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Supplementary Materials for

Nanoparticle-induced neutrophil apoptosis increases survival in sepsis and alleviates neurological damage in stroke

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/11/eaax7964/DC1)

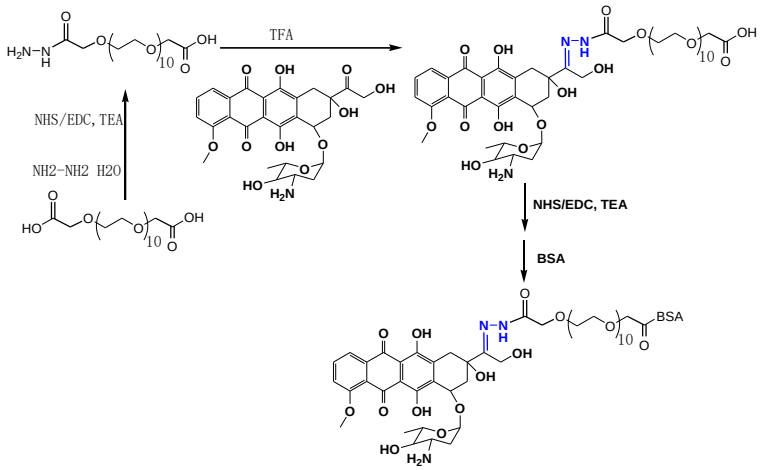

Fig. S1. Synthetic pathways of DOX-hyd-BSA.

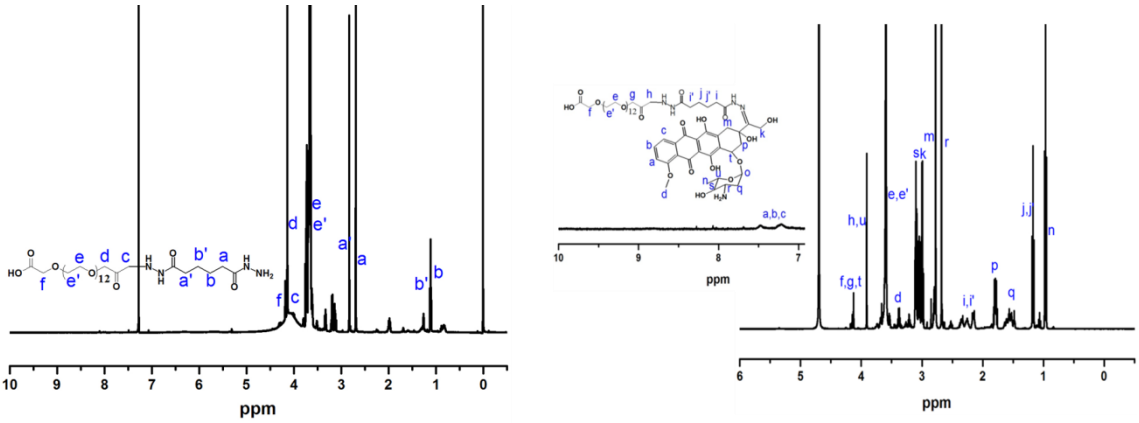

Fig. S2. ^{1}H NMR analysis. ^{1}H NMR spectra of HOOC-PEG-hyd in *d*-$CDCl_3$ (left) and HOOC-PEG-hyd-DOX in D_2O (right).

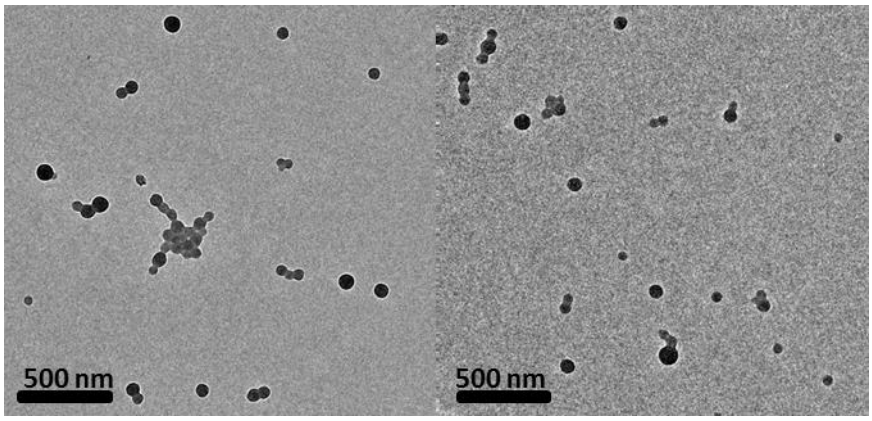

Fig. S3. TEM images of BSA NPs (left) and DOX-ab-BSA NPs (right).

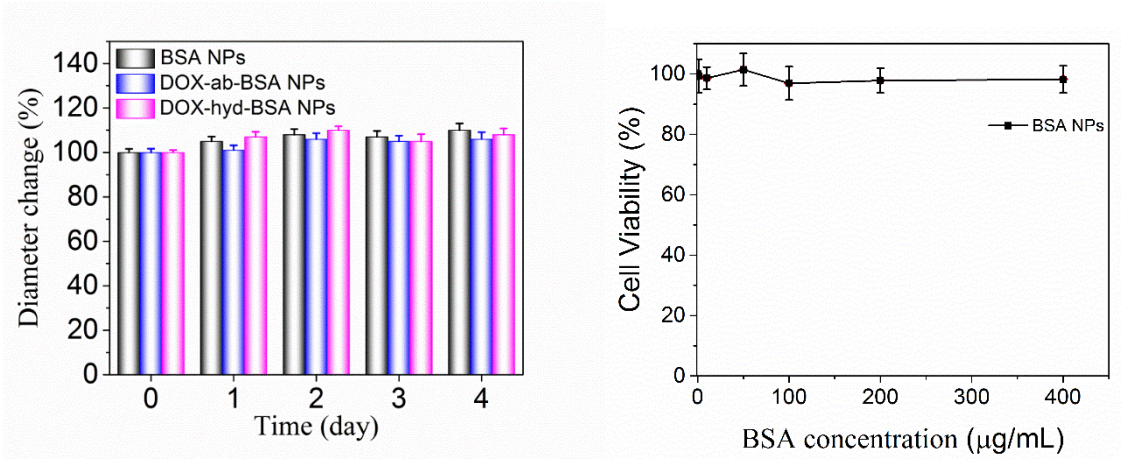

Fig. S4. The stability of BSA NPs in serum. Stability of BSA NPs, DOX-ab-BSA NPs, and DOX-hyd-BSA NPs in PBS with 20 % FBS at pH 7.4 (left). Cytotoxicity of BSA NPs to HL60 cells (right). The cells were incubated with BSA NPs for 24 h at different concentrations of NPs. Data are shown as mean ± s.d. (n = 6 independent experiments).

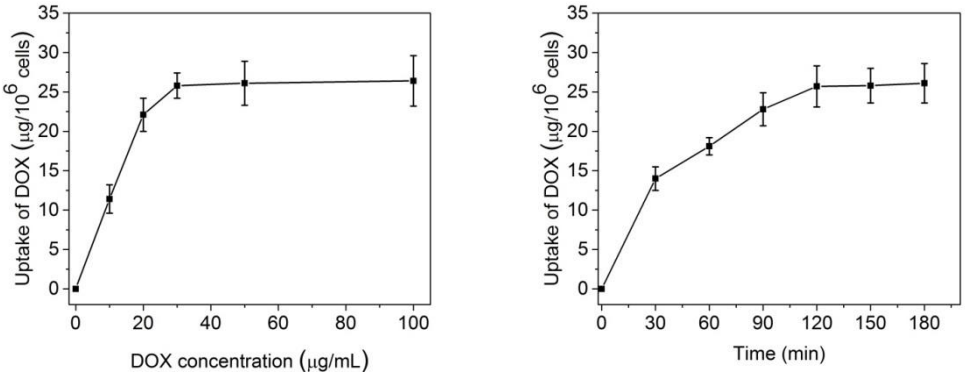

Fig. S5. Quantitative analysis of cellular uptake of DOX by differentiated HL-60 cells. Uptake of DOX by differentiated HL-60 cells after they (at 10^6 cells/ml) were incubated with DOX-hyd-BSA NPs at different concentrations of DOX for 2 hours (left). Time course of DOX uptake by differentiated HL60 cells after they (at 10^6 cells/ml) were incubated with DOX-hyd-BSA NPs (at 30 μg/ml of DOX) (right).

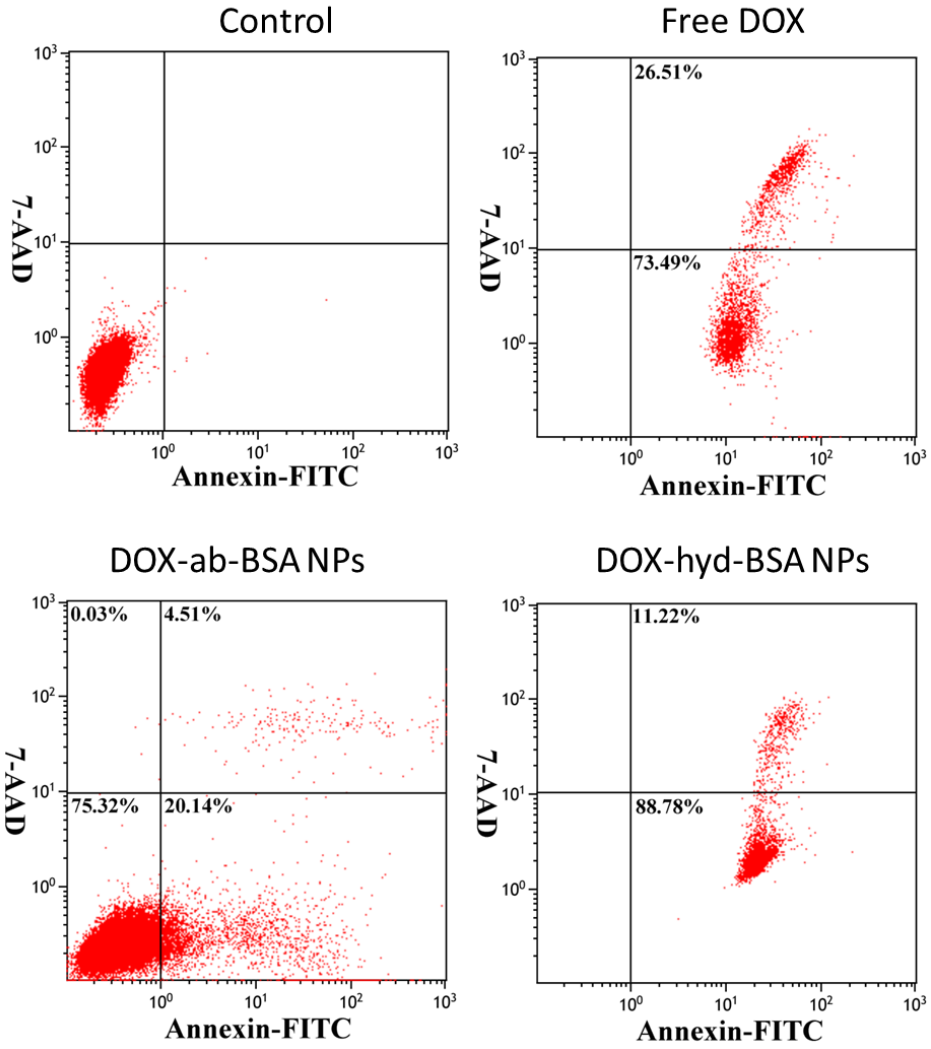

Fig. S6. Flow cytometric analysis on apoptosis of differentiated HL-60 cells 24 hours after treatments with free DOX (0.2 mg/kg), DOX-ab-BSA, or DOX-hyd-BSA NPs (equal to DOX of 0.2 mg/kg), respectively.

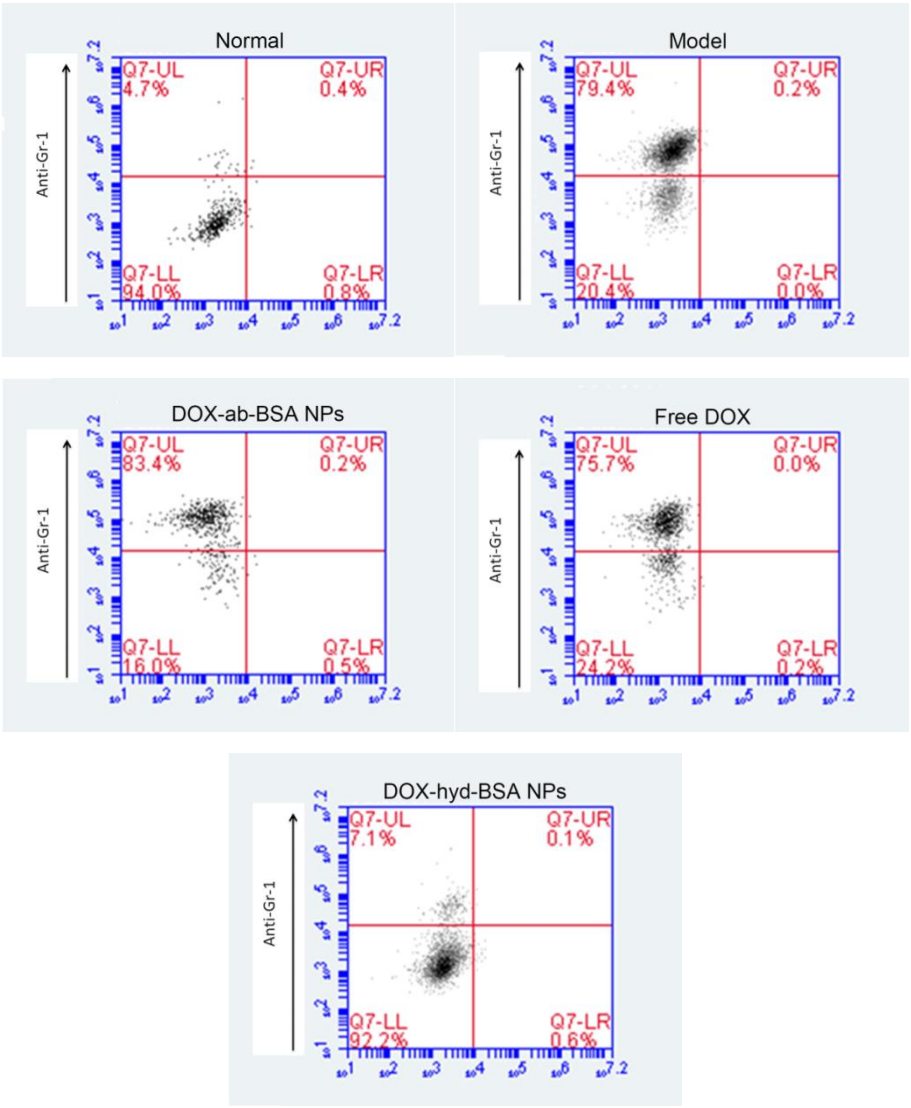

Fig. S7. Flow cytometric analysis of neutrophils in BALF. 4 h after *i.t.* LPS challenge (10 mg/kg), PBS, free DOX (0.2 mg/kg), DOX-ab-BSA or DOX-hyd-BSA NPs (DOX at 0.2 mg/kg) were *i.v.* administrated in mice, respectively. BALF was collected 20 h later, and then flow cytometry was performed. Normal represents healthy mice: Model represents LPS-challenged mice; Free DOX represents free DOX treatment; DOX-ab-BSA NPs represents DOX-ab-BSA NPs treatment; DOX-hyd-BSA NPs represents DOX-hyd-BSA NPs treatment. All data expressed as mean ± s.d. (3 mice per group).

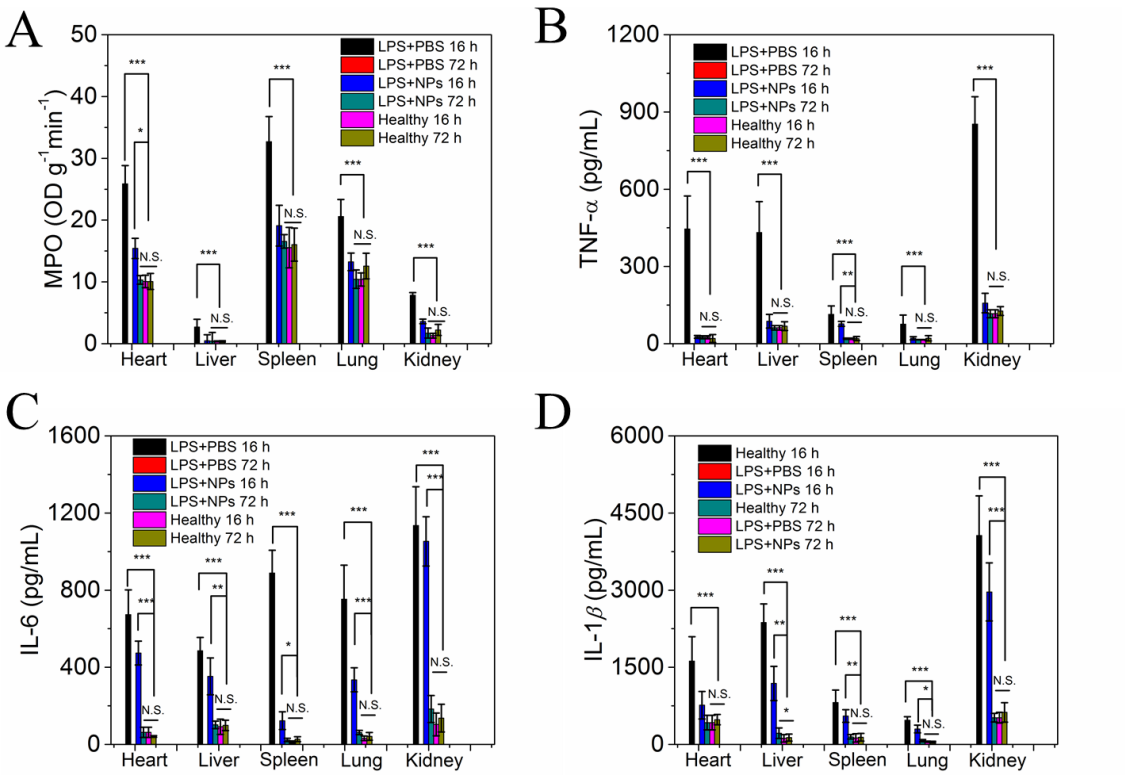

Fig. S8. DOX-hyd-BSA NPs decrease systemic inflammation in the mouse sepsis model. 4 h after (*i.p.*) LPS-challenge (50 mg/kg), the mice were treated with PBS or DOX-hyd-BSA NPs. MPO activity (a), TNF-*α* (b), IL-6 (c) and IL-1*ß* (d) in the major organs were measured at 16 h and 72 h post-LPS challenge. All data expressed as mean ± s.d. (5 mice per group at 16 h were used in the experiment, but 4 mice in NPs-treated group were used and all mice in PBS-treated group died at 72 h). Lung tissues were assessed after removal of BALF. * p value < 0.05, ** p value < 0.01, ***p value < 0.001.

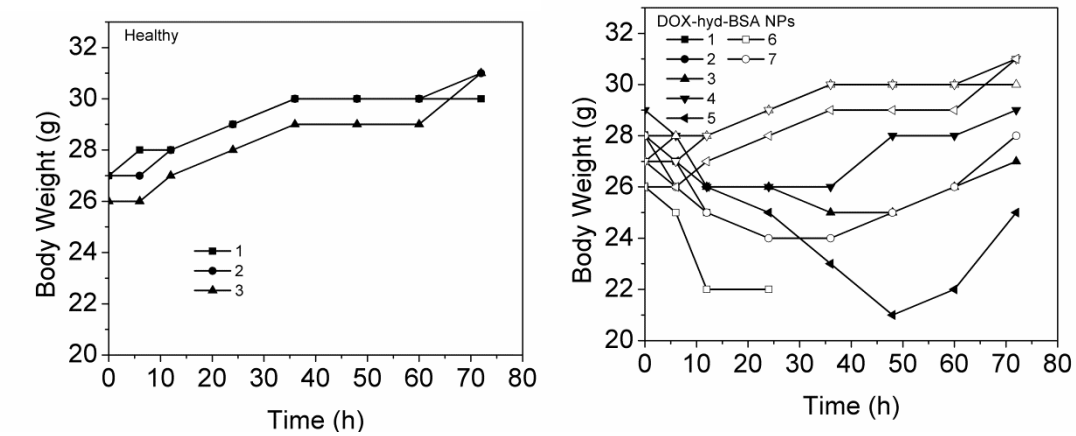

Fig. S9. Mouse body weights were monitored following the experiment as shown in Fig. 5M. 3 Mice (left) were the control group (healthy mice) and 7 mice (right) were the DOX-hyd-BSA NPs-treatment group (at 0.2 mg (DOX)/kg).

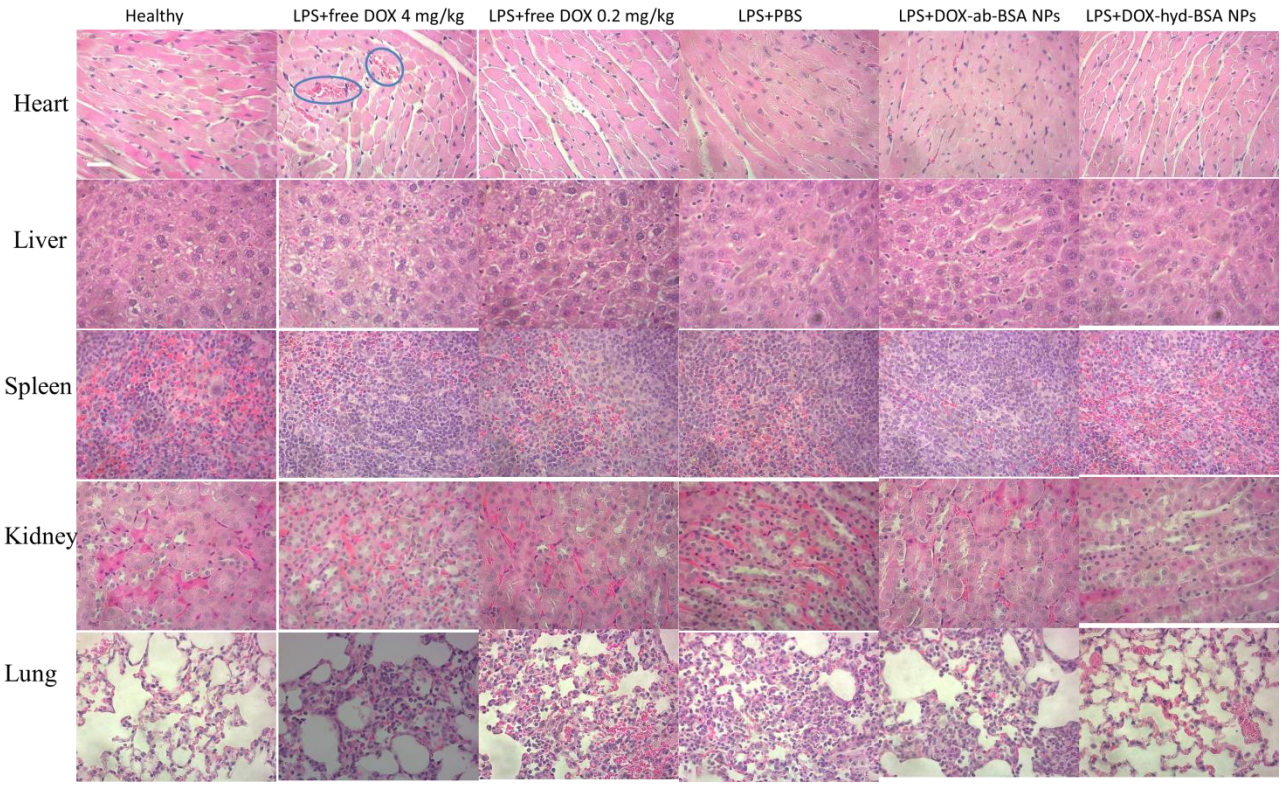

Fig. S10. Toxicity of DOX-conjugated BSA NPs evaluated by histological analysis. H&E-stained sections of major organs of healthy and LPS-challenged mice after treatments with PBS, free DOX (4 or 0.2 mg/kg), DOX-ab-BSA NPs, or DOX-hyd-BSA NPs (equal to 0.2 mg/kg at DOX). Scale bar, 20 μm. It was observed that DOX-conjugated NPs and free DOX (0.2 mg/kg DOX) did not show any noticeable signs of tissue or cellular damage. In contrast, the high dose of DOX (4.0 mg/kg) induced myocardial damage as shown by vacuolization and myofibril loss highlighted by the circles. Furthermore, the damage of liver, spleen, kidney and lung by DOX in 0.2-4.0 mg/kg was not observed.