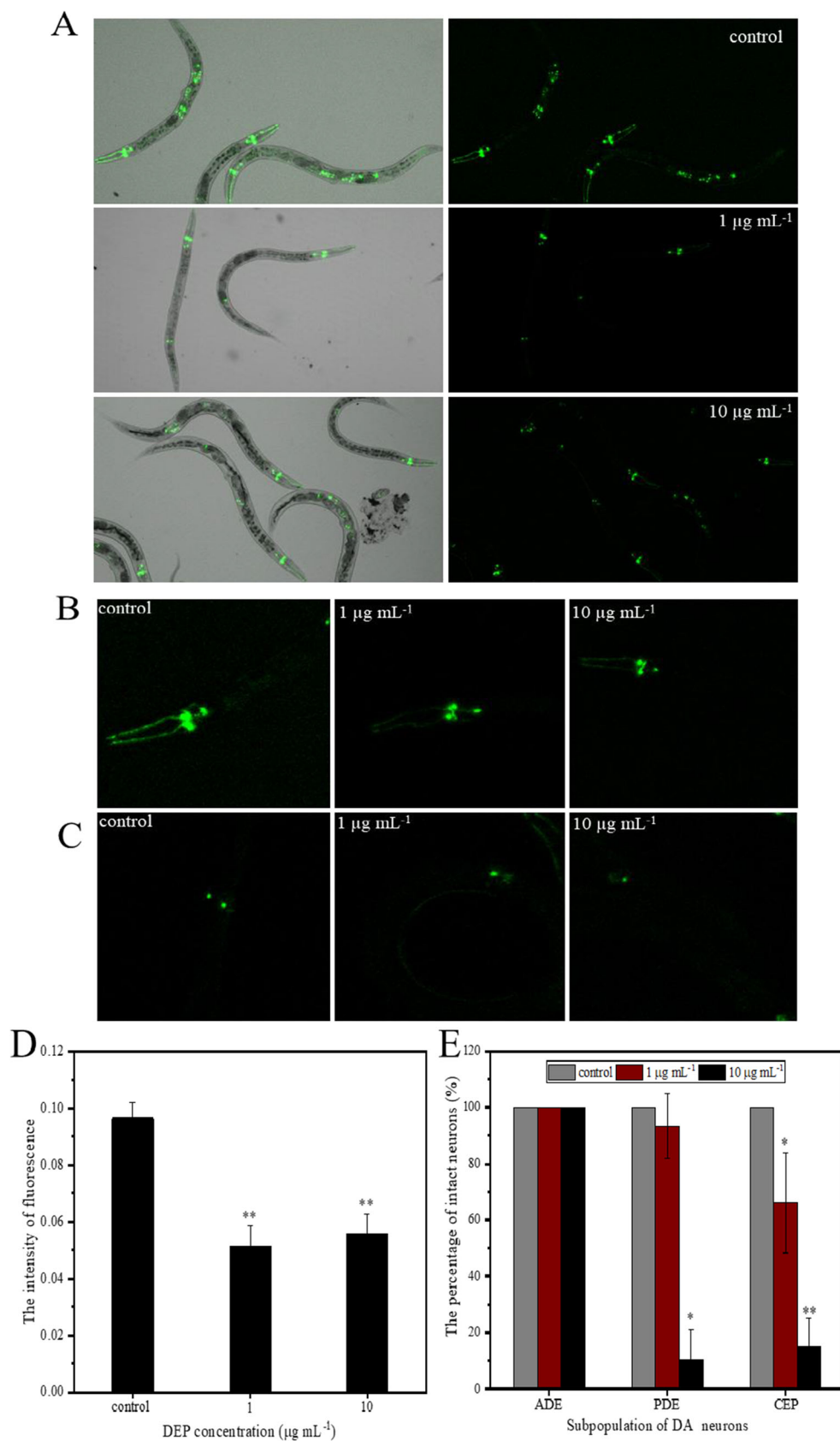
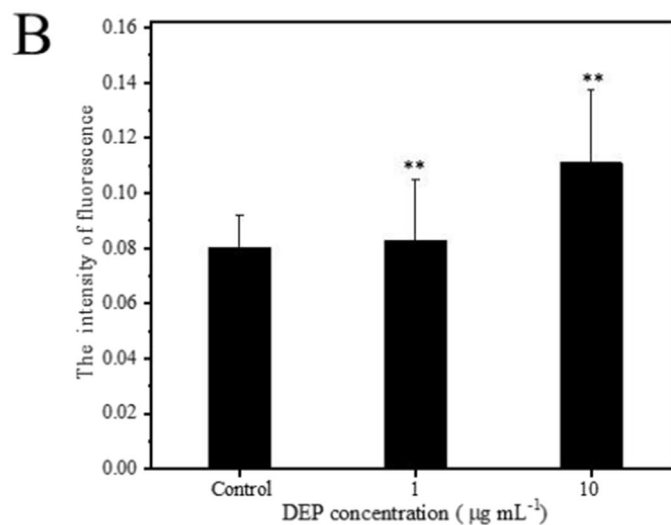
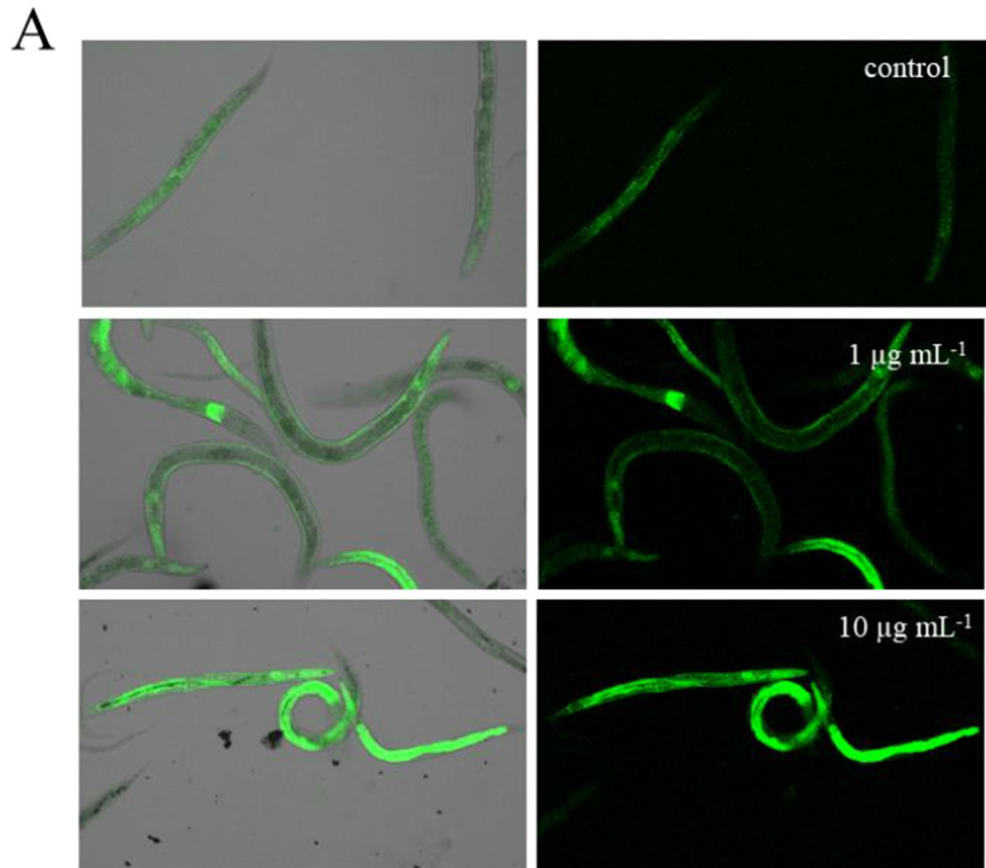


Fig. 4 Toxicity evaluation of the oxidative stress after exposure to DEP. **(A)** The morphology of *hsp-16.2::GFP* animal (1:100); **(B)** The intensity of the fluorescence of TJ375; Data (mean \pm SD) were expressed as the percentage value compared to the control group. * $p < 0.05$; ** $p < 0.01$



dismutase (SOD) activities can be impaired by exposure to PM (Deng et al. 2013). Other evoked protective pathways were probably responsible for the inconspicuous change of *sod-3* expression (Chirino et al. 2010; Deng et al. 2013).

DEP-induced cytotoxicity in BEAS-2B cell line

Furthermore, BEAS-2B cell line was applied to evaluate the cytotoxicity induced by DEP exposure in vitro to better

explore the responses to DEP at the single cell level. As shown in Fig. 5A, the cell line displayed a normal morphology under the exposure scenarios of 0.1 and 1 µg mL⁻¹. Savary et al. once reported that human bronchial epithelial cells exposed to DEP that of environmental concentrations (2 µg mL⁻¹) also showed little impact on the phenotype of cell line (Savary et al. 2018). However, the accumulation of DEP appeared at the cellular cytoplasm in the presence of 10 and 50 µg mL⁻¹ DEP. Similar with black carbon and other particles (Gao et al.

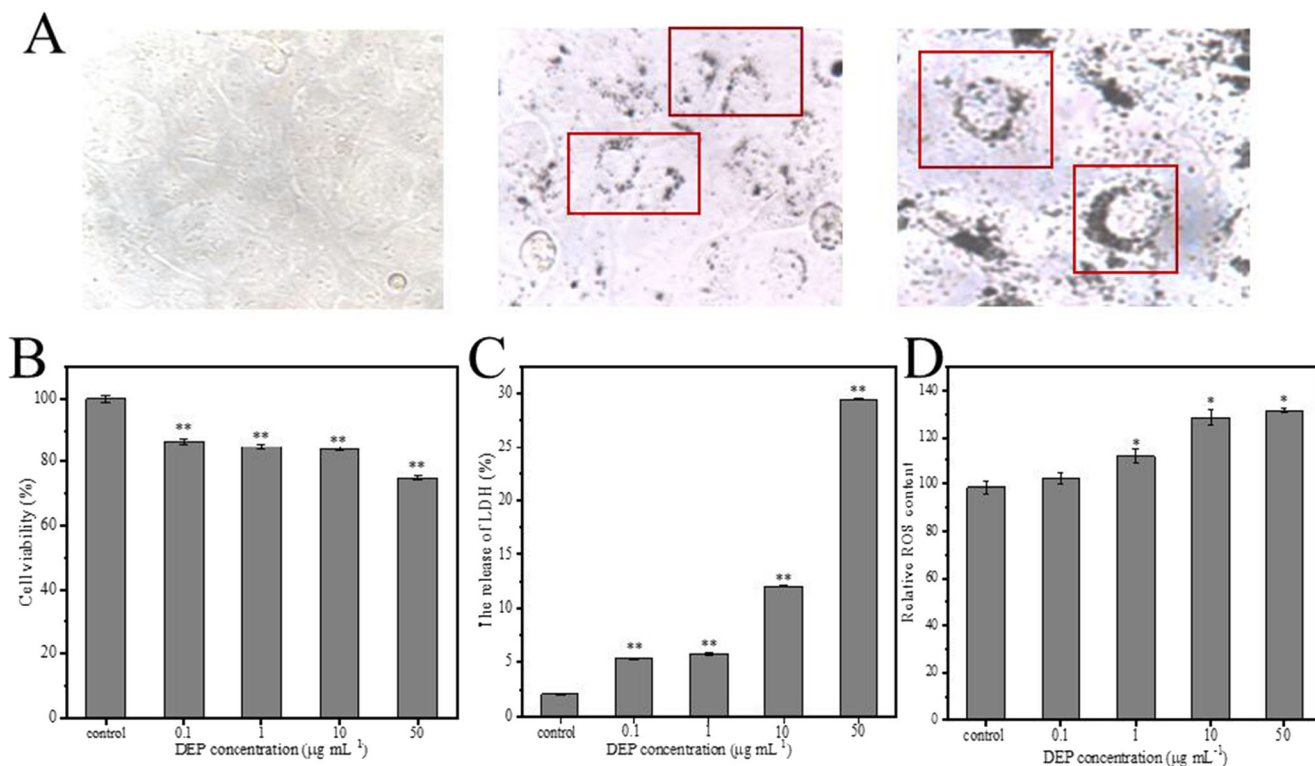


Fig. 5 Cell's response to DEP exposure. (A) Representative pictures of DEP accumulation in cell under the dose of $10 \mu\text{g mL}^{-1}$ (1:400). (B) Cell viability; (C) The release of LDH; (D) The relative ROS level. * indicated statistical significance in comparison with control group. * $p < 0.05$; ** $p < 0.01$

2016; Nel et al. 2009), the characteristic of small size made DEP more potentially penetrate into cell membranes and further transferred to the cellular cytoplasm. Such result is also obtained by Zhao et al. (2018) that nanoparticles enter into cell through the function of endocytosis (Zhao et al. 2018). Although Hiura et al. (1999) has emphasized that only phagocytic cell lines possessed accumulative effect of particles for its immune function that of clearance (Hiura et al. 1999), other types of cell could further infected by the DEP through the ability of endocytosis.

As the consequences of intracellular response to DEP, the cell viability and release of LDH were also applied to determine the final adverse outcome affected by DEP exposure in BEAS-2B cell line. As seen, the results suggested that cell viability displayed a significant reduction in the presence of DEP with concentrations higher than $0.1 \mu\text{g mL}^{-1}$ (Fig. 5B, $p < 0.01$). Specifically, at $50 \mu\text{g mL}^{-1}$ DEP exposure medium, the lethality of cells reached up to 25.2% compared with the control group. To further decipher the negative impact on cell line, lactate dehydrogenase analysis (LDH) was also applied to evaluate the cell viability. Results from Fig. 5C demonstrated that DEP exposure induced significant increase of LDH release of cells, indicating an injured integrity of the cell's membranes. The invasion of DEP exhibited a dose-dependent relationship for LDH release under DEP exposure. LDH released from cell serves as an evidence for penetration

of particles into the cell and the damage of cell membranes (Sayes et al. 2005). The decrease of viability can be attributed to the increasing release of LDH (Deng et al. 2013). Obviously, the release of LDH, which displayed negative correlation with cell viability, was also detected in the present study.

DEP-induced ROS generation in BEAS-2B cell line

The molecular mechanisms of increased cell death were further proceeded by cellular responses to DEP. As shown in Fig. 5D, the detected signal of ROS in BEAS-2B cell line increased significantly at dose of $1 \mu\text{g L}^{-1}$ DEP, and continuously increased with the increasing DEP concentrations, exhibiting a concentration-response relationship. The disrupt equilibrium of ROS level could be a main indicator in case of nanoparticles exposure (Wang et al. 2009), which was also applicable for the toxicity of air particles. It was widely accepted that the ROS generation is highly related with the stability of system and always considered as a sensitive indicator for the toxicity of particulate pollutions (Wang et al. 2009). Actually, the abnormal formation of ROS as initial response for the invasion of xenobiotic compound, can lead to a series of consequences for DEP exposure. Meanwhile, ROS elevation in cells is also suggested to be attributable to the

regulation of cell survival and cell death (Azad et al. 2008; Deng et al. 2013), in agreement with the results in the present study.

DEP-induced DNA damage and apoptosis in BEAS-2B cell line

To explore the potential toxicity of biological macromolecules induced by DEP exposure, the apoptosis and the integrity of DNA were recorded in the present study. Comet assay was used to monitor DNA repairing as well as DNA damage. The results from Fig. 6 displayed that the proportion of tail DNA, as directive of damaged DNA fragments, increased dose-

dependently with DEP concentrations, indicating a severe interference of DEP to macromolecules. The breakage of DNA strand can be attributed to the increased risk of tumorigenesis and carcinogenesis for air pollution (Schins and Knaapen 2007). Previous studies have emphasized the apoptosis processes is highly regulated by genes, and the appearance of shattered DNA fragments showed potential to influence those programs. For that purpose, the proportion of apoptosis was simultaneously recorded after exposing animals to DEP. The presence of DEP significantly triggered to the increase of cell distribution in Q2 zone, late apoptosis. As reported, a dose-dependent increase of apoptosis in reproductive system was also observed in *C. elegans* in the presence of DEP (Wang

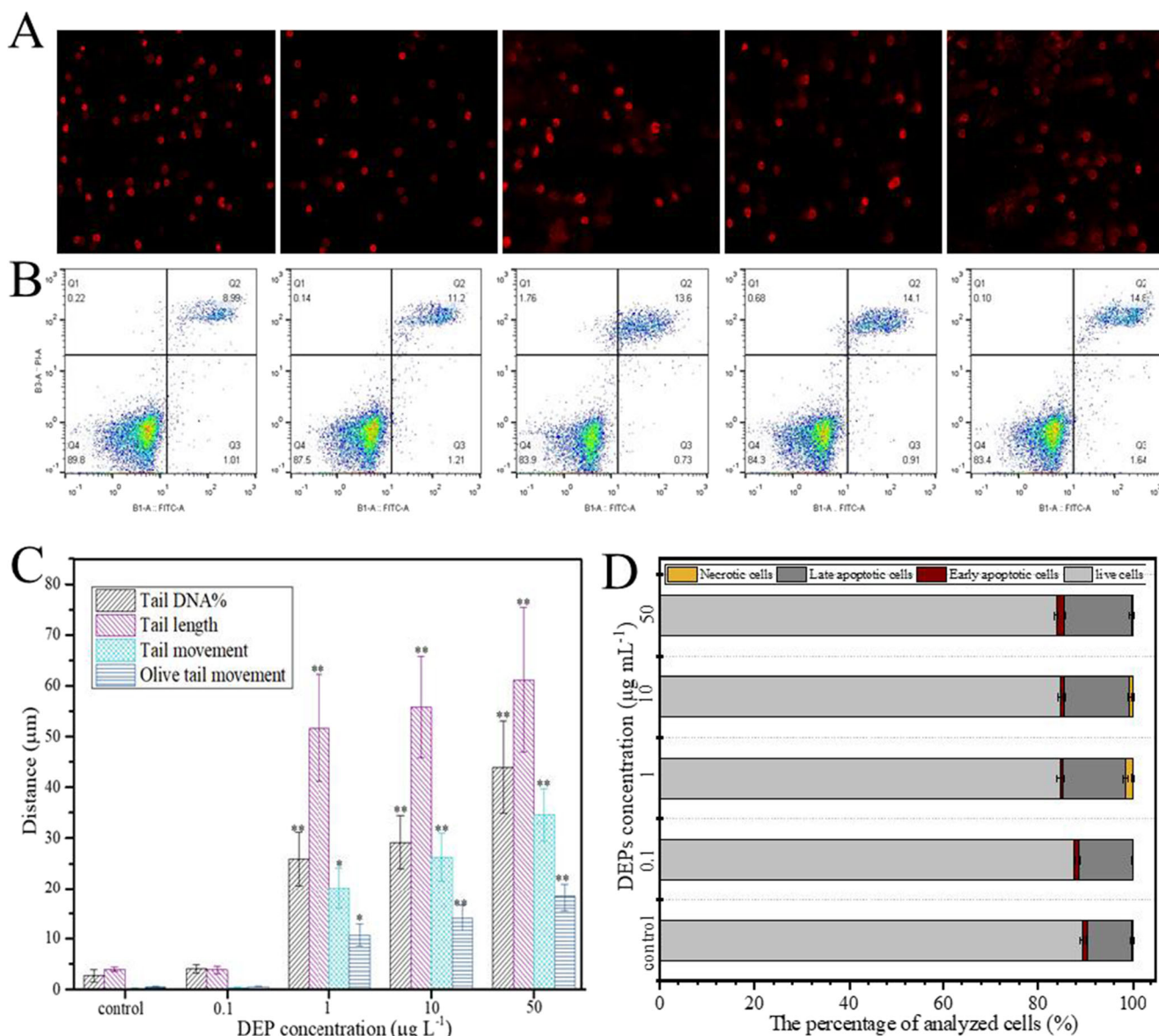


Fig. 6 Toxicity evaluation of cell line after exposure to DEP. (A) The representative images of comet assay; (B) The represent images of apoptosis; (C) The statistics results of comet assay; (D) The statistics

results of apoptosis. Data (mean \pm SD) were expressed as the percentage value compared to the control group. * $p < 0.05$; ** $p < 0.01$

et al. 2019). It was previously emphasized that the apoptosis can be introduced at different fine particles exposure (Guo et al. 2014; Osornio-Vargas et al. 2003). Apparently, DEP could cause a universal disturbance on the gene regulation in control of apoptosis. Moreover, acting as the second messengers in intracellular signalling cascades, excessive ROS may further induce the cellular apoptosis due to the invasion of particles (Long et al. 2007).

In this study, both *C. elegans* and BEAS-2B cell line were applied to investigate how DEP participated in toxic outcomes at different levels from the entire bioassay and cellular processes. The cell responses to DEP partly reflected the possible influence on single cell level. Simultaneously, the worm's response to DEP implied certain denouement that were adjusted by information transmission among different cell lines. As for the issue of the toxic contribution, DEP as a complex mixture which was made up of different fractions, was difficult to specifically quantify in those two models. Highly bio-persistent property of elemental core results in the most damaged injuries limited to primary target organ, while blocks expanded toxicity of particles for other tissues (Steiner et al. 2016). However, the chemicals absorbed on the surface of carbon core can play an important role in the unbalance of biological regulation (Geller et al. 2006). Apparently, the long retention of DEP in tissues could increase risk the release of PAHs which were partly confirmed to be carcinogenic. The accumulation of DEP in tissues or cells could be the original cause for a series of negative responses detected in this study. Hence, the application of *C. elegans* together with human cell lines as the supplement of DEP assessments could be more objective to reflect the DEP toxicity.

Conclusion

In this contribution, the present study examined the toxic effects of DEP on human BEAS-2B cell line and *C. elegans*. DEP exposure induced negative responses of *C. elegans*, in which the abnormal regulation of dopamine and stress responses were inescapably responsible for the alteration of locomotive activities of nematodes and its offspring. Furthermore, in the aspect of cell level, DEP could inevitably enter into the cytoplasm of cell and participate in various biological processes which eventually triggered the cell death. This study demonstrated that in vitro and in vivo adverse outcomes caused by DEP exposure could be as a complement to each other, which provided faster and more accurate comprehension for DEP toxicity.

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Author contribution Chenzhi Yan analyzed the data and write the manuscript. Xuan Wu performed the cell-related experiments. Xue Cao was a major contributor in modifying the manuscript and worm-related experiments. Meng Li assisted the part of worm-related experiment, Lei Zhou helped to corrected errors in manuscript, Guangli Xiu provided the idea of this manuscript and financial support. Jiayi Zeng helped to complete parts of worm-related experiments. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

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