

# Emodin Protects SH-SY5Y Cells Against Zinc-Induced Synaptic Impairment and Oxidative Stress Through ERK1/2 Pathway

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## Research Article

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# Abstract

**Background:** Zinc is an essential trace element important for the physiological function of the central nervous system. The abnormal accumulation of zinc inside neurons may induce mitochondrial dysfunction and oxidative stress which contributes to many brain diseases. We hypothesized that natural anthraquinone derivative emodin can protect against neurotoxicity induced by pathological concentrations of zinc via Extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway, and alleviate the oxidative stress and mitochondria dysfunction.

**Results:** Human neuroblastoma (SH-SY5Y cells) was treated with zinc sulfate and different concentrations of emodin, and was examined in the changes in the levels of ERK1/2 expressions, oxidative stress (DCFH-DA staining), mitochondrial function (JC-1 staining), and lipid peroxidation (4-hydroxynonenal staining), and DNA oxidation (8-Hydroxy-2-deoxyguanosine staining). Emodin ameliorated zinc-induced altered expression level of phosphorylated-ERK1/2 (not total-ERK1/2), and synaptic proteins (presynaptic SNAP25, synaptophysin and postsynaptic PSD95) in SH-SY5Y cells, in a dose-dependent manner. Moreover, emodin inhibited the generation of reactive oxygen substrates, oxidative stress, facilitated the collapse of mitochondrial membrane potential ( $\Delta\Psi_m$ ) in SH-SY5Y cells.

**Conclusion:** Our results indicated that emodin exerts neuroprotective effects against zinc by normalizing synaptic impairment via decreasing the phosphorylation of ERK1/2, reducing reactive oxygen substrates and protecting mitochondrial function.

## Background

Zinc is an essential trace element obtained from the diet that regulates the expression of many biological molecules and activation of signalling pathways. Zinc deficiency affects up to 2 billion people worldwide and has profound effects on immune and neurological system functions[1]. In the central nervous system zinc is one of the most abundant oligoelements, involves in the balance of excitatory and inhibitory signals of synapse[2]. During neuronal activity, zinc is released in the form of free ionic ( $Zn^{2+}$ ) from synaptic vesicles. Maintaining the homeostasis of zinc is thus essential for the physiological function of the brain[3]. Excessive zinc in the extracellular fluid has been shown to increase neurotoxicity, induce mitochondrial dysfunction, and oxidative stress. Abnormal increase in the levels of metal ions including  $Zn^{2+}$ , have been found in  $A\beta$ , form  $A\beta$ -Zn complexes[4], which results in a loss of zinc modulatory activity and cognitive deficits in animal models of Alzheimer's disease (AD)[5].

In addition, zinc accumulation has been shown to cause mitochondrial dysfunction and oxidative stress in AD[6, 7] as well as in ischemic stroke models[8, 9]. Mitochondrial  $Zn^{2+}$  accumulation is a possible trigger of hippocampal ischemic injury[8]. The synergistic interaction between  $Zn^{2+}$  and reactive oxygen species (ROS) has been shown to amplify the ischemic brain injury in rodent model[9] through direct ROS generation or through, mitochondrial  $Ca^{2+}$  uniporter[10]. Recent study indicates that zinc status introduce through Inflammation through NLRP3-mediated pathway[11].

Emodin, an anthraquinone derivative, is a major active ingredient of many herbs including *Rheum palmatum*, *Polygonum cuspidatum*, *Aloe Vera*, and *Cassia obtusifolia* etc[12]. Emodin shows neuroprotective, anti-inflammatory effects in animal models of cerebral ischemia stroke, traumatic brain injury, AD, and Parkinson's disease[13–15]. Different signaling pathways have been reported that mediate the effect of emodin such as the Nrf2, Phosphatidylinositol 3-Kinase/Beclin-1/B-Cell Lymphoma 2, and AMP-activated protein kinase signaling pathway[16–18]. Previous studies from our and other groups have reported that emodin demonstrates neuroprotective effect, and can inhibit the neurotoxic effect of NaF on SH-SY5Y cells via reducing reactive oxygen substrates (ROS) overproduction and oxidative stress [19].the underlying mechanism of emodin remains to be elucidated.

The Extracellular signal-regulated kinase 1/2 (ERK1/2) is activated by neurotrophins and other chemicals and plays an important role in differentiation, survival, structural plasticity, and long-term potentiation of neurons, as well as memory formation in animal models[20]; Emerging evidence suggests that ERK1/2 signaling pathway is implicated in a number of neurodegenerative diseases with oxidative stress[21]. Aberrant accumulation of activated ERK1/2 in neurons has been reported in AD brains[22, 23]. Here, we hypothesized that emodin can protect against neurotoxicity induced by pathological concentrations of zinc via ERK1/2 signaling pathway, and alleviate the oxidative stress and mitochondria dysfunction. We exposed human neuroblastoma SH-SY5Y cells to high dose of zinc sulfate, and assessed the effect of emodin on attenuating synaptic impairment, mitochondria function and oxidative stress damage.

## Methods

### Antibodies and reagents

Emodin with purity > 96% was purchased from the National Institutes for Food and Drug Control (China). Zinc sulfate was purchased from Sigma-Aldrich (USA). The total ERK (1:1000) and phosphorylated ERK (1:1000) antibodies were purchased from Cell Signaling Technology (Boston, Massachusetts). The PSD95 (1:1000), SNAP25 (1:1000), synaptophysin (1:1000), 4-Hydroxynonenal (4-HNE, 1:100), and 8-hydroxy-2'-deoxyguanosine (8-OHdG, 1:200) antibodies were bought from Abcam (USA). anti-mouse and anti-rabbit secondary antibodies (1:5000) were purchased from Bio-rad (USA). anti-rabbit DyLight-546 and anti-mouse DyLight 488 secondary antibodies were from Invitrogen (California, USA); Reactive oxygen species assay Kit with 2'-7'-dichlorofluorescein diacetate (DCFH-DA) for detecting intracellular hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and oxidative stress and mitochondrial membrane potential assay kit with JC-1 were bought from Beyotime (China).

### Cell culture and treatment

SH-SY5Y cells were cultured in Dulbecco's modified eagle medium (DMEM)/ nutrient mixture F-12 (F12) supplemented with 10% Fetal Bovine Serum (FBS) at 37°C. At 80% confluence, cells were seeded into 6-well culture plates. After serum deprivation overnight, different concentrations of emodin (10, 25, and 40µM) were applied to pre-treat the cells for 2 hours in serum-free media. 300µM zinc sulfate was applied for an additional 4 hours.

# Cell extraction and western blotting analysis

After being washed with cold Phosphate-buffered saline (PBS, pH7.4), the cells were lysed with 100 $\mu$ L preheated sodium dodecyl sulfate (SDS) sample buffer and scraped with a rubber policeman. The extract was placed in an Eppendorf tube and boiled for 5min, cooled on ice immediately. cell lysates (10 $\mu$ L) were run on TGX Stain-Free-FastCast Acrylamide gels (Bio-Rad). The gels were Stain-Free activated for 45 sec and imaged utilizing the ChemiDoc MP imaging system (Bio-Rad, US). The separated proteins were transferred to the polyvinylidene difluoride (PVDF) membranes utilizing the Trans-Blot Turbo Transfer System (Bio-Rad), and the membranes were blocked with 5% non-fat dried milk in Tris-buffered saline (TBS) for 1hour. The block solution was then removed from the incubation solution and specific antibody was added for overnight incubation at 4°C. Immunoblot images were obtained by ChemiDoc MP imaging system. The stain-free signals and immunoblots were evaluated using the ImageLab software (Bio-Rad). Protein expression levels were normalized to the Stain-Free total protein lane density obtained from each gel .

## ROS measurements

For detecting intracellular H<sub>2</sub>O<sub>2</sub> and oxidative stress, DCFH-DA staining was used. Cells in 24-well plates at a density of 5 $\times$ 10<sup>4</sup>/well were incubated in control media or 300 $\mu$ M zinc sulfate for 4hours with or without 2hours pre-treatment of emodin (10,25, and 40 $\mu$ M). DCFH-DA staining was used to determine changes in intracellular reactive oxygen species (ROS) levels according to the manufacturers' instructions. Briefly, the cells were maintained in DCFH-DA at 37°C for 20 minutes, then removed the staining solution and the cells were washed with PBS pH7.4 twice gently. Image analysis was performed using a confocal microscope (Leica SP8, Leica, Germany) and the Image J 1.49V software.

## Mitochondrial membrane potential detection

Mitochondrial membrane potential was measured using a mitochondrial membrane potential probe JC-1 staining dye in SH-SY5Y cells. Briefly, cells in 24-well plates were treated with 300 $\mu$ M zinc sulfate for 4hours with or without pretreatment of emodin (10, 25, and 40 $\mu$ M) for 2hours. After adding F12 medium/JC-1 working solution (1:1), cells were maintained in a CO<sub>2</sub> incubator for 20minutes. The staining solution was removed, then the cells were washed twice gently with JC-1 staining buffer. The fluorescence was detected with confocal microscopy (Leica SP8). and the pictures were captured in five fields of each sample in triplicate. The  $\Delta\Psi_m$  of SH-SY5Y cells was represented by the ratio of monomeric JC-1 to aggregated JC-1.

## Immunofluorescence staining

SH-SY5Y cells grown on coverslips were treated with 300 $\mu$ M zinc-sulfate for 4hours with or without pretreatment of emodin (10, 25, and 40 $\mu$ M) for 2hours. The cells were washed with PBS pH7.4 and fixed with 4 % paraformaldehyde for 20 minutes. After permeated with TBS containing 0.1% Triton X-100 for 5 minutes, the cells were blocked with 5% bovine serum albumin (BSA) in TBS for 30 minutes. The cells were then incubated with primary antibodies against 4-HNE (1:100) or 8-OHdG (1:200) overnight at 4°C,

anti-rabbit DyLight-546 and anti-mouse DyLight-488 secondary antibodies (1:200) were incubated for 1 hour in the dark. Nuclei were counter-stained with 4',6-diamidino-2-phenylindole (DAPI). Image analysis was performed using a confocal microscope (Leica SP8) and the Image J 1.49V (NIH, US).

## Statistical analysis

One-way ANOVA and Bonferroni post-hoc analysis was used for group comparisons using GraphPad Prism 8 (GraphPad Prism). Data was presented as the mean  $\pm$  standard error (SEM) (n = 3). Significant was set at  $p < 0.05$ .

## Results

### Emodin attenuated the phosphorylation of ERK1/2 in SH-SY5Y cells

We hypothesized that the emodin effect on the protection of SH-SY5Y cells against zinc via ERK1/2 pathway. We examined the phosphorylated and total expression levels of ERK in SH-SY5Y cell lysate by using western blotting. 300  $\mu$ M zinc sulfate significantly increased the expression levels of phosphorylated ERK1/2 ( $p = 0.03$ ) but not the total ERK1/2 in the treated SH-SY5Y cells compared to control group (Fig. 1a-d). A dose-dependent effect of emodin pre-treatment on phosphorylated ERK1/2 was observed: pre-treatment with emodin at 40 $\mu$ M (but not at 10 or 25 $\mu$ M dose) totally abolished the increase in the levels of phosphorylated ERK1/2 induced by zinc sulfate ( $p = 0.02$ , vs. Zn-treated group) (Fig. 1a, c). No difference was observed in the level of total ERK1/2 by pre-treatment with emodin at different dose.

### Emodin ameliorated synaptic impairment in zinc sulfate-induced SH-SY5Y cells

To investigate the possible effects of emodin on synaptic function related proteins that affected by the presence of zinc, we examine the changes in the levels of presynaptic terminal proteins (SNAP25 and synaptophysin) and postsynaptic density protein (PSD95). We found that 300 $\mu$ M zinc sulfate treatment significantly reduced the expression levels of presynaptic SNAP25 ( $p = 0.04$ ), synaptophysin ( $p = 0.01$ ) and postsynaptic PSD95 (approximately 50%,  $p = 0.04$ ) in the SH-SY5Y cells compared to control group, respectively (Fig. 2c, d,e). A dose-dependent protection effect of emodin pre-treatment against synaptic damage caused by zinc treatment was observed: Pre-treatment with emodin at 40 $\mu$ M (but not at 10 or 25  $\mu$ M) significantly ameliorated the reduction in the levels of synaptophysin ( $p = 0.04$ ), and SNAP25 ( $p = 0.03$ ) induced by zinc sulfate compared to zinc-treated control group (Fig. 2c, d). Pre-treatment with emodin at 10 $\mu$ M (but not at 25 or 40 $\mu$ M) significantly ameliorated the zinc-induced reduction in the levels of PSD95 by approximately 50% ( $p = 0.01$ ), compared to zinc-treated control group (Fig. 2e).

# Emodin inhibited ROS generation in zinc-induced SH-SY5Y cells

To detect the zinc-induced oxidative stress and the antioxidant effect of emodin, we assessed ROS production by using DCFH-DA fluorescent staining in SH-SY5Y cells. 300 $\mu$ M zinc sulfate significantly increased the intracellular level of ROS indicated by DCFH-DA fluorescent intensity in the treated SH-SY5Y cells compared to control group ( $p = 0.009$ ). Pre-treatment with emodin showed a dose-dependent effect: at 40  $\mu$ M (but not at 10 or 25 $\mu$ M) significantly decreased the zinc-induced increase in DCFH-DA fluorescent intensity by approximately 50% ( $p = 0.04$  vs. zinc-treated control group) (Fig. 3a,b).

# Emodin reestablished the loss of mitochondrial membrane potential ( $\Delta\Psi_m$ ) in zinc-induced SH-SY5Y cells

Next we examined whether emodin can ameliorate the mitochondrial dysfunction induced by zinc treatment. We measured the mitochondrial membrane potential  $\Delta\Psi_m$  in zinc-treated SH-SY5Y cells by using JC-1 staining. JC-1 aggregate in the normal mitochondrial matrix emits red fluorescence. Green fluorescence is produced in JC-1 staining of cells following loss of  $\Delta\Psi_m$ . The ratio of green and red fluorescence was used to indicate the toxicity introduced by zinc in mitochondria and the protective effect of emodin. In control groups, JC-1 aggregated in mitochondria and the green/red fluorescence intensity ratio was  $0.77 \pm 0.11$ . Exposure of SH-SY5Y cells to 300 $\mu$ M zinc sulfate for 4 hours increase green/red fluorescence intensity ratio to  $1.85 \pm 0.14$  ( $p = 0.0008$ , vs. control), implying the collapse of  $\Delta\Psi_m$  (Fig. 4). In the presence of increasing concentrations of emodin, the green fluorescence gradually weakened, while the red fluorescence remained the same, resulting in a reduced green/red fluorescence intensity ratio. The green/red fluorescence intensity ratio from JC-1 staining was  $1.57 \pm 0.03$  at 25 $\mu$ M ( $p = 0.04$ , vs. zinc-treated control group), and  $1.44 \pm 0.03$  at 40 $\mu$ M ( $p = 0.004$ , vs. zinc-treated control group), implying the reestablishment of  $\Delta\Psi_m$ .

# Emodin attenuated oxidative stress damage in zinc-induced SH-SY5Y cells

To measure the potential oxidative damage following zinc-treatment, products of oxidative stress such as 4-hydroxynonenal (4-HNE) from lipid peroxidation and 8-hydroxy-2'-deoxyguanosine (8-OHdG) from DNA oxidation were analyzed via immunostaining (Figs. 5 and 6). The fluorescence intensity of 4-HNE and 8-OHdG staining in the SH-SY5Y cells were quantified among the control, zinc-treated group and emodin pretreated zinc group. 300 $\mu$ M high dose of zinc significantly increased the intensity of 4-HNE ( $p = 0.0002$ , vs. controls). Pre-treatment with emodin at 25 and 40 $\mu$ M (but not 10 $\mu$ M) significantly reduced the zinc-induced increase in the fluorescence intensity of 4-HNE in SH-SY5Y cells by approximately 50% ( $p = 0.006$ , vs. zinc treated control group) and 70% ( $p = 0.0006$ , vs. zinc treated control group), respectively, in a dose-dependent manner (Fig. 5a, b). The effect of emodin treatment at 40 $\mu$ M (but not 25 $\mu$ M) was significantly higher compared to that that 10 $\mu$ M dose ( $p = 0.005$ )

High dose of zinc significantly increased the intensity of 8-OHdG ( $p = 0.002$ , compared to the controls. Pre-treatment with emodin at 25, 40 $\mu$ M (but not 10 $\mu$ M) significantly reduced the zinc-induced increase in the fluorescence intensity of 8-OHdG in SH-SY5Y cells by approximately 68% ( $p = 0.005$ , vs. zinc treated control group) and 74% ( $p = 0.002$ , vs. zinc treated control group), respectively, with a dose-dependent manner (Fig. 6a, b). The effects of emodin treatment at 25 and 40 $\mu$ M were significantly greater compared to that that 10 $\mu$ M dose ( $p = 0.03$  and  $p = 0.01$ , respectively).

## Discussion

Our results revealed that treatment with pathological dose (300 $\mu$ M) of zinc cause increase p-ERK1/2 expression, synaptic impairment, mitochondrial dysfunction and oxidative stress in SH-SY5Y cells. Pretreatment with emodin showed a protective effect against these changes to induced by 300 $\mu$ M zinc in SH-SY5Y cells in a dose dependent manner. The concentration of zinc is about 150 $\mu$ M in the brain from healthy controls, whereas the zinc concentrations in A $\beta$  plaques from brain of AD patients are increased by 3-fold compared with controls (reaching more than 400 $\mu$ M) [24]. Thus in the present study we used 300 $\mu$ M zinc to mimic the concentration under pathological conditions, which is characterized in detail in previous study[25].

Zinc is also involved in several important signaling pathways, inhibits the proteinphosphatase 2A (PP2A) [26] and activates Glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ), ERK1/2 and c-Jun N-terminal kinase (JNK) [27–29]. Here we found that zinc-treatment increased the expression of p-ERK but not the total ERK, which was entirely abolished by pretreatment with emodin. This implied that emodin may have potential as a therapeutic drug for decreasing high zinc level-induced neurotoxicity via suppressing p-ERK1/2. *In vivo* studies in animal models have shown that the chelator-driven perturbation of Zn<sup>2+</sup> in brain decreased the levels of brain-derived neurotrophic factor, PSD95, and dendritic spine density[30]. Reduced levels of synaptic-related proteins indicated a declined synaptic function in response to zinc exposure. our results indicated a protective effect exerted by emodin on synaptic impairment, in line with earlier study[19]. However the optimal dosage of emodin against pre- and post- synaptic protein differs in our observation. The highest dose 40 $\mu$ M of emodin significantly alleviated the zinc induced reduction in presynaptic proteins, but did not ameliorated the reduction in post-synaptic PSD95.

In addition to affecting synaptic function, zinc has been shown linked with oxidative stress as well as neuroinflammation that are important in AD disease development[11]. In the present study, pretreatment of SH-SY5Y cells by emodin suppressed the zinc-induced ROS generation, reduced the formation of lipid peroxidation product (4-HNE), and decreased the level of DNA damage marker (8-OHdG). These results suggest that emodin has an antioxidant effect again zinc-induced ROS generation. The decrease of mitochondrial  $\Delta\Psi_m$  is a sign of early apoptosis. In the present study, Zinc-induced ROS generation resulted in a dissipation of  $\Delta\Psi_m$ , indicating mitochondria dysfunction. The Zinc-induced neurotoxicity has been reported associating with ROS generation[31]. The mitochondrial respiratory chain which regulates apoptosis is susceptible to Zn<sup>2+</sup>. Accumulation of Zn<sup>2+</sup> further induces mitochondrial dysfunction and oxidative stress[32]. Free Zn<sup>2+</sup> induces mitochondrial permeability transition and

generation of ROS[33]. ROS can, in turn, increase the detrimental amount of zinc release from metallothioneins[34], forming a vicious circle. Liu et al have previously reported that emodin inhibited the influx of  $Zn^{2+}$  (200 $\mu$ M) into neuronal cells, thereby preventing the consumption of NAD<sup>+</sup> and ATP, inhibiting the generation of ROS and ER stress, and inactivating AMPK/ACC signaling pathways to exert neuroprotective effects[35].

## Conclusions

In conclusion, we demonstrated a protective effect of emodin against zinc-induced neurotoxicity in SH-SY5Y cell line. Emodin pretreatment normalized the zinc-induced synaptic impairment, reduced the oxidative stress through the inhibition of phosphor-ERK1/2 signal pathway, and inhibited mitochondrial dissipation in a dose-dependent manner. Further studies are needed to investigate neuroprotective effects of emodin and pathways involved in animal models with pathological accumulation of zinc in brain.

## Abbreviations

ERK1/2: Extracellular signal-regulated kinase 1/2;  $\Delta\Psi_m$ : Mitochondrial membrane potential; AD: Alzheimer's disease; ROS: Reactive oxygen species; 4-HNE:4-hydroxynonenal; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; PP2A: Proteinphosphatase 2A; GSK-3 $\beta$ : Glycogen synthase kinase-3 $\beta$ ; JNK: c-Jun N-terminal kinase; FBS: Fetal Bovine Serum; PBS: Phosphate-buffered saline; SDS: Sodium dodecyl sulfate; PVDF: polyvinylidene difluoride; TBS: Tris-buffered saline; DAPI: 4',6-diamidino-2-phenylindole.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

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

### Competing interests

The authors declare no conflict of interest.

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### Authors' contributions

QC and ZT contributed to the conception and study design. QC, CC and FC performed the experiments. CC, FC, YT and YY contributed to data collection and data analysis. QC, CC, RN and ZT wrote the manuscript. All authors approved the final manuscript before submission.

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Not applicable.

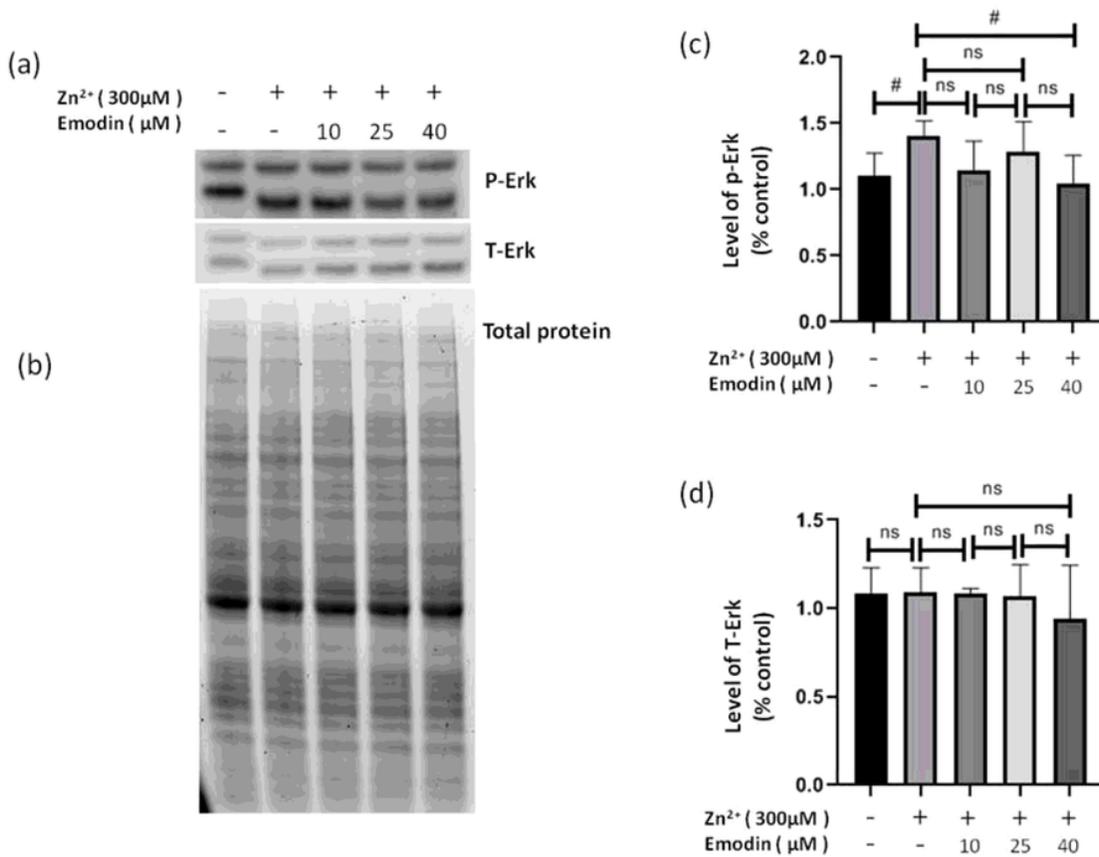
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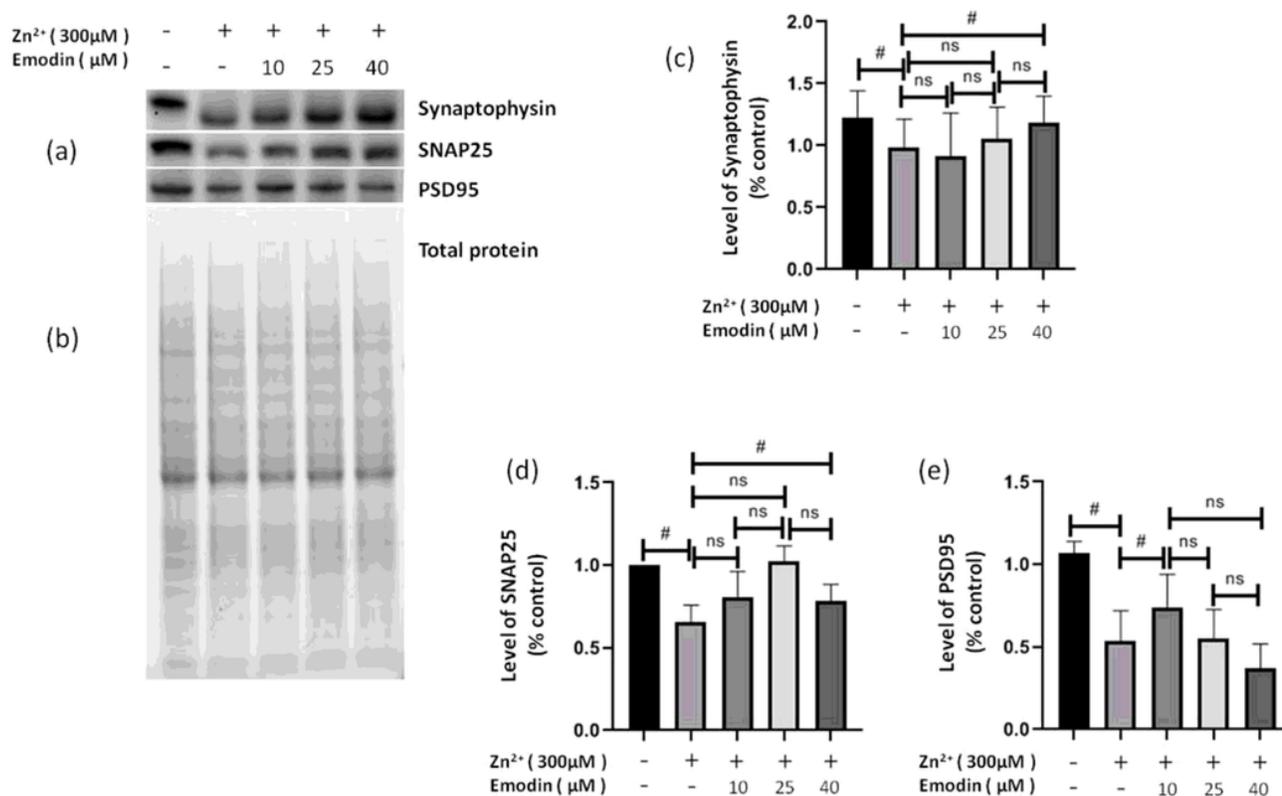
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## Figures



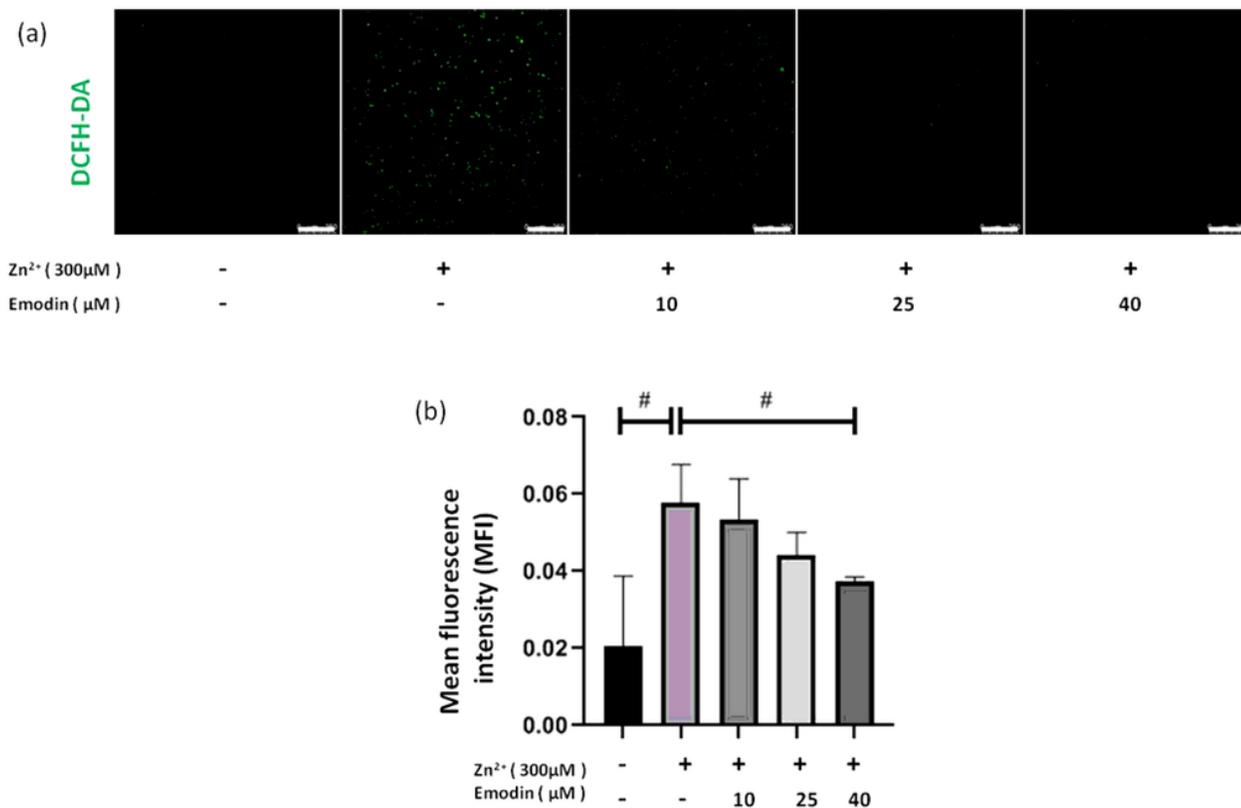
**Figure 1**

Emodin reversed the activation of the phosphorylated ERK1/2 in SH-SY5Y cells exposed to 300 μM zinc sulfate. (a) Immunoblot image of phosphorylated ERK1/2 (p-Erk) and total ERK1/2 (t-ERK) in SH-SY5Y cells; (b) Stain-Free image of SH-SY5Y cell lysate; (c-d) Immunoblot analysis of phosphorylated ERK1/2 and total ERK1/2. Different concentrations of emodin (10, 25, and 40 μM) was applied. One-way ANOVA and Bonferroni post-hoc analysis, #*p* < 0.05; ns, not significant. Data as mean ± SEM.



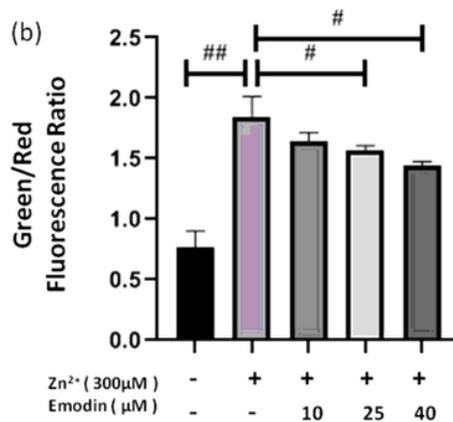
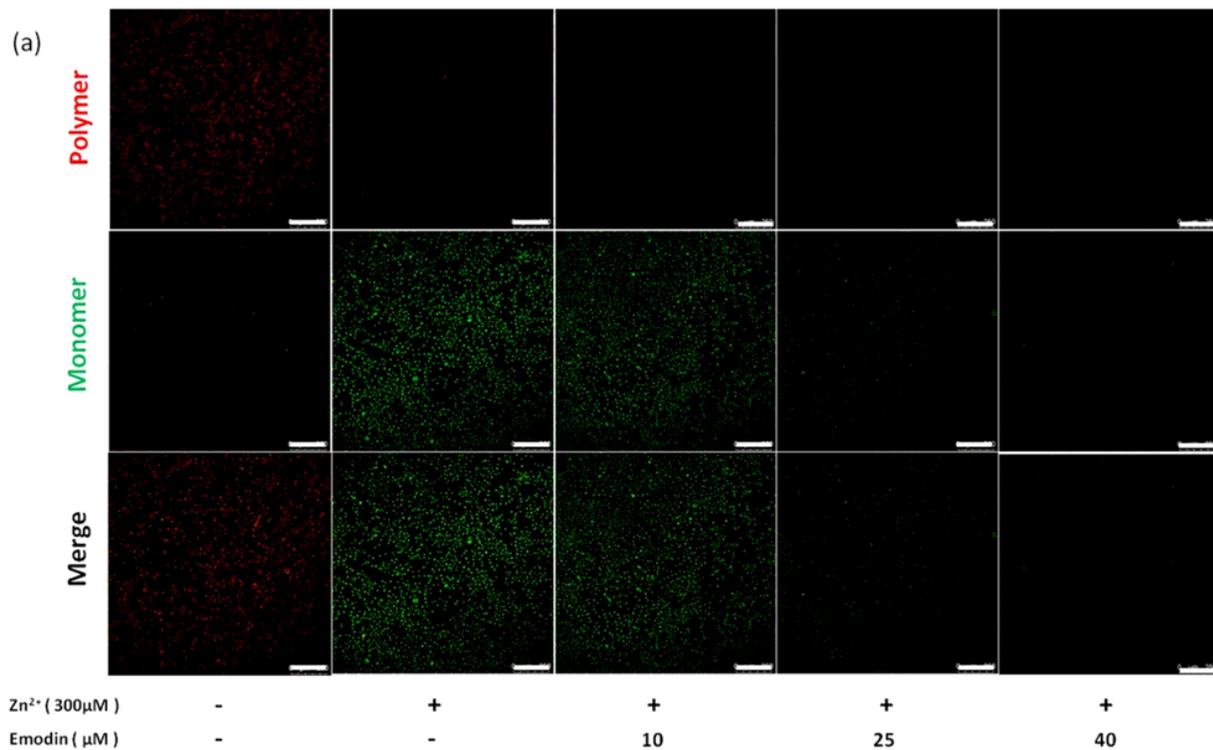
**Figure 2**

The dose-dependent effect of emodin on synapse-related proteins in SH-SY5Y cells exposed to 300 μM zinc sulfate. (a) Immunoblots of Synaptophysin, SNAP25, and PSD95 in SH-SY5Y cells; (B) Stain-Free image of SH-SY5Y cell lysate; (c-e) Quantitation of the immunoblots. Different concentrations of emodin (10, 25, and 40 μM) was applied. One-way ANOVA and Bonferroni post-hoc analysis, #p < 0.05; ns, not significant; Data as mean ± SEM.



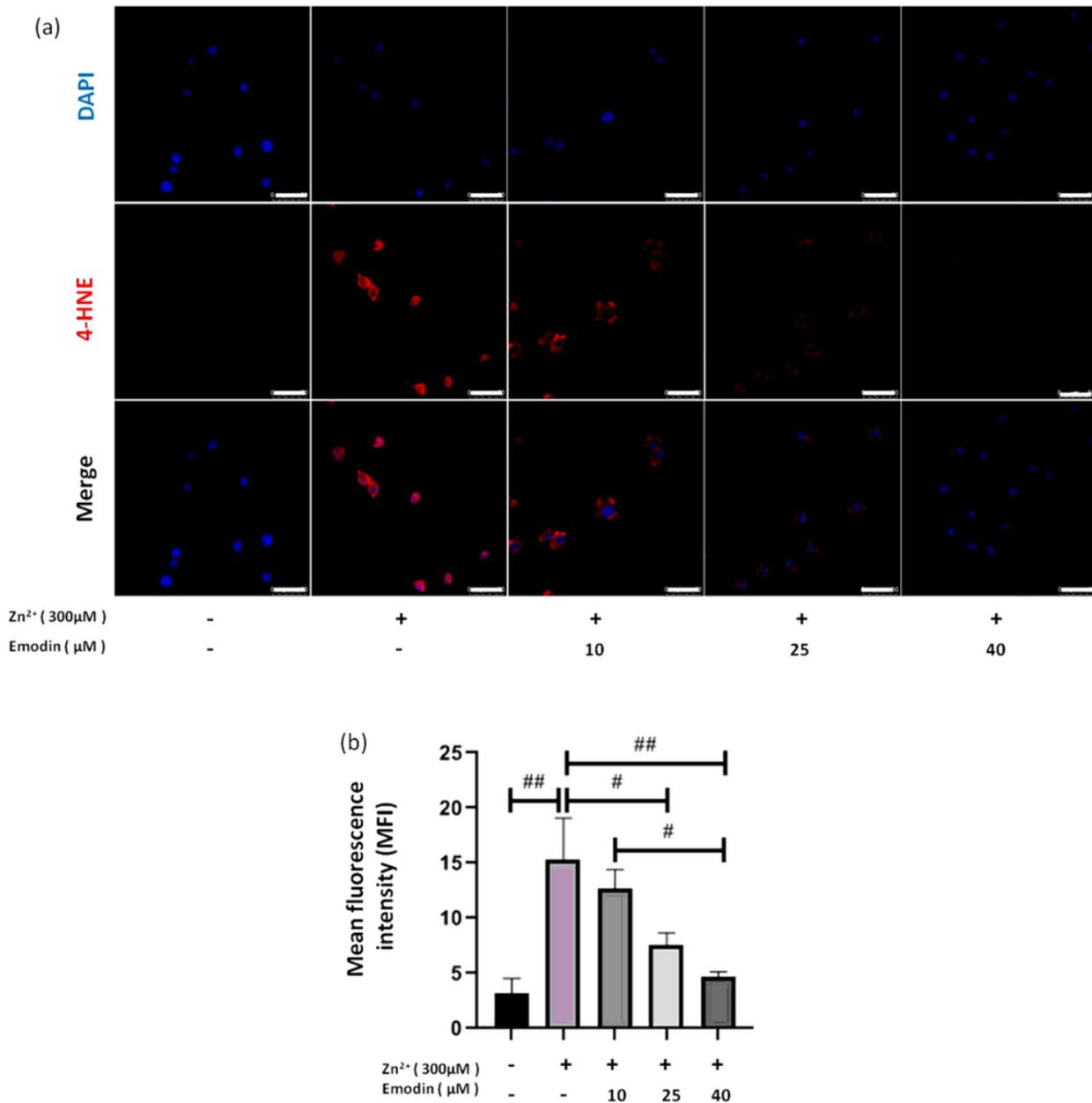
**Figure 3**

Emodin decreased the zinc-induced reactive oxygen species in SH-SY5Y cells. (a) fluorescence staining of DCFH-DA fluorescent staining in SH-SY5Y cells, from left to right are untreated cells, 300 μM zinc sulfate-treated cells without emodin pretreatment (10, 25, and 40 μM). scalebar = 250 μm. (b) the mean fluorescent intensity analysis. One-way ANOVA and Bonferroni post-hoc analysis, #p < 0.05; ns, not significant. Data as mean ± SEM.



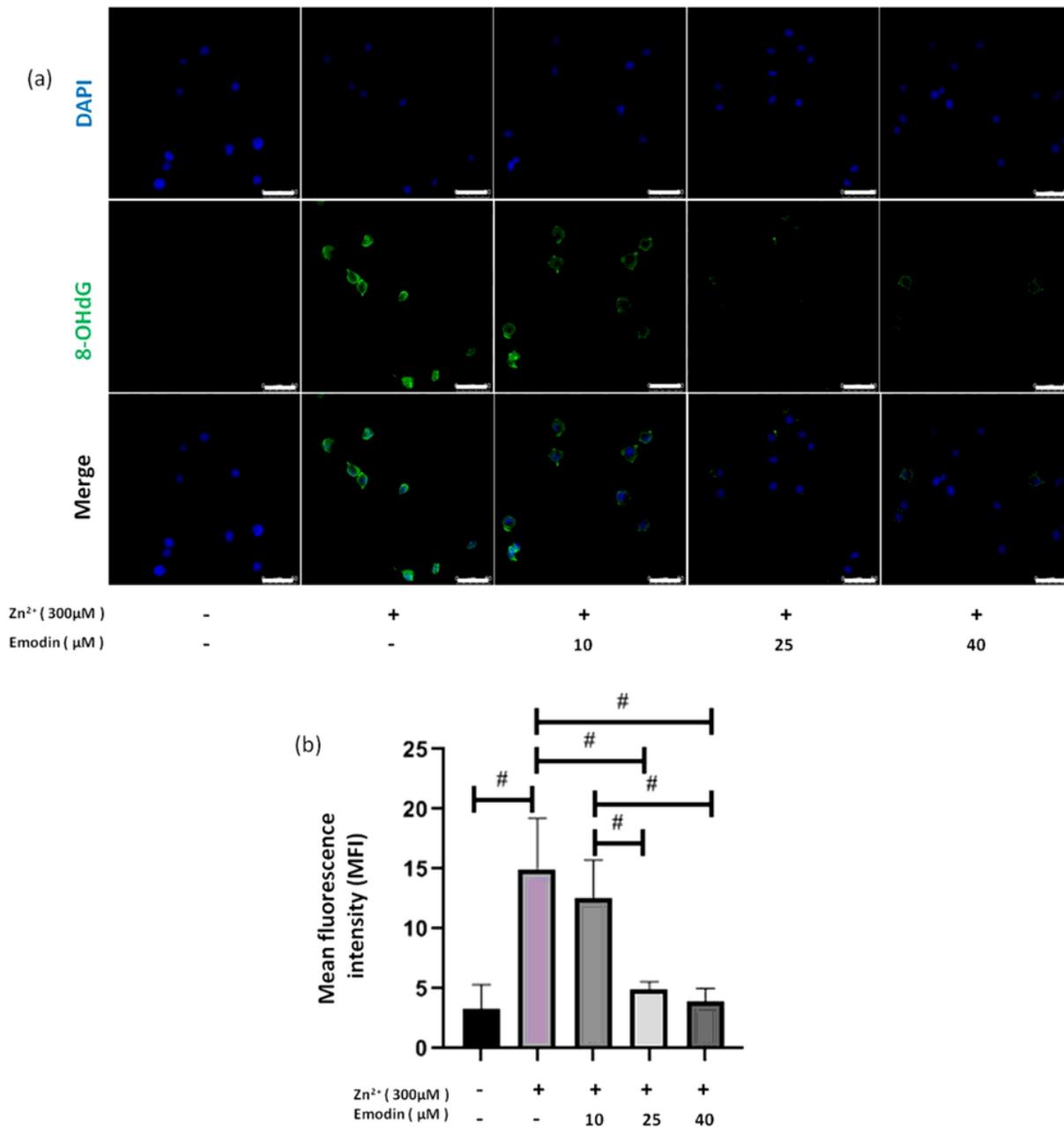
**Figure 4**

Emodin corrected the effects of zinc on depolarization of  $\Delta\Psi_m$  in SH-SY5Y cells. (a) fluorescence staining of JC-1 in untreated and zinc-treated SH-SY5Y cells with or without pretreatment with emodin (10, 25, and 40  $\mu\text{M}$ ). Mitochondrial aggregate, polymer form of JC-1 (red) indicating normal  $\Delta\Psi_m$ , and monomeric form of JC-1 (green) indicating dissipation of  $\Delta\Psi_m$ . scale bar = 250  $\mu\text{m}$ . (b) the ratio of green fluorescence to red fluorescence. One-way ANOVA and Bonferroni post-hoc analysis; # $p < 0.05$ , ## $p < 0.001$  significant. Data as mean  $\pm$  SEM



**Figure 5**

Emodin repressed lipid peroxidation in zinc-treated SH-SY5Y cells. (a) immunofluorescence-based confocal images of 4-Hydroxynonenal (4-HNE, red) in untreated and 300 μM zinc sulfate -treated cells, with or without pretreatment with different concentrations of emodin with or without pretreatment with emodin (10, 25, and 40 μM). scalebar = 50 μm; Nuclei was stained by DAPI (blue); (b) the mean fluorescent intensity analysis. One-way ANOVA and Bonferroni post-hoc analysis, #p < 0.05, ##p < 0.001; Data as mean ± SEM



**Figure 6**

Emodin suppressed lipid peroxidation DNA oxidation in zinc-treated SH-SY5Y cells. (a) immunofluorescence-based confocal images showing the 8-hydroxy-2'-deoxyguanosine (8-OHdG, green) in untreated and 300 μM zinc sulfate -treated cells with or without pretreatment with emodin (10, 25, and 40 μM). scalebar = 50 μm; Nuclei was stained by DAPI (blue); (b) the mean fluorescent intensity analysis. One-way ANOVA and Bonferroni post-hoc analysis, #p < 0.05; Data as mean ± SEM.

## Supplementary Files

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- [originaldata.pdf](#)