Supporting Information

Surface-Engineered Carbon Dots as Green and Efficient Nanoscavengers with Dual Protective Functions against Oxidative Stress

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Materials. All chemicals used in this study were obtained directly from commercial suppliers and used without further purification. Tea polyphenols were purchased from Zhongshan Huazhong Food Additives Co., Ltd. (Zhongshan, China). Dopamine hydrochloride and $ABTS^{+}$• were purchased from Sigma-Aldrich (Shanghai, China). DPPH• was supplied by Shanghai Yuanye Biotechnology Co., Ltd. 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) was purchased from Aladdin Industrial Corporation (Shanghai, China). H_2O_2 and potassium persulfate ($K_2S_2O_8$) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All experiments were conducted using ultrapure water produced by a Millipore purification system.

Synthesis of TP-CDs. Tea polyphenols (1.00 g) and dopamine hydrochloride (1.00 g) dissolved in 35 mL of ultrapure water and sonicated for 5 minutes. The resulting solution was transferred into a 50 mL Teflon-lined stainless-steel autoclave and heated at 180 °C for 6 hours. After the reaction, the autoclave was allowed to cool naturally to room temperature. The resulting solution was centrifuged at 10,000 rpm for 10 minutes to remove large aggregates. The supernatant was filtered through a 0.22 μm water-based filter membrane, and the filtrate was collected. Further purification was performed via dialysis using a dialysis membrane (molecular weight cut-off: 500–1000 Da) for 60 hours. The final product was obtained by freeze-drying the dialyzed solution, yielding a dark powder.

Characterization. The morphology of TP-CDs was characterized by transmission

electron microscopy (TEM, JEM-2100). Surface functional groups, elemental composition, and structural properties were analyzed using Fourier transform infrared spectroscopy (FTIR, Nexus 870), X-ray diffraction (XRD, SmartLab 9kW), Raman spectroscopy (LabRAM HR800), and X-ray photoelectron spectroscopy (XPS, ESCALAB 250). Electron paramagnetic resonance (EPR) spectra were acquired using an X-band benchtop spectrometer (EMXnano, Bruker, Germany) to evaluate free radical scavenging activity.

UV-visible absorption spectra were measured with a UV-1800PC spectrophotometer, while fluorescence spectra were recorded using an F-7000 fluorescence spectrophotometer. Fluorescence lifetime and absolute quantum yield were determined respectively with a fully integrated steady-state and time-resolved fluorometer (FluoroMax-4P, HORIBA, France) and an ultrafast time-resolved fluorescence spectrometer (FLSP920, Horiba, Japan).

Determination of antioxidant properties. Determination of DPPH scavenging ability (D-RS) by TP-CDs: Measure out 2 mL of different concentrations of TP-CDs aqueous solution (10-50 μg mL^{-1}) taken separately, and 2 mL of DPPH ethanol solution (2 mmol L^{-1}), mixed well, and left in the dark for 30 minutes. Determine the absorbance of the reaction solution at 520 nm and record it as A_1. Use ultrapure water to replace TP-CDs and record its absorbance value (A_0) under the same conditions. The rate at which the samples scavenge DPPH radicals was determined using the equation below:

$$\text{D - RS (\%)} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (1)$$

Determination of 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) ($ABTS^{+}\bullet$) scavenging ability (A-RS) by TP-CDs: 7.4 mmol L^{-1} $ABTS^{+}\bullet$ and 2.6 mmol L^{-1} potassium persulfate were mixed (1:1, v/v) thoroughly and then left overnight in the dark at 4 °C. The mixture was diluted with ultrapure water until the absorbance at 734 nm was 0.7 ± 0.2 and used. Subsequently, 1.0 mL of TP-CDs solution with different concentrations (10-50 μg mL^{-1}) and 4.0 mL of the above $ABTS^{+}\bullet$ working solution

were taken. The absorbance values at 734 nm were determined after 30 min of standing. Record the absorbance as A_1. Use ultrapure water to replace TP-CDs, with the other conditions being the same, and record the absorbance value as A_0. The free radical scavenging rate was calculated from the equation:

$$\text{A - RS (\%)} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (2)$$

Determination of hydroxyl radical scavenging ability (H-RS) by TP-CDs: In a 20 mL sample bottle, add 2.0 mL of different concentrations (50-250 μg mL^{-1}) of TP-CDs solution, 2.0 mL of 6.0 mmol L^{-1} ferrous sulfate aqueous solution, 2.0 mL of 6.0 mmol L^{-1} salicylic acid ethanol solution, and finally add 2.0 mL of 6.0 mmol L^{-1} hydrogen peroxide solution. After keeping the reaction solution in the dark for 30 minutes, the absorbance value at 510 nm was measured and recorded as A_1. Use ultrapure water to replace TP-CDs, with the other conditions being the same, and record the absorbance value as A_0. The •OH radicals scavenging rate was calculated from the equation:

$$\text{H - RS (\%)} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (3)$$

Determination of superoxide anion scavenging ability (S-RS) by TP-CDs: $\bullet O_2^-$ is produced by mixing xanthine (5 μL, 560 mM) and xanthine oxidase (5 μL, 1:10 dilution), then 50 μL of different concentrations of TP-CDs aqueous solution (50-250 μg mL^{-1}) are added separately, followed by the addition of the color reagent and thorough mixing. The entire reaction system turns red. After 15 minutes of standing, the absorbance of the solution at 550 nm is measured and recorded as A_1. Use ultrapure water to replace TP-CDs, with the other conditions being the same, and record the absorbance value as A_0. The $\bullet O_2^-$ radicals scavenging rate was calculated from the equation:

$$\text{H - RS (\%)} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (4)$$

EPR signal test of TP-CDs: Prepare a reaction solution by mixing 30% H_2O_2 and DMPO in a volume ratio of 10:1, and then irradiate it with an LED lamp (400-500 nm, 15 mW cm^{-2}) for 3 minutes to obtain the positive control group of •OH. Take 50 μL of

the positive control group solution, then add 50 μL of different concentrations (50-250 μg mL^{-1}) of TP-CDs solution, mix them evenly in the dark and react for 2 minutes, and then measure the EPR signal to evaluate the ability of TP-CDs to remove •OH free radicals. In addition, prepare 2 mL of different concentrations of TP-CDs aqueous solution (10-50 μg mL^{-1}) and 2 mL of DPPH ethanol solution (2 mmol L^{-1}), mix them thoroughly and shake well, and then leave them in the dark for 30 minutes to measure the EPR signal to evaluate the ability of TP-CDs to remove DPPH free radicals.

Cell culture. In this study, human normal liver cells (QSG7-701) were used as the model for cell experiments. The cells were placed in cell culture medium and cultured in a 37 °C incubator with 5% CO_2 for normal temperature and humidity. The cells were digested and passaged using trypsin, and the cell state was maintained during the experiment.

Cell viability assay (CCK-8 assay). QSG7701 cells were seeded into 96-well culture plates and incubated at 5% CO_2 for about 12 h to adhere to the wall. The culture medium was aspirated, and then a different concentration of TP-CDs solution diluted in culture medium was added to each well and incubated for another 24 h. Then, 110 μL of CCK-8/DMEM (1:10, v/v) mixture solution was added to each well in the dark and incubated for another 1 h. Finally, the absorbance at 450 nm was measured using an enzyme-linked immunosorbent assay (ELISA) reader. Each sample concentration was set up with 6 replicates in each 96-well plate, and the experiment was repeated three times.

Detection of Intracellular ROS. Using DCFH-DA as a fluorescent probe, QSG7701 cells were seeded at a density of 10^5 cells per well in a 6-well plate and incubated in a culture incubator for 24 h. After discarding the culture medium, the cells were washed with PBS twice. Among them, 4 wells were replaced with 300 μg mL^{-1} TP-CDs solution, and the other 2 wells were replaced with blank DMEM culture medium. After co-culturing for 0.5 hours, a blank well was set as a negative control, and the rest were added with an appropriate amount of H_2O_2 (the final concentration was 250 μmol mL^{-1}). The cells were co-cultured for another 4 hours. Then, the materials were removed and the cells were washed with PBS twice to remove any excess samples which did

not enter the cells. After that, 100 μL of DCFH-DA was added for 20 minutes to stain the cells. After staining, the cells were observed and images captured under an inverted fluorescence microscope.

Set blank, control, and experimental groups, with each group having 4 wells. After incubating QSG7701 cells in a 24-well plate at a constant temperature in a culture for 24 hours, discard the culture medium and add 300 μg mL^{-1} of TP-CDs to the experimental group. Control and blank groups were treated with an equal volume of culture medium. Incubate for 4 hours after washing with PBS 3 times, then add 600 μL of 250 μmol mL^{-1} H_2O_2 solution to the control and experimental groups, and an equal volume of culture medium to the blank group. Continue incubation for 2 hours after washing with PBS 3 times. Finally, collect the cell lysate, and determine the malondialdehyde (MDA), glutathione (GSH) content, and superoxide dismutase (SOD) activity of the cells.

Zebrafish cultivation and biotoxicity analysis. Zebrafish Cultivation: The zebrafish were purchased from Shanghai Feixi Biological Technology Co., Ltd. with the species being wild short-fin adult zebrafish. The water used in the system for raising zebrafish needs to be pre-treated with chlorine removal and oxygenation before use. In addition, activated carbon and biological cotton are placed in the tank to filter impurities in real time to prevent water pollution. The water temperature is set at 28 °C, and the daily light cycle is 14 hours of light and 10 hours of darkness. The fish are fed twice a day with granular, regular fish food and shelled surimi in the morning and evening.

Zebrafish egg laying: The day before egg laying, select life-active zebrafish with a ratio of male: female = 2: 1 and place them in a breeding box overnight, and use a partition to separate them. The next morning, the divider was removed and egg laying began, which lasted about 2 hours. The eggs were collected and the embryos were washed with Holt Buffer hatching solution (Holt Buffer solution consists of 3.5 g of sodium chloride, 0.05 g of potassium chloride, 0.1 g of calcium chloride, and 0.1 g of sodium bicarbonate dissolved in 1 L of deionized water, with 1 ml of 0.0001% methylene blue). Dilute TP-CDs with hatching liquid at different concentrations (50-250 μg mL^{-1}), then expose 1.5 h_{pf} (hour post fertilization) zebrafish embryos to

hatching liquid containing different concentrations of TP-CDs for cultivation, and the blank control group only adds hatching liquid and is continuously cultivated in a constant temperature incubator for 96 hours for subsequent evaluation of abnormal phenotypes of embryos. Replace 1/2 of the embryo hatching liquid every 24 hours, and remove dead embryos and young fish in time to avoid pollution.

Zebrafish embryotoxicity assay: The embryonic survival rate, heart rate, and body length of the zebrafish larvae at 96 h_{pf} were used to evaluate the biotoxicity of TP-CDs. The heart rate of zebrafish larvae was observed under an inverted microscope, and the number of heartbeats in 20 seconds of young fish is recorded. Each well had 6 larvae randomly selected, and the experiment was repeated three times. Body length was measured as the distance from the head to the tail tip, and 6 larvae were randomly selected from each well.

Zebrafish daily care and experimental procedures were carried out in accordance with the "Implementation Regulations for the Administration of Medical Animal Experiment" (Ministry of Health, China, No. 55, 1998). Animal welfare and experimental procedures were strictly reviewed and approved by the Animal Ethics Committee of Anhui University. (Approval No. IACUC(AHU)-2023-058)

Determination of antioxidant activity in the body. The oxidative stress-sensitive fluorescent probe DCFH-DA was used to detect the generation of ROS in zebrafish larvae. Expose 2-4 h_{pf} zebrafish larvae to a 5 mM H_2O_2 solution for 24 hours, and then rinse the larvae with the incubation solution after the experiment. Subsequently, expose the cleaned larvae to incubation solutions containing different concentrations of TP-CDs (50-250 μg mL^{-1}), with the control group being incubation solution without TP-CDs. After the culture ends (96 h_{pf}), rinse the larvae with E3 culture medium and then treat young zebrafish with DCFH-DA solution (20 μg mL^{-1}) at 28 °C in the dark for 1 hour. After incubation, the young fish were rinsed with ultrapure water three times to wash them thoroughly, followed by anesthetizing the young fish and observing the fluorescent state of the zebrafish young fish throughout their body.

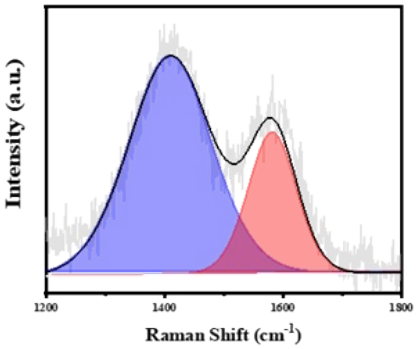

Figure S1. Raman spectrum of TP-CDs.

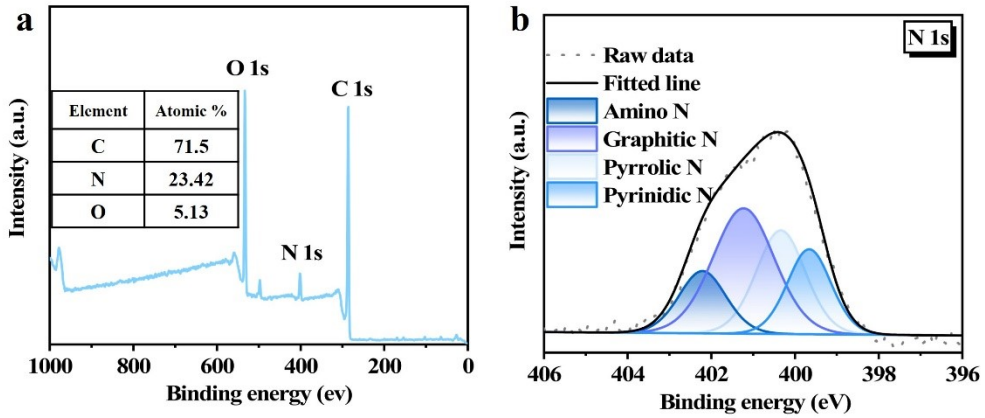

Figure S2. (a) XPS survey spectrum of TP-CDs (inset: atomic composition of TP-CDs). (b) High resolution N 1s XPS spectra of TP-CDs.

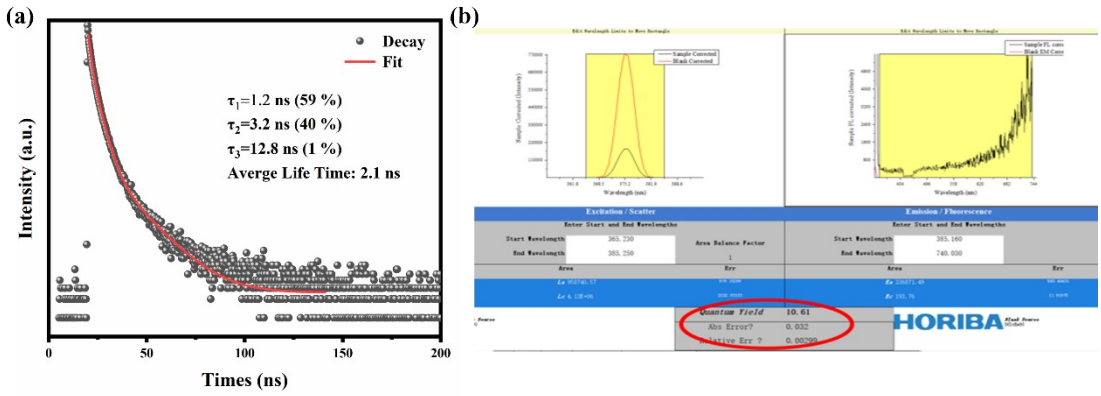

Figure S3. (a) Photoluminescence decay trace of TP-CDs recorded at 365 nm. (b) Absolute fluorescence quantum yield of TP-CDs.

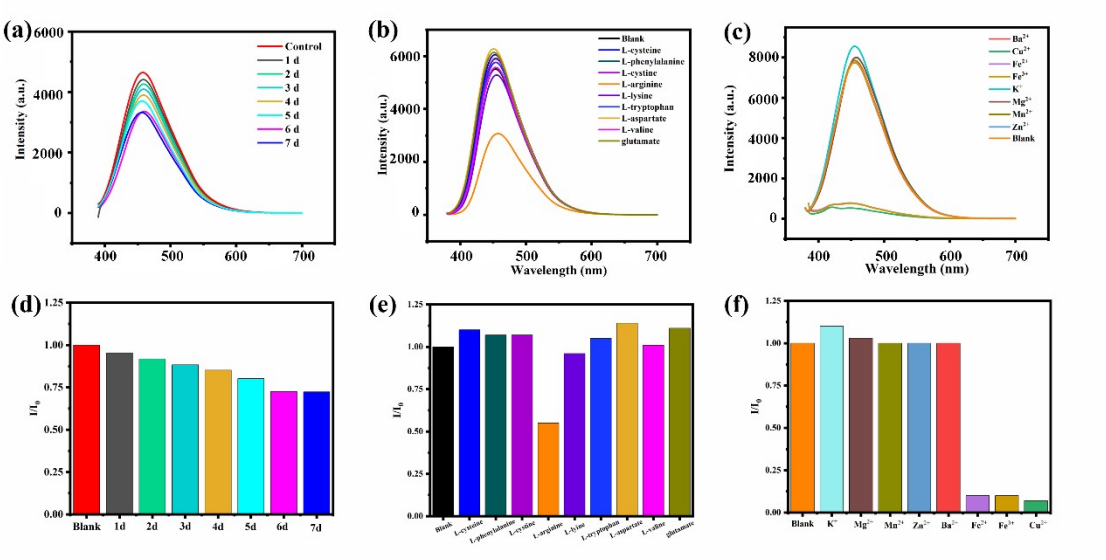

Figure S4. Stability and selectivity of TP-CDs in aqueous solution. (a) Time-dependent photoluminescence (PL) spectra of TP-CDs stored in the dark for 7 days (λ_{ex}=365 nm). (b) PL spectra of TP-CDs (250 μg mL^{-1}) incubated with 200 μM amino acids. (c) PL spectra of TP-CDs exposed to 200 μM metal ions. (d–f) Corresponding relative fluorescence intensity (I/I_0), where I_0 represents the initial fluorescence intensity of TP-CDs.

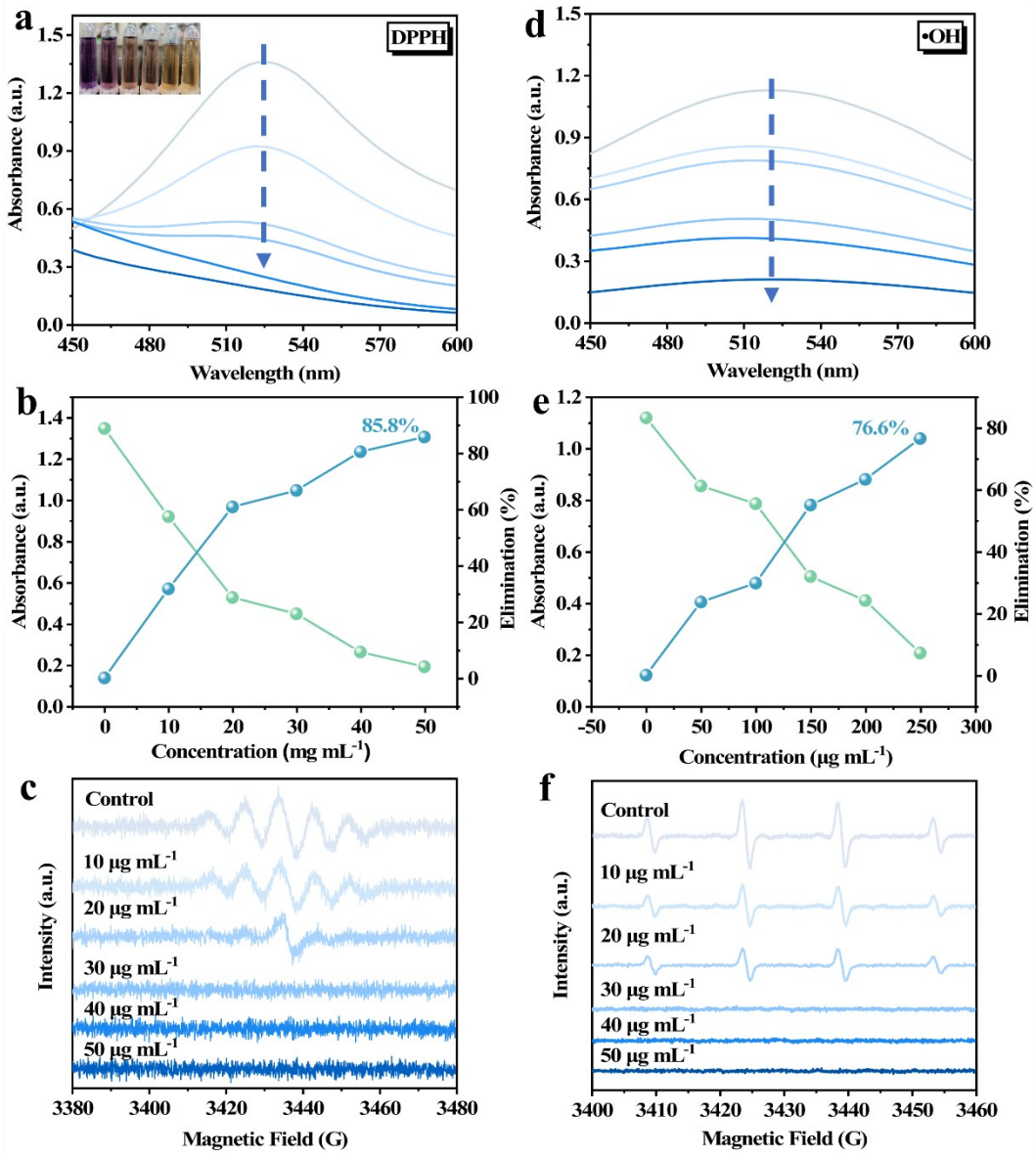

Figure S5. Antioxidant activity of TP-CDs. (a) UV-Vis absorption spectra of DPPH• solution (inset: visual color change from purple to orange after TP-CDs addition). (b) DPPH• scavenging efficiency at different TP-CDs concentrations (0-50 μg mL^{-1}). (c) EPR spectra of DPPH• species at different TP-CDs concentrations (0-50 μg mL^{-1}). (d) UV-Vis absorption spectra of hydroxyl radical (•OH) solution. (e) •OH scavenging efficiency at varying TP-CDs concentrations (0-250 μg mL^{-1}), (f) EPR spectra of •OH species at different TP-CDs concentrations (0-50 μg mL^{-1}).

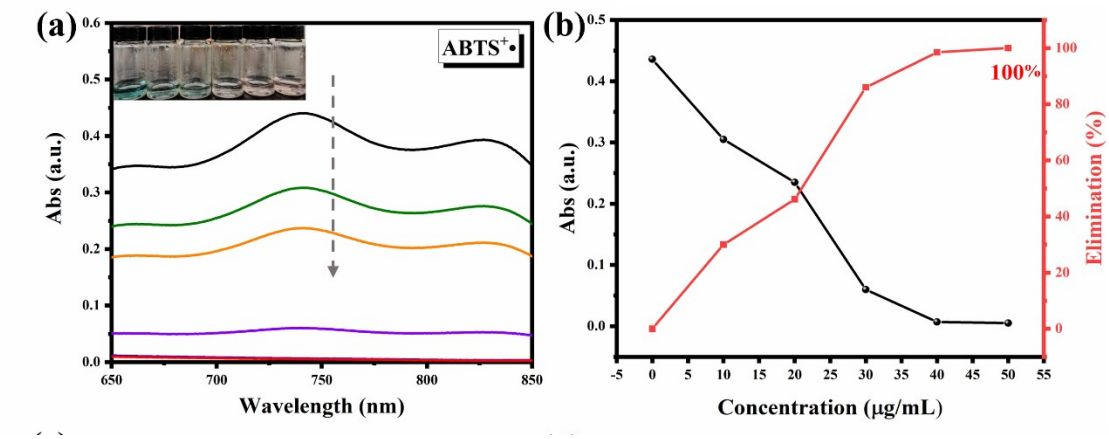

Figure S6. Antioxidant capacity of TP-CDs against $ABTS^{+}$• radicals. (a) UV-Vis absorption spectra of $ABTS^{+}$• solution (inset: photograph of $ABTS^{+}$• solution). (b) Scavenging rates of $ABTS^{+}$• by TP-CDs at varying concentrations.

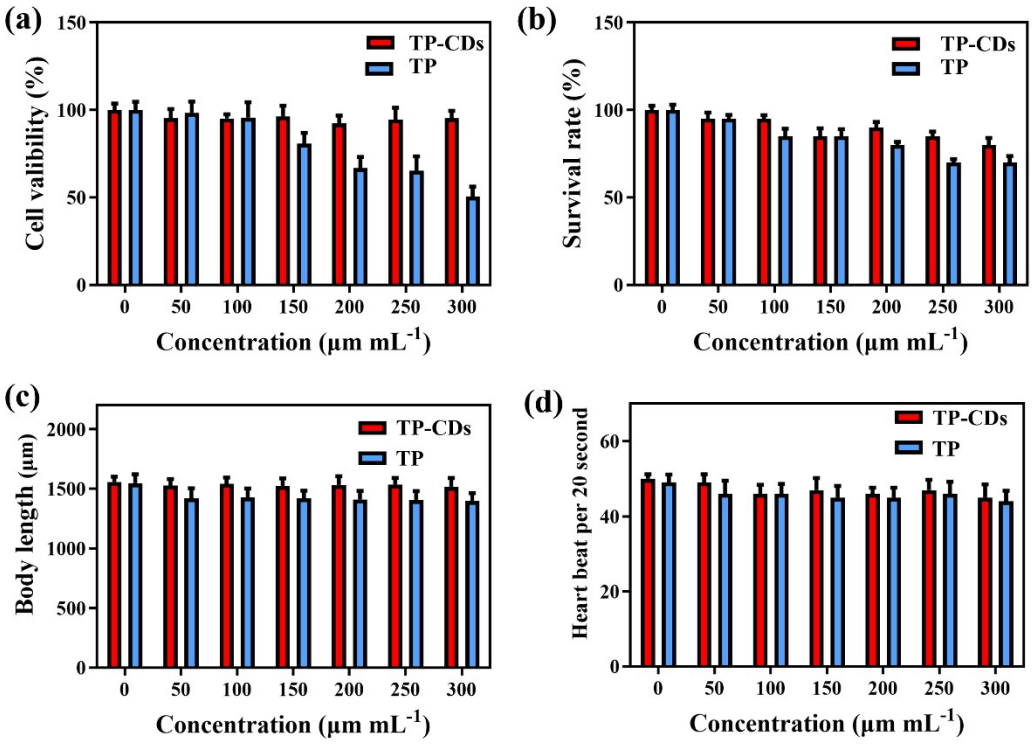

Figure S7. Biocompatibility assessment of TP-CDs and TP. (a) Viability of QSG7701 cells treated with TP-CDs or TP (50–300 μg mL^{-1}) for 24 h, measured by CCK-8 assay. (b-d) Toxicity evaluation in zebrafish embryos (96 hpf) exposed to TP-CDs/TP: (b) survival rate, (c) body length, and (d) heartbeat rate.

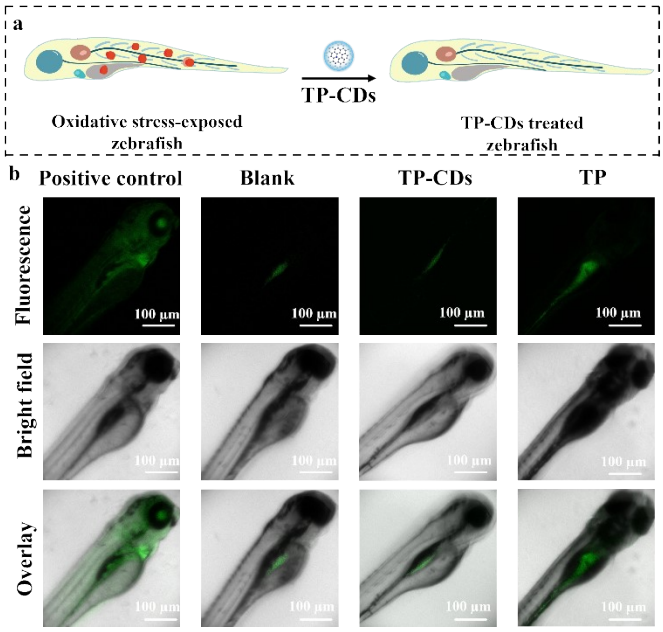

Figure S8. (a) Schematic diagram of TP-CDs therapeutic mechanism against oxidative stress in zebrafish. (b) Detection of intracellular ROS in 96 hpf zebrafish embryos via laser scanning confocal microscopy (LSCM). Embryos were stained with the DCFH-DA fluorescent probe (scale bar = 100 μm).