Neurochemical Research
https://doi.org/10.1007/s11064-018-2496-9

ORIGINAL PAPER

Gastrodin Suppresses Pentylenetetrazole-Induced Seizures Progression by Modulating Oxidative Stress in Zebrafish

Meng Jin[1,2] · Qiuxia He[1,2] · Shanshan Zhang[1,2] · Yixuan Cui[1,2] · Liwen Han[1,2] · Kechun Liu[1,2]

Received: 4 October 2017 / Revised: 1 February 2018 / Accepted: 5 February 2018

Abstract
Pentylenetetrazole (PTZ)-induced seizures in Zebrafish models are now widely accepted for investigating human disease epilepsy. In epilepsy, the generation of oxidative stress contributes to the brain injury. Although Gastrodin (GAS) has been reported to have anticonvulsant activities, its effects on zebrafish seizure models and the underlying mechanism remain unclear. In this study, we evaluated the effects of GAS pretreatment on PTZ-induced seizures in zebrafish larvae and investigated the underlying mechanism related to its anti-oxidative defense. We found for the first time that GAS significantly decreased seizure-like behavior and extended the latency period to the onset of seizures. In addition, after exposure to GAS, anti-oxidative activity was observed in PTZ-induced seizures by measurement of antioxidant enzymes activities and oxidative stress-related genes expression. The overall results indicate that GAS attenuates PTZ-induced seizures in a concentration-dependent manner and modulates oxidative stress to potentially protect larval zebrafish from further seizures. Furthermore, our results have provided novel insights into GAS related therapy of seizures and associated neurological disorders.

Keywords Gastrodin · Seizures · Oxidative stress · Zebrafish

Introduction

Epilepsy is a group of neurological disorders characterized by recurrent and unprovoked seizures, which occurs due to genetic, developmental, metabolic and unknown reasons [1]. In 2016, World Health Organization (WHO) reported that about 50 million people have epilepsy, making it one of the most common neurological diseases globally. To date, animal models of seizures and epilepsy have significantly contributed to the basic understanding of epilepsy and discovery of anticonvulsant therapies [2–5]. Based on these models, multiple levels of analyses ranging from behavioral, electrophysiological to molecular have been successfully performed [6–9]. In addition to the rodent seizure models, zebrafish are now widely accepted as an extremely attractive system for the rapid screening of anticonvulsant drugs and early evaluation of anti-seizure activities because of its remarkable advantages including low cost of generation and maintenance, simple genetic manipulations, and easy phenotype assessments [10]. Moreover, zebrafish have vertebrate neural architecture and exhibit behavioral, electrographic, and molecular changes that would be expected from a seizure episode after exposure to convulsant agents [6].

Gastrodin (GAS) is a phenolic glucoside derived from the traditional Chinese herb "Tian ma" (*Gastrodia elata Blume*), which has been used for thousands of years in oriental countries to treat different kinds of neurological diseases including epilepsy, brain ischemia, Alzheimer's disease, and Parkinson's disease [11–17]. GAS has been shown to have the activities of anticonvulsant, anti-inflammatory, analgesic, anti-oxidative, and etc. [14, 18–23]. Regarding to its anticonvulsant properties, previous studies reported that

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11064-018-2496-9) contains supplementary material, which is available to authorized users.

✉ Liwen Han
hanlw@sdas.org

✉ Kechun Liu
hliukch@sdas.org

[1] Biology Institute, Qilu University of Technology (Shandong Academy of Sciences), 28789 East Jingshi Road, Jinan 250103, Shandong Province, People's Republic of China

[2] Key Laboratory for Drug Screening Technology of Shandong Academy of Sciences, 28789 East Jingshi Road, Jinan 250103, Shandong Province, People's Republic of China

Published online: 07 February 2018

GAS attenuates seizures and reduces recovery time in a gerbil epilepsy model [23]. GAS decreases seizure severity by modulating the mitogen-activated protein kinase-associated inflammatory responses [24]. Co-administration of GAS and phenytoin enhances the anticonvulsant effects and reduces the side effects of phenytoin [25]. In addition, a recent study showed that GSA reduces the severity of status epilepticus in the rat pilocarpine model of temporal lobe epilepsy by inhibiting Nav1.6 sodium currents [26]. Furthermore, previous studies have demonstrated that oxidative stress plays a key role in the progression and recovery of epilepsy [27–29], suggesting GAS may protect against oxidative stress, which would potentially protect pentylenetetrazole (PTZ)-induced larval zebrafish from further seizures.

Oxidative stress, which is involved in the pathogenesis of a number of neurologic conditions and neurodegenerative disorders [30–35], has been suggested as a possible mechanism of epileptic activity [36–38]. The excess generation of reactive oxygen species (ROS) trigger lipid peroxidation (LPO) and DNA damage, thus causing the damage of organisms. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) have been shown to protect against oxidative stress and scavenge the excess generation of ROS. Therefore, the activities of antioxidant enzymes are chosen as indicators of anti-oxidative properties. Considering the fact that seizures are associated with the anti-oxidative mechanism, it is of great interest to explore the anticonvulsant agents that may ameliorate the damage of oxidative stress due to seizures.

Although it has been reported that GAS has anticonvulsant effects on rodent models of seizures and epilepsy, the evaluation of GAS on zebrafish seizure models is lacking. Moreover, the precise mechanism underlying its anticonvulsant activity remains largely unknown. This study is initiated to investigate the anticonvulsant effects of GAS on a zebrafish model of seizures and its underlying mechanism. We found that GAS ameliorated PTZ-induced epileptic seizures with increase of seizure latency and *c-fos* expression and decrease of seizure-like behavior. In addition, anti-oxidative defense were observed on GAS pretreated animals that were suffering from seizures, indicating that GAS has positive effects on suppression of seizures progression, likely due to its anti-oxidative activity.

Materials and Methods

Animals and Maintenance

Zebrafish (*Danio rerio*) of the wild-type AB strain were maintained according to standard procedures [39]. Fish were kept under a 14 h light/10 h dark cycle photoperiod and fed twice a day with commercial flake fish food supplemented with live brine shrimp. Zebrafish embryos were obtained from natural mating of adult zebrafish bred and maintained in an automated re-circulating system with charcoal-filtered tap water. Fertilized eggs were collected, washed and transferred to sterile cell culture plates. Plates were maintained in an incubator at 28 ± 0.5 °C and monitored daily until 6 days post fertilization (dpf).

Materials

The drugs gastrodin (GAS), pentylenetetrazole (PTZ), apocynin, diphenyleneiodonium (DPI), nicotinamide adenine dinucleotide phosphate (NADPH), and lucigenin were purchased from Sigma (St. Louis, USA). The stock solutions were prepared in double-distilled water (ddH_2O), and serial dilutions were made in normal bathing medium (reverse osmosis water equilibrated with instant ocean salts) before the experiments. All other chemicals and reagents utilized in this study were of analytical grade.

Drug Pretreatments and PTZ-Induced Seizures

This study investigated the effects of GAS pretreatment on PTZ-induced seizures in 7 dpf Zebrafish larvae. All animals received the GAS treatment 24 h before the PTZ exposure. GAS was tested in three different concentrations, which were selected based on preliminary studies conducted in our laboratory. To generate PTZ-induced zebrafish model of seizures, a common convulsant agent PTZ, was added to aquarium water. The final concentration of PTZ is 15 mM. As described in [6], seizures were induced until stage III behavioral seizure activity was observed (loss of posture with rolling to the side and clonic convulsive movements).

Locomotor Activity and Latency Score

Zebrafish larvae at 7 dpf were transferred to 48-well plate (1 per well, with 300 µl of aquarium water) for 30 min to minimize any interference in the test. Following acclimation, aquarium water was disposed and 300 µl of 15 mM PTZ solution was added to each well. After that, the locomotor activity for each larva was recorded during 20 min using an automated computerized video-tracking system. The detailed behavioral seizure profile characterization was performed during the same time frame each day (from 10:00 a.m. to 4:00 p.m.) and in a silent room. Zeblab software (Viewpoint, Lyon, France) was used to analyze the digital tracks and the average speed was analyzed every 2 min. A total of eight zebrafish larvae (n = 8) were used to compose each experimental group.

The seizure latency period was defined as the time from initial exposure to PTZ until zebrafish reached each convulsion stage [40]. Stage I, dramatically increased swimming

activity; Stage II, whirlpool swimming behavior; Stage III, wild jump, clonus-like seizures followed by loss of posture [6]. The video was observed to score the convulsion stages and to quantify the latency of each fish. Each set of experiments was repeated at least three times using animals from different batches and a minimum of 8 zebrafish per data point. If animals did not show both clonic movements and loss of posture, they were considered to be seizure-free and were not included in the data analysis.

Measurement of Reactive Oxygen Species (ROS) Generation

Zebrafish larvae at 6 dpf were pretreated with GAS 24 h prior to PTZ exposure. 7 dpf zebrafish larvae were exposed to PTZ for 1 h, then transferred to a 24-well plate, treated with 30 µM DCFH-DA solution (Sigma, St. Louis, USA) and incubated for 40 min in the dark at 28 °C. After incubation, the larvae were washed with aquarium water for three times. If animals did not show both clonic movements and loss of posture, they were considered to be seizure-free and were not included in DCFH-DA staining. Photography was carried out using an Olympus confocal microscope using the excitation wavelength of 488 nm and emission wavelength of 530 nm, and then processed with Image J software. The fluorescence intensity of individual larva was quantified by using the Image J software.

Determinations of Oxidative Stress-Related Parameters

All animals received the GAS treatment 24 h before the PTZ traetment. 7 dpf Zebrafish larvae (n = 30) with 1 h PTZ exposure were collected, washed with PBS and homogenized in cold saline. The homogenates were centrifuged at 2500 rpm for 10 min at 4 °C, and the supernatants were used for the following assays. The Coomassie blue protein binding method was used to determine protein concentrations [41], using bovine serum albumin (BSA) as a standard control. The activity of superoxide dismutase (SOD) was assayed with SOD commercial detection kit (DOJINDO, Shanghai, China) following the manufacturer's instruction [42]. The assay used the xanthine–xanthine oxidase system to produce superoxide ions, which reacted with 2-(4-iodophenyl)-3-(4-nitrophenol-5-phenlyltetrazolium chloride) to form a red formazan dye, and the absorbance at 550 nm was determined. The activities of catalase (CAT), glutathione peroxidase (GPx), and the malondialdehyde (MDA) content were determined using their respective commercial kits (Nanjing Jiancheng Biotechnology Institute, Nanjing, China) following the manufacturer's instruction. CAT activity was determined spectrophotometrically by measuring the disappearance rate of hydrogen peroxide (H_2O_2) at 240 nm, as described by [43]. GPx activity was measured by determination of the reduced GSH using H_2O_2 as substrate [44]. A series of enzymatic reactions was activated by GPx in the homogenates which subsequently led to the conversion of GSH (reduced glutathione) to oxidized glutathione (GSSG). The change in absorbance during the conversion of GSH to GSSG was recorded spectrophotometrically at 412 nm. MDA level was determined by the thiobarbituric acid (TBA) method [45]. Briefly, after mixing TBA with the homogenates and centrifuging, the supernatants were obtained, and TBA was added. The developed red color of the resulting reaction was measured at 532 nm. OD values were read by Bio-Rad 680 microplate reader (Bio-Rad Laboratories).

For determination of NADPH oxidase activity, NADPH oxidase inhibitors were added after 5 min of PTZ exposure. The concentration of NADPH oxidase inhibitors are 200 µM of apocynin and 10µM of DPI, respectively. Lucigenin-dependent enhanced chemiluminescence was used to determine NADPH oxidase activity, measured as NADPH-dependent superoxide production [46]. After treatments (1 h after PTZ exposure), the homogenates were prepared as mentioned above and incubated with dark-adapted lucigenin (5 µM) at 37 °C for 15 min. Superoxide production was started by the addition of the NADPH oxidase substrate NADPH (100 µM) and the chemiluminescence signal was measured every 3 s for 3 min with an AutoLumat LB953 Multi-Tube Luminometer (Berthold Technologies, Bad Wildbad, Germany). NADPH-dependent superoxide production is presented as % chemiluminescence (CL) signal/min of the untreated control.

Real-Time Quantitative PCR

Zebrafish larvae at 6 dpf were pretreated with GAS 24 h before the PTZ exposure. At 7 dpf, all groups were transferred to a 6-well plate (each group for per well) for 1 h, and then collected for real-time quantitative PCR (qPCR) analysis. For PTZ and GAS pretreatments, animals were exposed to PTZ instead fish water for 1 h. If animals did not show both clonic movements and loss of posture, they were considered to be seizure-free and were not included in qPCR analysis. qPCR was carried out to investigate the transcript levels of *c-fos, Mn-sod, Cu/Zn-sod, cat, gpx 1a, nrf2*, and *keap 1*. Total RNA extraction, reverse transcription, and qPCR were performed according to the manufacturer's protocols. Briefly, total RNA from 30 larval zebrafish was extracted from each group samples using TRIzol® (Invitrogen, Carlsbad, USA). cDNA was generated using the PrimeScript™ RT Master Mix (Takara, Tokyo, Japan). qPCR was performed using SYBR® Premix DimerEraser™ (Takara, Tokyo, Japan) and the Bio-Rad CFX96 Real-Time System. Runs were carried out in triplicate using the housekeeping gene *rpl13a* to normalize the mRNA level of target

genes. Data were analyzed using the Bio-Rad CFX Manager software to quantify the relative gene expression. Primer sequences are available on request.

Statistical Analysis

The results were analyzed by one-way ANOVA followed by Dunnett's post-hoc test and expressed as mean ± SEM. $P < 0.05$ was considered as significant. All GAS pretreatments were compared with the untreated controls and with the PTZ-induced treatments.

Results

Behavioral Seizure Response Following GAS Pretreatment

To investigate the behavioral seizure response, we analyzed locomotion plots by measuring the total distance moved (in cm) for each video recording. Previous study revealed that after 1 h of PTZ exposure there is no difference in locomotor activity between PTZ and the control group [38]. The decline of the locomotor activity observed in longer periods of PTZ exposure might be due to the fact that the larvae begin to undergo seizures with more frequency and spending more time on seizure stage III (loss of posture), which, as consequence, promotes a decrease of the locomotor activity. Moreover, our pilot experiments revealed that GAS at three concentrations significantly reduced larval locomotor activity within the first 5 min after PTZ induction, which is consistent with previous study of antiepileptic drugs [47]. Therefore, we evaluated distance moved during 20 min of PTZ exposure for convenience. As shown in Fig. 1a, pretreatment of GAS inhibited the PTZ-induced increase in locomotor activity. Distance traveled of the GAS groups was lower in comparison to that of the PTZ group and exhibited a concentration-dependent profile. For example, the total distance moved was shown to be down-regulated by approximately 1.5-fold at GAS concentration of 600 µM, whereas nearly 2.5-fold and 3.3-fold at 800 and 1000 µM GAS, respectively. Moreover, distance moved of the 1000 µM GAS pretreated group was similar to that found to the untreated group (Ctl group), suggesting the seizures has been fully suppressed at this concentration. Furthermore, animals that were pretreated with 1000 µM GAS but without PTZ exposure (hereafter referred to as GAS alone group) significantly reduced locomotor activity compared with control. This is possible due to an inhibitory effect of the sedation of GAS on the overall amount of movement, although the net change and absolute level of movement seen with PTZ treatment makes this less likely [48].

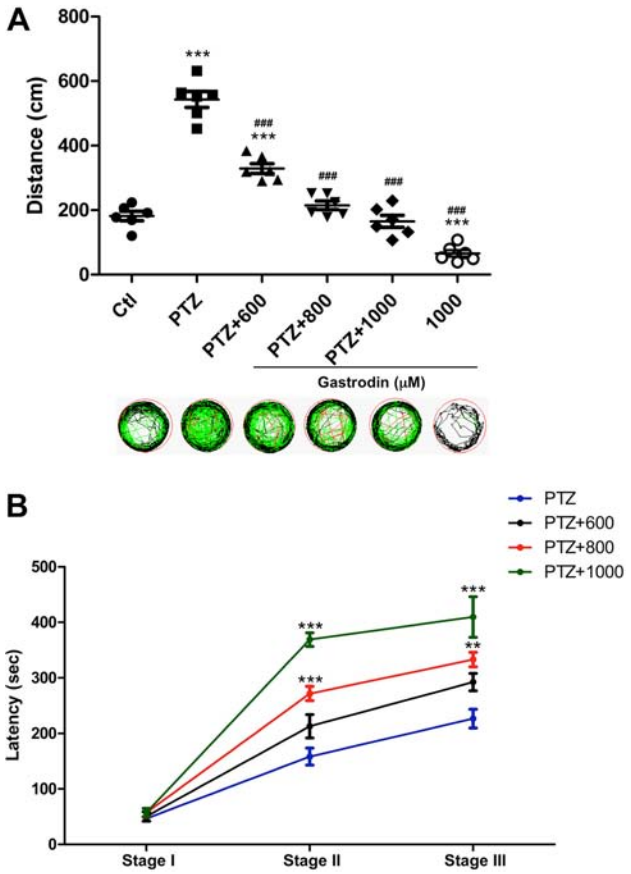

Fig. 1 Effects of GAS on locomotor activity response during PTZ exposure. **a** The total distance moved. Animals were exposed to 600, 800 and 1000 µM of GAS for 24 h prior to 15 mM PTZ exposure. Animals in the control group (Ctl) and PTZ group (PTZ) were handled identically but included exposure to fish water (no PTZ or GAS treatments) and to PTZ (no GAS treatments), respectively. The distance moved for each larva from each group was analyzed using Zeblab software. n = 8 per group, ***$P < 0.001$ versus Ctl, ###$P < 0.001$ versus PTZ. In the digital tracks map (at the bottom of **a**), the red lines are associated with fast movement; green lines are associated with medium movement; and black lines indicate slow movement. **b** Latency to the first behavioral manifestation of seizure stage I, II, and III during PTZ exposure. The blue represents PTZ group; black line represents 600 µM GAS with exposure of PTZ; red line represents 800 µM GAS with exposure of PTZ; green line represents 1000 µM GAS with exposure of PTZ. Data were expressed as mean ± SEM of at least 8 animals for each group. **$P < 0.01$, ***$P < 0.001$ versus PTZ. (Color figure online)

In order to investigate seizure development and zebrafish larvae response to GAS, we monitored the latencies to first sign of seizure stage I, II, and III, which is another indicator of seizure behavior. As shown in Fig. 1b, the latency to each seizure stage was prolonged in animals pretreated with GAS. Specifically, zebrafish larvae at 7 dpf pretreated with 800 and 1000 µM GAS showed significantly extended latencies compared with animals exposed to PTZ at stage II and III. However, there were no

significant differences in the latencies to reach stage (I, II and III) between larval zebrafish pretreated with 600 μM GAS and their respective control groups. Moreover, larval zebrafish pretreated with GAS at three different concentrations reached stage I with similar latencies when compared to respective control group. Our results suggested that GAS pretreatment extended the latency periods to the onset of seizure stages, especially stage II and III, in a concentration-dependent manner. This is the first report to demonstrate that GAS decreases seizure-like behavior and extends the latency period to the onset of seizures on zebrafish seizure models.

Effects of GAS on the Neuronal Activity Marker *c-fos*

To investigate whether GAS pretreatment alters the expression of *c-fos* gene after PTZ exposure, its relative mRNA expression levels were measured. c-Fos has been shown to be induced transiently in response to seizures induction [49]. Expression of *c-fos* mRNA was low in untreated group, and greatly up-regulated in animals that were exposed to PTZ. Pretreatment of GAS was able to inhibit the seizure-induced increase of *c-fos* expression. In addition, significant concentration-dependent suppression in *c-fos* expression was also detected in GAS pretreatment zebrafish larvae. Animals pretreated with the medium and high concentration of GAS (800 and 1000 μM) showed similar *c-fos* expression compared with untreated zebrafish larvae. There was no difference in expression of *c-fos* between control and GAS alone group, indicating GAS alone does not affect the expression of this seizure marker under normal conditions (Fig. 2).

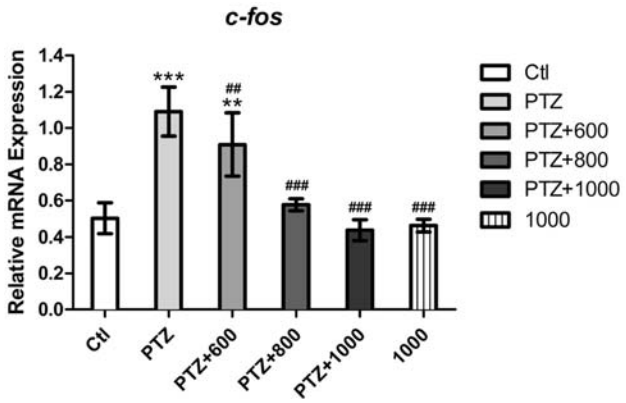

Fig. 2 Effects of GAS on gene expression of *c-fos* in zebrafish submitted to PTZ-induced seizures. Relative mRNA expression of *c-fos* in control, PTZ, GAS pretreatments (600, 800 and 1000 μM), and GAS alone group after 1 h of PTZ exposure. n = 5 per group, $^{**}P < 0.01$, $^{***}P < 0.001$ versus Ctl, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus PTZ

PTZ-Induced Seizures Result in ROS Production via NADPH Oxidase Activation

In the present study, we found that features of seizures emerged earlier than ROS generation (Supplementary Fig. 1 and Fig. 3a), suggesting seizures causing ROS. However, the mechanism of seizure-induced ROS generation is unclear. Previous studies shown that NADPH oxidase is a major enzymatic source of cellular ROS generation and seizure activity results in ROS production via NADPH oxidase activation in cell culture [50]. To investigate if this is the case in zebrafish seizure models, we inhibited the activity of NADPH oxidase after seizure onset (seizure stage III) and tested the ROS production. We found that two widely used NADPH oxidase inhibitors [51], apocynin and DPI, significantly decreased NADPH oxidase activity induced by PTZ (Fig. 3d), thereby effectively reduced ROS generation (Fig. 3b, c). These findings suggested that seizure caused ROS accumulation by activating NADPH oxidase in larval zebrafish.

Down-Regulation of PTZ-Induced ROS Production by GAS in Zebrafish Larvae

Previous studies have demonstrated that antioxidants attenuate brain injury and play a pivotal role in recovery of epilepsy [27–29]. Therefore, we speculated that GAS might suppress PTZ-induced seizures progression via an anti-oxidative pathway. To that end, the inhibitory effect of GAS on seizure-induced ROS accumulation was evaluated by DCFH-DA staining in zebrafish larvae 1 h after PTZ exposure. As shown on Fig. 4a, the control, which was untreated, generated only background fluorescence whereas the larvae exposed to PTZ markedly generated fluorescence images, suggesting that ROS was generated in the presence of PTZ in the zebrafish seizure models. When the zebrafish larvae were treated with GAS prior to PTZ exposure, the generation of ROS was effectively reduced in a concentration-dependent manner (Fig. 4b). In addition, the highest concentration of GAS pretreatment (1000 μM) without PTZ-induction showed no difference in ROS accumulation compared with control group.

Effects of GAS on Activities of Antioxidant Enzymes and MDA Content in Zebrafish Seizure Models

To investigate whether the antioxidant defense system was activated by GAS pretreatment after PTZ exposure, the activities of SOD, CAT and GPx, as well as the content of MDA in zebrafish seizure models were assayed. When the concentration of GAS reached 1000 μM, SOD in PTZ-exposure animals was completely restored to near normal level (Fig. 5a). Similar alterations were observed for the

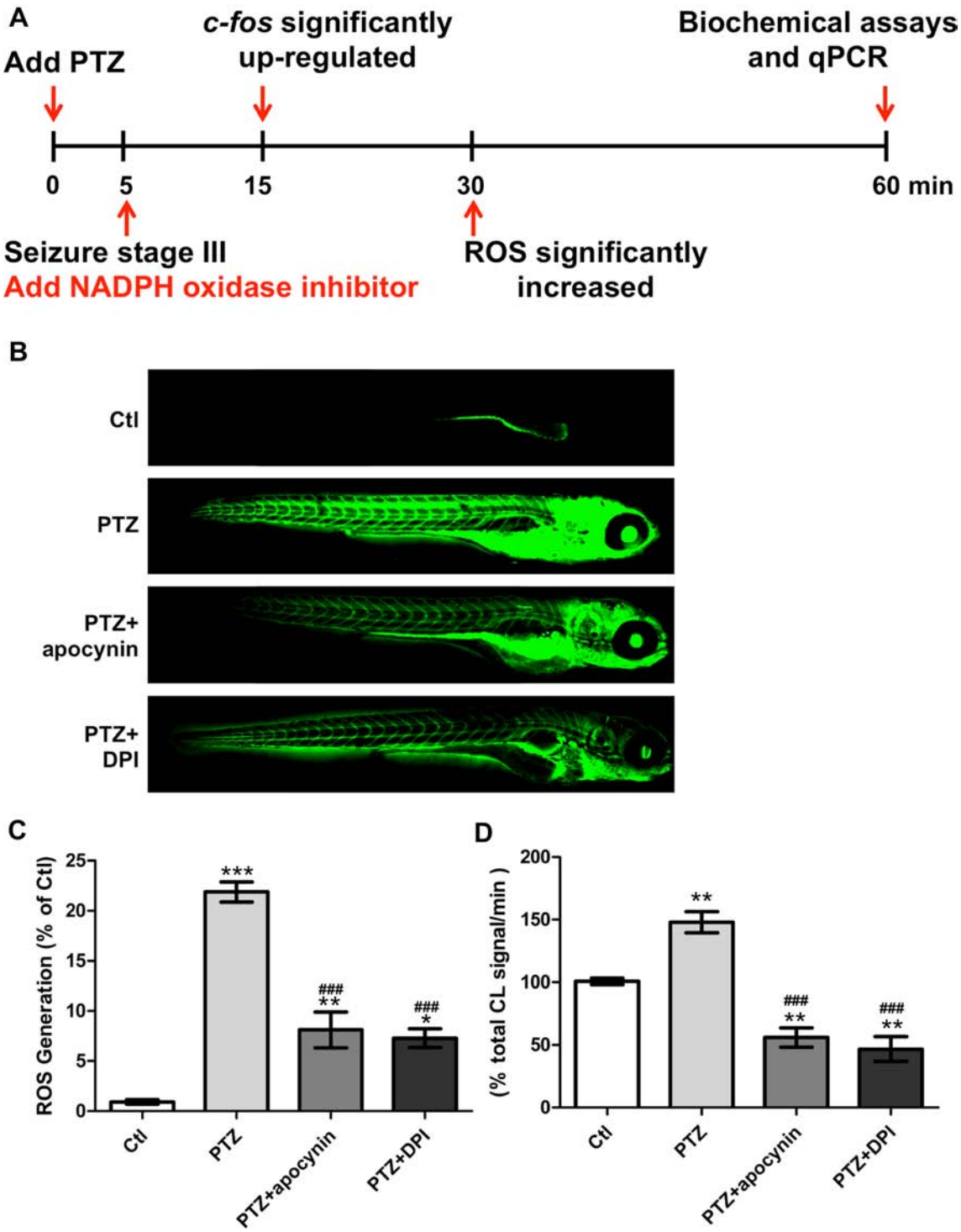

Fig. 3 The role of NADPH oxidase in seizure-caused ROS generation. **a** A schematic diagram summarizing the different time points for different assays and seizure features. Zebrafish larvae usually take 3–5 min to reach seizure stage III after PTZ exposure. *c-fos* mRNA and ROS generation significantly increase after 15 and 30 min of PTZ exposure, respectively. Therefore, biochemical assays including measurement of ROS and oxidative stress-related parameters, and qPCR were performed using the animals after 1 h exposure of PTZ to ensure that we could properly observe the seizures process. **b** Fluorescence images of ROS generation in zebrafish larvae treated with: control; PTZ; 200 μM apocynin with exposure of PTZ; and 10 μM DPI with exposure of PTZ. **c** The fluorescence intensities of ROS levels in individual zebrafish larvae were quantified, and values represent the means ± SEM of 5 independent experiments. **d** NADPH oxidase activity in control, PTZ; 200 μM apocynin with exposure of PTZ; and 10 μM DPI with exposure of PTZ. NADPH-dependent superoxide production is presented as % chemiluminescence (CL) signal/min of the untreated control. n = 5 per group, *P < 0.05, **P < 0.01, ***P < 0.001 versus Ctl, $^{###}$P < 0.001 versus PTZ

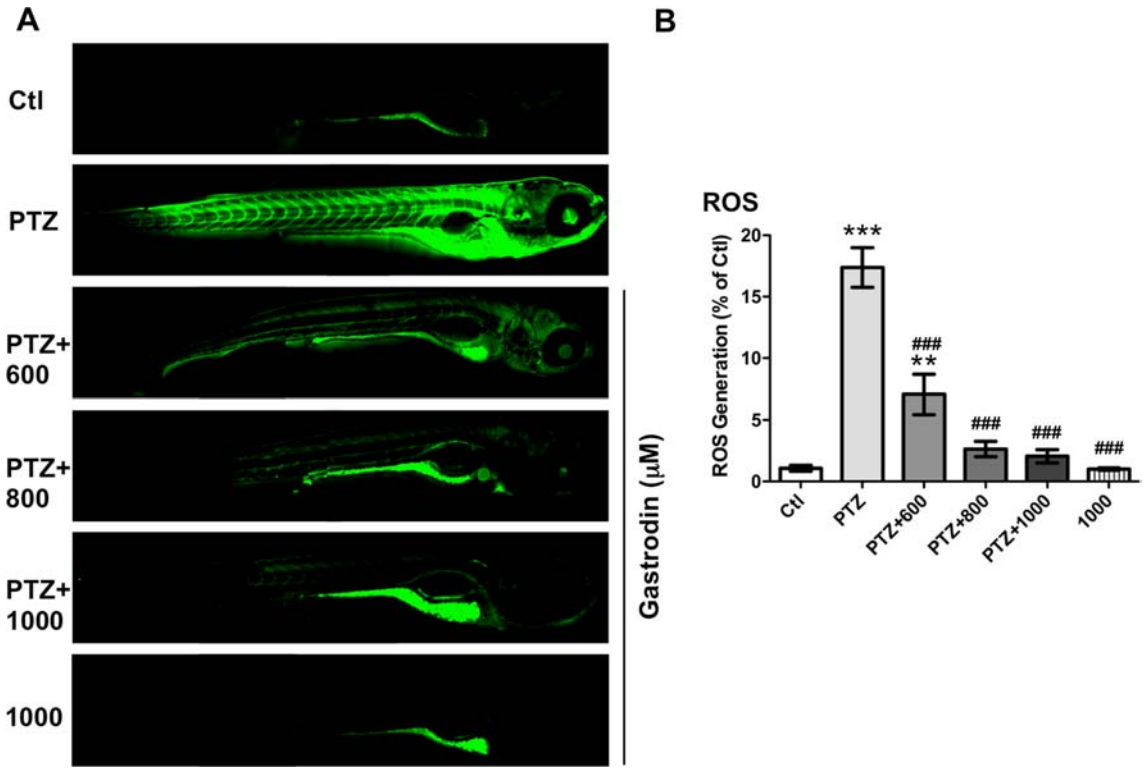

Fig. 4 The ROS scavenging ability of GAS on PTZ-induced ROS generation in zebrafish larvae. **a** Fluorescence images of ROS generation in zebrafish larvae treated with: control; PTZ; 600 μM GAS with exposure of PTZ; 800 μM GAS with exposure of PTZ; 1000 μM GAS with exposure of PTZ; and GAS alone. **b** The fluorescence intensities of ROS levels in individual zebrafish larvae were quantified, and values represent the means ± SEM of 5 independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ versus Ctl, $^{###}P < 0.001$ versus PTZ

CAT activity (Fig. 5b). PTZ markedly decreased the activity of CAT, which was concentration-dependently increased by pretreatment of GAS. However, no significant change in GPx activity between PTZ group and GAS pretreatments, whereas its activity effectively decreased in PTZ-induced animals compared with untreated group (Fig. 5c). The MDA content, an indicator to evaluate the LPO level induced by oxidative stress in organisms [34], was also measured. As a result, a significant induction in MDA content in PTZ-exposure group, which was reversed by pretreatment of GAS in a concentration-dependent manner. No apparent induction of MDA was observed in 800 and 1000 μM GAS pretreated groups compared with control (Fig. 5d). Furthermore, the activities of SOD and CAT significantly elevated in GAS alone group compared with control, which are consistent with previous reports that GAS has the anti-oxidative activity [52–54].

Effects of GAS on Expression of Oxidative Stress-Related Genes

In addition to activities of antioxidant enzymes, we measured transcriptional responses of antioxidant enzymes. As shown in Fig. 6, the expression of manganese superoxide dismutase (Mn-Sod) (Fig. 6a), copper/zinc superoxide dismutase (Cu/Zn-Sod) (Fig. 6b), catalase (Cat) (Fig. 6c), and glutathione peroxidase 1a (Gpx1a) (Fig. 6d) dramatically decreased with PTZ exposure, and gradually up-regulated with the increase of GAS concentration. When the concentration of GAS reached 1000 μM, mRNA of *Mn-sod, Cu/Zn-sod, cat*, and *gpx1a* in PTZ-induced animals were completely restored to near normal level. Moreover, the expression of nuclear factor erythroid-derived 2-like 2 (Nrf2) and kelch-like ECH-associated protein 1 (Keap1) were measured. The transcription factor Nrf2 and its negative regulator Keap1 play an important role in the regulation of major antioxidant enzymes and antioxidant genes [55–57]. Our results revealed a significant and concentration-dependent up-regulation of Nrf2 expression in the zebrafish larvae with pretreatment of GAS (Fig. 6e). In contrast, Keap1 expression was significantly suppressed in GAS pretreated animals in a concentration dependent manner (Fig. 6f). Transcriptional levels of all stress-related genes assayed in our study were increased in GAS alone group as compared to untreated larval zebrafish. In summary, expression of *nrf2, Mn-sod, Cu/Zn-sod, cat* and *gpx1a* mRNA significantly increased in GAS pretreatments compared with PTZ group, which suggested that pretreatment of GAS was able to prevent the decrease of oxidative

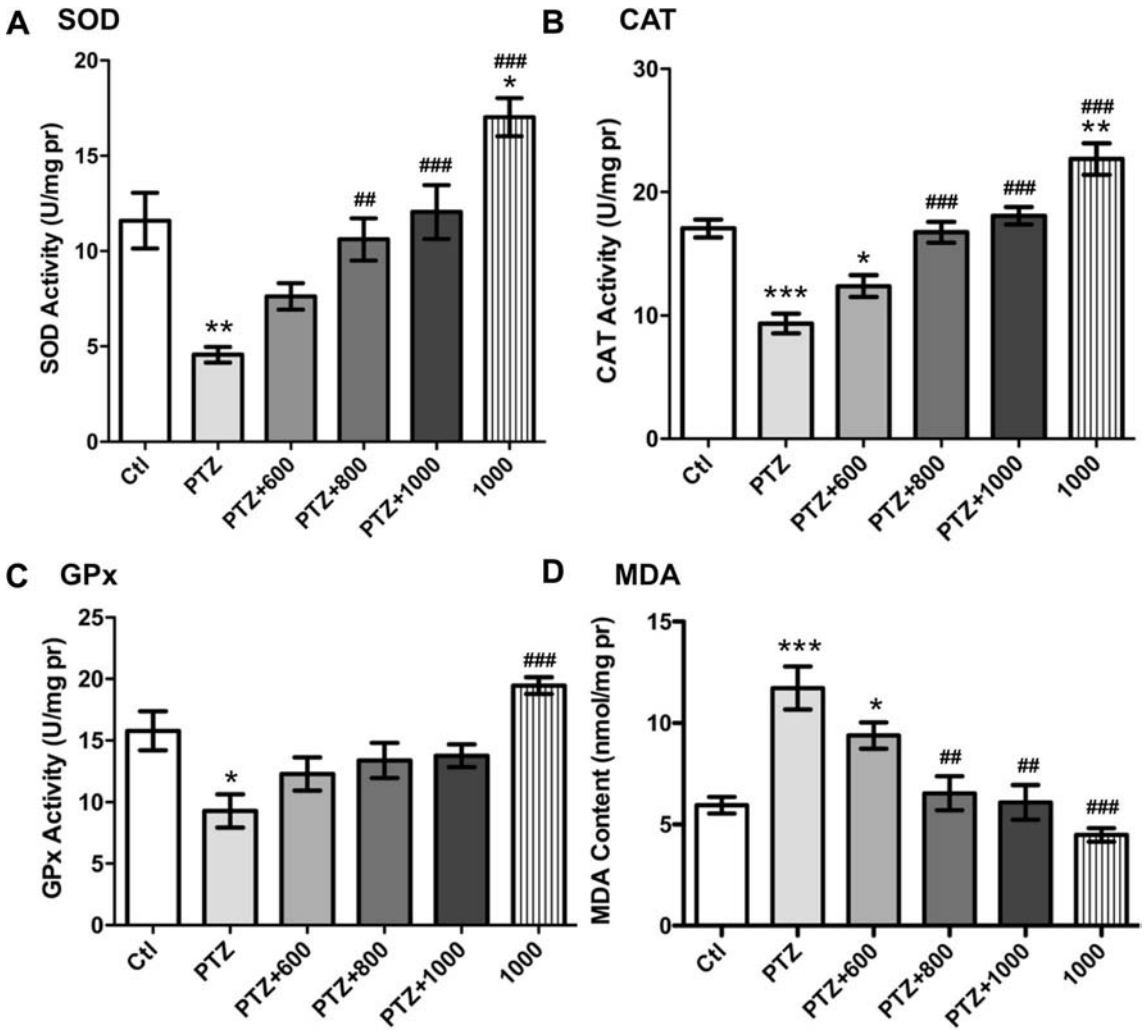

Fig. 5 Antioxidant enzymes activities and MDA content in GAS-pretreated zebrafish seizure models. SOD (**a**) and CAT (**b**), and GPx (**c**) activities and MDA (**d**) content in control, PTZ, GAS pre-treatments (600, 800 and 1000 μM), and GAS alone group. n = 5 per group, *P < 0.05, **P < 0.01, ***P < 0.001 versus Ctl, ##P < 0.01, ###P < 0.001 versus PTZ

stress-related genes expression caused by PTZ-induced seizures.

Discussion

Epilepsy is one of the most common chronic brain disorders, characterized by frequently repeated seizures, emotional and cognitive dysfunctions [58, 59]. Moreover, epilepsy has significant economic implications in terms of health care needs, premature death and lost work productivity. Therefore, more efficacious treatment and mechanism investigation for epilepsy are in desperate need. Gastrodin (GAS), a major bioactive component of the traditional Chinese herb "Tianma", has a long history as an anti-epilepsy drug. However, the anticonvulsant mechanism of GAS and its effect on zebrafish seizure models have not been clearly demonstrated. In the present study, we demonstrated that GAS improved PTZ-induced seizures and modulated oxidative stress to potentially protect seizures from deterioration in zebrafish larvae.

Zebrafish Is a New Seizure Model

Animal models for seizures and epilepsy have played a fundamental role in understanding of basic mechanism underlying epileptogenesis and discovery and evaluation of antiepileptic drugs [2–5]. Zebrafish, are small freshwater teleosts emerging as a promising model organism in study of behavior and neurological diseases including seizures. Physiologically and behaviorally, acute seizures induced in wild-type zebrafish closely resemble those induced in mammals [6, 60]. The underlying rules governing initiation and termination of electrical seizure events were universal in mice, zebrafish and humans [61]. Variation in the genetic

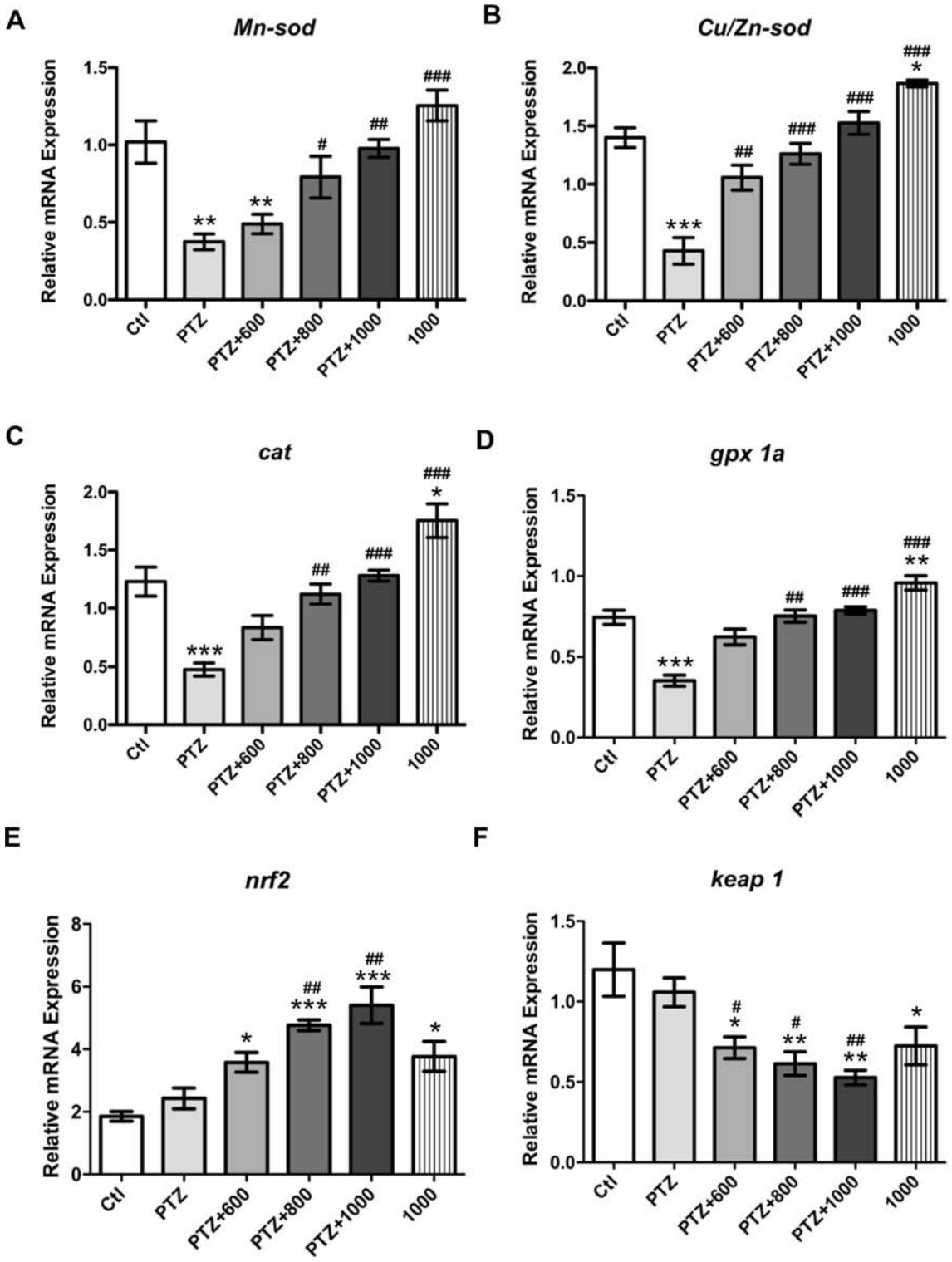

Fig. 6 Expression of oxidative stress-related genes in GAS-pretreated zebrafish seizure models. The mRNA level of *Mn-sod* (**a**), *Cu/Zn-sod* (**b**), *cat* (**c**), *gpx 1a* (**d**), *nrf2* (**e**), and *keap 1* (**f**) in control, PTZ, GAS pretreatments (600, 800 and 1000 μM), and GAS alone group. n = 5 per group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus Ctl, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus PTZ

background of rodent seizure models lead to opposing or contradictory results, confounding experimental studies [62]. Therefore, zebrafish seizure models are deployed as a complementary model for epilepsy research. In addition to animal seizure models, in vitro seizure models, chemically and electrically-induced, are useful to investigate the cellular mechanisms that underlie epileptogenesis and the spontaneous recurrent epileptiform discharge activity associated with epilepsy [63]. It was reported that GAS has no effect on NMDA receptor-mediated seizures by using low magnesium medium induced epileptiform discharges in hippocampal slice preparations [64]. NMDA receptors are key factors involved in low magnesium-induced epileptiform activity in hippocampal slices. These findings provide further evidence that GAS exhibits anticonvulsive effects on $GABA_A$ receptor-mediated seizures [23–25]. Indeed, PTZ, a non-competitive GABA antagonist, induced seizures are suppressed by GAS in vivo.

Previous findings revealed that antiepileptic drugs at low and high concentration induce different locomotion in larval zebrafish. Antiepileptic drugs at high concentration usually cause sedative effects and sedated animals exhibit slow movement or barely move [47, 65]. Admittedly, the reduced locomotion in our GAS alone group maybe due to the sedation of high concentration of GAS. However, we did not observe sedative effects (slow or no movement) in those fishes pretreated with GAS and then induced with PTZ, which suggested the decrease in larval activity is not an inhibitory effect of the sedation of GAS in GAS pretreatments. In addition, it must be emphasized that increased locomotion is one of indicators of seizures. Other indicators such as increased *c-fos* expression, abnormal electroencephalography (EEG) recordings, and seizure-like behaviors improve the accuracy of seizure recognition [47]. In the recent study, a more subtle PTZ convulsion progression scale is defined in adult zebrafish, providing a rapid way in assessing seizures [65]. Therefore, it is more convincing to assure that the animal have or not a seizure by determination of more than one indicator of seizures.

Seizures Induce ROS Generation by Activating NADPH Oxidase

It is known increased ROS generation during seizure development [50, 66] and an imbalance between production of ROS and cellular antioxidant defenses cause oxidative stress [67–70]. In our study, a significant increase in ROS generation was found after the occurrence of seizures, which was reversed by inhibition of NADPH oxidase, suggesting that seizure caused ROS accumulation by activating NADPH oxidase. A schematic diagram summarizing the proposed mechanism is shown in Fig. 7. Our results are consistent with previous cell culture-based studies that seizures result

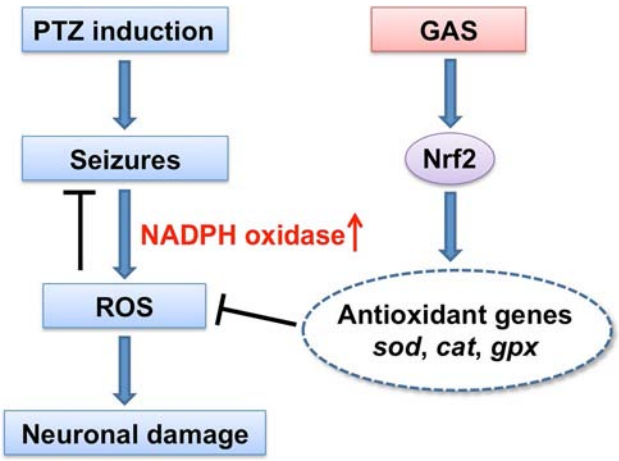

Fig. 7 Schematic of proposed role of GAS in attenuating PTZ-induced seizures associated with oxidative stress modulation. Following PTZ-induced seizures, there is increased production of ROS by activating NADPH oxidase, which causes neuronal damage. GAS activates the transcription factor Nrf2, a key oxidative stress sensor, leading to further activation of antioxidant genes such as *sod, cat*, and *gpx*. Upregulation of antioxidant genes blocks ROS production, resulting in attenuation of seizures

in ROS production via NADPH oxidase activation [42]. To ensure the inhibition of NADPH oxidase, two different NADPH oxidase inhibitors, apocynin and DPI, were used respectively. Both inhibitors were able to reduce ROS accumulation caused by PTZ-induced seizures.

GAS Upregulates Activities of Antioxidant Enzymes and Expression of Oxidative Stress-Related Genes to Eliminate ROS During PTZ-Induced Seizures

The generation of ROS was effectively reduced by GAS pretreatment, indicating that GAS play important roles in oxidative stress modulation after PTZ-induced seizures. We found that GAS was able to induce activities of antioxidant enzymes and expression of oxidative stress-related genes in larval zebrafish, which are undergoing seizure stage III. SOD, CAT and GPx are key antioxidant enzymes in fish to counter oxidative stress. SOD catalyzes superoxide anion radical into H_2O_2, while CAT and GPx trigger the subsequent degradation of H_2O_2 thereby protects the organisms against oxidative damage [52–54]. Our studies showed that both SOD and CAT activities were significantly elevated in animals suffering from seizures that were pretreated with GAS, indicating the importance of these enzymes to combat oxidative stress generated in PTZ-induced seizures. However, no apparent up-regulation in GPx activity was observed, suggesting that GPx play a less important role in resisting the oxidative stress. This phenomenon is possible because the action of one antioxidant enzyme is replaced or compensated by others. Indeed, GPx activity could be

partially compensated by elevation in CAT activity [71]. Moreover, the change trend of *sod* and *cat* mRNA coincide with their activities. The up-regulation of *Mn-sod* and *Cu/Zn-sod* in GAS pretreatments was probably to activate SOD activity for the elimination of superoxide anion radical induced by seizures, and the increase in mRNA levels of *cat* was to activate CAT for H_2O_2 detoxification. Surprisingly, while GPx activity was relatively stable, its mRNA level significantly decreased in PTZ-induced larvae, which was reversed by pretreatment of GAS in a concentration dependent manner. The mismatch between GPx activity and its transcriptional levels might be related to the translational and posttranslational regulations and the presence of multiple gene copies in zebrafish. These observations support previous findings that protecting against oxidative stress would potentially protect patients from further epilepsy [72]. Furthermore, the activities of SOD, CAT, and GPx significantly increased in GAS alone group compared with control, which is in agreement with previous findings that GAS has the ability to activate anti-oxidative pathways.

GAS Activates Antioxidant Genes via Upregulation of Nrf2

The transcription factor Nrf2 is recognized as a key oxidative stress sensor that binds to DNA antioxidant response elements leading to the activation of antioxidant genes such as *sod, cat*, and *gpx*. Keap1, a negative regulator of Nrf2, suppresses Nrf2 in cytoplasm [55, 73, 74]. Here, enhanced Nrf2 expression in GAS pretreatments indicated that antioxidant defenses were activated. This is consistent with previous findings reported in mammals [12, 16, 19, 53]. Notably, Nrf2 expression was restored to the level that is significantly higher than normal. Together with previous studies that the anti-oxidative activity of GAS is closely related to the induction of Nrf2 pathway, our findings implied that PTZ and GAS might synergistically regulate the expression of Nrf2 expression; which could account for the overstimulation of Nrf2. In addition, in contrast to down-regulation effect of PTZ on mRNA levels of antioxidant enzymes, unchanged effect of PTZ on Nrf2 expression might be due to the fact that PTZ regulates other Nrf2-involved pathways. Therefore, the expression level of Nrf2 was not down-regulated. Indeed, Nrf2 is a major regulator for several cytoprotective factors such as anti-apoptosis factors, anti-inflammatory factors and transcriptional factors. Previous studies showed that GAS could up-regulate gene expression of Nrf2 to protect primary cultured rat hippocampal neurons against neurotoxicity [27]. GAS could also alleviate proinflammatory response and inhibit apoptosis by activating Nrf2 signaling pathway [19, 54]. However, Nrf2-regulated pathways are not the sole effect of GAS when it attenuates epilepsy and seizures. For example, GAS exerts neuroprotective effects by suppressing the expression of inducible NO synthase (iNOS), cyclooxygenase-2 and proinflammatory cytokines in cultured LPS-stimulated microglia via MAPK pathways [75]. GAS strongly attenuates LPS-induced acute inflammatory responses by inhibition of NF-κB and MAPKs activation via the phosphatidylinositol 3-kinase signaling [76]. Moreover, GAS has been found to reduce seizure severity and regulate GABA neurotransmitter levels by inhibiting GABA degrading enzymes [23, 77].

Seizure-Induced Oxidative Damage Is Alleviated by GAS

To investigate oxidative damage, the content and transcriptional level of MDA was determined. MDA is often used to monitor LPO which has been known as a major contributor to the impairment of cell function under oxidative stress. In the current study, the content and mRNA level of MDA were increased in zebrafish larvae following exposure to PTZ, indicating that the zebrafish in seizures suffered from oxidative damage. These observations are in agreement with previous findings that epilepsy progression causes oxidative damage [27–29]. The fact that up-regulation was suppressed by GAS pretreatment suggests that oxidative damage is alleviated by GAS.

In conclusion, the novel findings of our study were that GAS suppresses PTZ-Induced seizures progression by modulating oxidative stress in a concentration dependent manner in zebrafish. These results support previous evidence that zebrafish is a suitable alternative for studying seizures and anti-oxidation.

Acknowledgements We thank Ximin Wang for zebrafish maintenance. We are grateful to Dr. Ming Fa for critical reading and editing of manuscript, Dr. Wenlong Sheng and Dr. Rongchun Wang for technical help. We thank the Youth Fund of Shandong Academy of Sciences (No. 2018QN0024) for financial support.

Author Contributions MJ, KCL, LWH, and QXH conceived the project and designed the experiments. MJ and YXC performed the experiments and analyzed the data. MJ wrote the manuscript. SSZ provided expertise on antiepileptic drugs and seizure induction experiments.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

1. Moshe SL, Perucca E, Ryvlin P, Tomson T (2015) Epilepsy: new advances. Lancet 385(9971):884–898. https://doi.org/10.1016/S0140-6736(14)60456-6
2. Loscher W (2016) Fit for purpose application of currently existing animal models in the discovery of novel epilepsy therapies.

Epilepsy Res 126:157–184. https://doi.org/10.1016/j.eplepsyres.2016.05.016
3. Jobe PC, Mishra PK, Ludvig N, Dailey JW (1991) Scope and contribution of genetic models to an understanding of the epilepsies. Crit Rev Neurobiol 6(3):183–220
4. Schwartzkroin PA, Roper SN, Wenzel HJ (2004) Cortical dysplasia and epilepsy: animal models. Adv Exp Med Biol 548:145–174
5. Grone BP, Baraban SC (2015) Animal models in epilepsy research: legacies and new directions. Nat Neurosci 18(3):339–343. https://doi.org/10.1038/nn.3934
6. Baraban SC, Taylor MR, Castro PA, Baier H (2005) Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. Neuroscience 131(3):759–768. https://doi.org/10.1016/j.neuroscience.2004.11.031
7. Auvin S, Pineda E, Shin D, Gressens P, Mazarati A (2012) Novel animal models of pediatric epilepsy. Neurotherapeutics 9(2):245–261. https://doi.org/10.1007/s13311-012-0119-8
8. Loscher W, Honack D (1993) Profile of ucb L059, a novel anticonvulsant drug, in models of partial and generalized epilepsy in mice and rats. Eur J Pharmacol 232(2–3):147–158
9. Hardy BT, Patterson EE, Cloyd JM, Hardy RM, Leppik IE (2012) Double-masked, placebo-controlled study of intravenous levetiracetam for the treatment of status epilepticus and acute repetitive seizures in dogs. J Vet Internal Med 26(2):334–340. https://doi.org/10.1111/j.1939-1676.2011.00868.x
10. Baraban SC, Loscher W (2014) What new modeling approaches will help us identify promising drug treatments? Adv Exp Med Biol 813:283–294. https://doi.org/10.1007/978-94-017-8914-1_23
11. Chen PZ, Jiang HH, Wen B, Ren SC, Chen Y, Ji WG, Hu B, Zhang J, Xu F, Zhu ZR (2014) Gastrodin suppresses the amyloid beta-induced increase of spontaneous discharge in the entorhinal cortex of rats. Neural Plast 2014:320937. https://doi.org/10.1155/2014/320937
12. Wang XL, Xing GH, Hong B, Li XM, Zou Y, Zhang XJ, Dong MX (2014) Gastrodin prevents motor deficits and oxidative stress in the MPTP mouse model of Parkinson's disease: involvement of ERK1/2-Nrf2 signaling pathway. Life Sci 114(2):77–85. https://doi.org/10.1016/j.lfs.2014.08.004
13. Wang Y, Wu Z, Liu X, Fu Q (2013) Gastrodin ameliorates Parkinson's disease by downregulating connexin 43. Mol Med Rep 8(2):585–590. https://doi.org/10.3892/mmr.2013.1535
14. Kumar H, Kim IS, More SV, Kim BW, Bahk YY, Choi DK (2013) Gastrodin protects apoptotic dopaminergic neurons in a toxin-induced Parkinson's disease model. Evid Based Complement Altern Med 2013:514095. https://doi.org/10.1155/2013/514095
15. Hu Y, Li C, Shen W (2014) Gastrodin alleviates memory deficits and reduces neuropathology in a mouse model of Alzheimer's disease. Neuropathology 34(4):370–377. https://doi.org/10.1111/neup.12115
16. Jiang G, Hu Y, Liu L, Cai J, Peng C, Li Q (2014) Gastrodin protects against MPP(+)-induced oxidative stress by up regulates heme oxygenase-1 expression through p38 MAPK/Nrf2 pathway in human dopaminergic cells. Neurochem Int 75:79–88. https://doi.org/10.1016/j.neuint.2014.06.003
17. Ojemann LM, Nelson WL, Shin DS, Rowe AO, Buchanan RA (2006) Tian ma, an ancient Chinese herb, offers new options for the treatment of epilepsy and other conditions. Epilepsy Behav 8(2):376–383. https://doi.org/10.1016/j.yebeh.2005.12.009
18. Fang H, Zhang JC, Yang M, Li HF, Zhang JP, Zhang FX, Wang QY, Wang RR, Liu J (2016) Perfusion of gastrodin in abdominal aorta for alleviating spinal cord ischemia reperfusion injury. Asian Pac J Trop Med 9(7):688–693. https://doi.org/10.1016/j.apjtm.2016.05.007
19. Peng Z, Wang S, Chen G, Cai M, Liu R, Deng J, Liu J, Zhang T, Tan Q, Hai C (2015) Gastrodin alleviates cerebral ischemic damage in mice by improving anti-oxidant and anti-inflammation activities and inhibiting apoptosis pathway. Neurochem Res 40(4):661–673. https://doi.org/10.1007/s11064-015-1513-5
20. Qiu F, Liu TT, Qu ZW, Qiu CY, Yang Z, Hu WP (2014) Gastrodin inhibits the activity of acid-sensing ion channels in rat primary sensory neurons. Eur J Pharmacol 731:50–57. https://doi.org/10.1016/j.ejphar.2014.02.044
21. Sun W, Miao B, Wang XC, Duan JH, Ye X, Han WJ, Wang WT, Luo C, Hu SJ (2012) Gastrodin inhibits allodynia and hyperalgesia in painful diabetic neuropathy rats by decreasing excitability of nociceptive primary sensory neurons. PloS ONE 7(6):e39647. https://doi.org/10.1371/journal.pone.0039647
22. Xu X, Lu Y, Bie X (2007) Protective effects of gastrodin on hypoxia-induced toxicity in primary cultures of rat cortical neurons. Planta Medica 73(7):650–654. https://doi.org/10.1055/s-2007-981523
23. An SJ, Park SK, Hwang IK, Choi SY, Kim SK, Kwon OS, Jung SJ, Baek NI, Lee HY, Won MH, Kang TC (2003) Gastrodin decreases immunoreactivities of gamma-aminobutyric acid shunt enzymes in the hippocampus of seizure-sensitive gerbils. J Neurosci Res 71(4):534–543. https://doi.org/10.1002/jnr.10502
24. Chen L, Liu X, Wang H, Qu M (2017) Gastrodin attenuates pentylenetetrazole-induced seizures by modulating the mitogen-activated protein kinase-associated inflammatory responses in mice. Neurosci Bull 33(3):264–272. https://doi.org/10.1007/s12264-016-0084-z
25. Zhou Z, Lin Y, Zheng H, He Y, Xu H, Zhang S, Weng W, Li W, Zhu L, Yang H (2015) Anticonvulsive and neuroprotective effects of synergetic combination of phenytoin and gastrodin on the convulsion induced by penicillin in mice. Fundam Clin Pharmacol 29(4):371–381. https://doi.org/10.1111/fcp.12127
26. Shao H, Yang Y, Qi AP, Hong P, Zhu GX, Cao XY, Ji WG, Zhu ZR (2017) Gastrodin reduces the severity of status epilepticus in the rat pilocarpine model of temporal lobe epilepsy by inhibiting Nav1.6 sodium currents. Neurochem Res 42(2):360–374. https://doi.org/10.1007/s11064-016-2079-6
27. Shao YY, Li B, Huang YM, Luo Q, Xie YM, Chen YH (2017) Thymoquinone attenuates brain injury via an anti-oxidative pathway in a status epilepticus rat model. Transl Neurosci 8:9–14. https://doi.org/10.1515/tnsci-2017-0003
28. Mazhar F, Malhi SM, Simjee SU (2017) Comparative studies on the effects of clinically used anticonvulsants on the oxidative stress biomarkers in pentylenetetrazole-induced kindling model of epileptogenesis in mice. J Basic Clin Physiol Pharmacol 28(1):31–42. https://doi.org/10.1515/jbcpp-2016-0034
29. Santos IM, Tome Ada R, Saldanha GB, Ferreira PM, Militao GC, Freitas RM (2009) Oxidative stress in the hippocampus during experimental seizures can be ameliorated with the antioxidant ascorbic acid. Oxid Med Cell Longev 2(4):214–221. https://doi.org/10.4161/oxim.2.4.8876
30. Migliore L, Fontana I, Colognato R, Coppede F, Siciliano G, Murri L (2005) Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. Neurobiol Aging 26(5):587–595. https://doi.org/10.1016/j.neurobiolaging.2004.10.002
31. Kikuchi A, Takeda A, Onodera H, Kimpara T, Hisanaga K, Sato N, Nunomura A, Castellani RJ, Perry G, Smith MA, Itoyama Y (2002) Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. Neurobiol Dis 9(2):244–248. https://doi.org/10.1006/nbdi.2002.0466
32. Perry G, Nunomura A, Hirai K, Zhu X, Perez M, Avila J, Castellani RJ, Atwood CS, Aliev G, Sayre LM, Takeda A, Smith MA (2002) Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? Free Radic Biol Med 33(11):1475–1479
33. Perry G, Taddeo MA, Nunomura A, Zhu X, Zenteno-Savin T, Drew KL, Shimohama S, Avila J, Castellani RJ, Smith MA

(2002) Comparative biology and pathology of oxidative stress in Alzheimer and other neurodegenerative diseases: beyond damage and response. Comp Biochem Physiol Toxicol Pharmacol 133(4):507–513
34. Zhang QF, Li YW, Liu ZH, Chen QL (2016) Exposure to mercuric chloride induces developmental damage, oxidative stress and immunotoxicity in zebrafish embryos-larvae. Aquatic Toxicol 181:76–85. https://doi.org/10.1016/j.aquatox.2016.10.029
35. Peternel S, Pilipovic K, Zupan G (2009) Seizure susceptibility and the brain regional sensitivity to oxidative stress in male and female rats in the lithium-pilocarpine model of temporal lobe epilepsy. Prog Neuro-psychopharmacol Biol Psychiatry 33(3):456–462. https://doi.org/10.1016/j.pnpbp.2009.01.005
36. Dal-Pizzol F, Klamt F, Vianna MM, Schroder N, Quevedo J, Benfato MS, Moreira JC, Walz R (2000) Lipid peroxidation in hippocampus early and late after status epilepticus induced by pilocarpine or kainic acid in Wistar rats. Neurosci Lett 291(3):179–182
37. Pazdernik TL, Emerson MR, Cross R, Nelson SR, Samson FE (2001) Soman-induced seizures: limbic activity, oxidative stress and neuroprotective proteins. J Appl Toxicol 21(Suppl 1):S87–S94
38. Lapin IP, Mirzaev SM, Ryzov IV, Oxenkrug GF (1998) Anticonvulsant activity of melatonin against seizures induced by quinolinate, kainate, glutamate, NMDA, and pentylenetetrazole in mice. J Pineal Res 24(4):215–218
39. Westerfield M (1993) The zebrafish book: a guide for the laboratory use of zebrafish (*Brachydanio rerio*). University of Oregon, Eugene
40. Siebel AM, Menezes FP, da Costa Schaefer I, Petersen BD, Bonan CD (2015) Rapamycin suppresses PTZ-induced seizures at different developmental stages of zebrafish. Pharmacol Biochem Behav 139(Pt B):163–168. https://doi.org/10.1016/j.pbb.2015.05.022
41. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
42. Elstner EF, Heupel A (1976) Inhibition of nitrite formation from hydroxylammoniumchloride: a simple assay for superoxide dismutase. Anal Biochem 70(2):616–620
43. Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126
44. Jaskot RH, Charlet EG, Grose EC, Grady MA, Roycroft JH (1983) An automated analysis of glutathione peroxidase, S-transferase, and reductase activity in animal tissue. J Anal Toxicol 7(2):86–88
45. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2):351–358
46. Li JM, Shah AM (2001) Differential NADPH- versus NADH-dependent superoxide production by phagocyte-type endothelial cell NADPH oxidase. Cardiovasc Res 52(3):477–486
47. Afrikanova T, Serruys AS, Buenafe OE, Clinckers R, Smolders I, de Witte PA, Crawford AD, Esguerra CV (2013) Validation of the zebrafish pentylenetetrazol seizure model: locomotor versus electrographic responses to antiepileptic drugs. PloS ONE 8(1):e54166. https://doi.org/10.1371/journal.pone.0054166
48. Berghmans S, Hunt J, Roach A, Goldsmith P (2007) Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants. Epilepsy Res 75(1):18–28. https://doi.org/10.1016/j.eplepsyres.2007.03.015
49. Herrera DG, Robertson HA (1996) Activation of c-fos in the brain. Prog Neurobiol 50(2–3):83–107
50. Kovac S, Domijan AM, Walker MC, Abramov AY (2014) Seizure activity results in calcium- and mitochondria-independent ROS production via NADPH and xanthine oxidase activation. Cell Death Dis 5:e1442. https://doi.org/10.1038/cddis.2014.390
51. Jaquet V, Scapozza L, Clark RA, Krause KH, Lambeth JD (2009) Small-molecule NOX inhibitors: ROS-generating NADPH oxidases as therapeutic targets. Antioxid Redox Signal 11(10):2535–2552. https://doi.org/10.1089/ARS.2009.2585
52. Du F, Wang X, Shang B, Fang J, Xi Y, Li A, Diao Y (2016) Gastrodin ameliorates spinal cord injury via antioxidant and anti-inflammatory effects. Acta Biochimica Polonica 63(3):589–593. https://doi.org/10.18388/abp.2016_1272
53. Li Y, Zhang Z (2015) Gastrodin improves cognitive dysfunction and decreases oxidative stress in vascular dementia rats induced by chronic ischemia. Int J Clin Exp Pathol 8(11):14099–14109
54. Qu LL, Yu B, Li Z, Jiang WX, Jiang JD, Kong WJ (2016) Gastrodin ameliorates oxidative stress and proinflammatory response in nonalcoholic fatty liver disease through the AMPK/Nrf2 pathway. Phytother Res 30(3):402–411. https://doi.org/10.1002/ptr.5541
55. Kobayashi M, Yamamoto M (2005) Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. Antioxid Redox Signal 7(3–4):385–394. https://doi.org/10.1089/ars.2005.7.385
56. Kaspar JW, Niture SK, Jaiswal AK (2009) Nrf2:INrf2 (Keap1) signaling in oxidative stress. Free Radic Biol Med 47(9):1304–1309. https://doi.org/10.1016/j.freeradbiomed.2009.07.035
57. Kobayashi M, Itoh K, Suzuki T, Osanai H, Nishikawa K, Katoh Y, Takagi Y, Yamamoto M (2002) Identification of the interactive interface and phylogenic conservation of the Nrf2-Keap1 system. Genes Cells Devot Mol Cell Mech 7(8):807–820
58. Realmuto S, Zummo L, Cerami C, Agro L, Dodich A, Canessa N, Zizzo A, Fierro B, Daniele O (2015) Social cognition dysfunctions in patients with epilepsy: evidence from patients with temporal lobe and idiopathic generalized epilepsies. Epilepsy Behav 47:98–103. https://doi.org/10.1016/j.yebeh.2015.04.048
59. Austin JK, Dunn DW (2002) Progressive behavioral changes in children with epilepsy. Prog Brain Res 135:419–427. https://doi.org/10.1016/S0079-6123(02)35039-8
60. Hunt RF, Hortopan GA, Gillespie A, Baraban SC (2012) A novel zebrafish model of hyperthermia-induced seizures reveals a role for TRPV4 channels and NMDA-type glutamate receptors. Exp Neurol 237(1):199–206. https://doi.org/10.1016/j.expneurol.2012.06.013
61. Jirsa VK, Stacey WC, Quilichini PP, Ivanov AI, Bernard C (2014) On the nature of seizure dynamics. Brain 137(Pt 8):2210–2230. https://doi.org/10.1093/brain/awu133
62. Schauwecker PE (2011) The relevance of individual genetic background and its role in animal models of epilepsy. Epilepsy Res 97(1–2):1–11. https://doi.org/10.1016/j.eplepsyres.2011.09.005
63. Deshpande LS, Lou JK, Mian A, Blair RE, Sombati S, Attkisson E, DeLorenzo RJ (2008) Time course and mechanism of hippocampal neuronal death in an in vitro model of status epilepticus: role of NMDA receptor activation and NMDA dependent calcium entry. Eur J Pharmacol 583(1):73–83. https://doi.org/10.1016/j.ejphar.2008.01.025
64. Wong SB, Hung WC, Min MY (2016) The role of gastrodin on hippocampal neurons after N-Methyl-D-Aspartate excitotoxicity and experimental temporal lobe seizures. Chin J Physiol 59(3):156–164. https://doi.org/10.4077/CJP.2016.BAE385
65. Torres-Hernandez BA, Del Valle-Mojica LM, Ortiz JG (2015) Valerenic acid and *Valeriana officinalis* extracts delay onset of pentylenetetrazole (PTZ)-Induced seizures in adult *Danio rerio* (Zebrafish). BMC Complem Altern Med 15:228. https://doi.org/10.1186/s12906-015-0731-3
66. Kim JH, Jang BG, Choi BY, Kim HS, Sohn M, Chung TN, Choi HC, Song HK, Suh SW (2013) Post-treatment of an NADPH oxidase inhibitor prevents seizure-induced neuronal death. Brain Res 1499:163–172. https://doi.org/10.1016/j.brainres.2013.01.007
67. Limon-Pacheco J, Gonsebatt ME (2009) The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutat Res 674(1–2):137–147. https://doi.org/10.1016/j.mrgentox.2008.09.015

68. Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101(1):13–30. https://doi.org/10.1016/j.aquatox.2010.10.006
69. Kohen R, Nyska A (2002) Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol 30(6):620–650. https://doi.org/10.1080/01926230290166724
70. Diaz-Ruiz A, Mendez-Armenta M, Galvan-Arzate S, Manjarrez J, Nava-Ruiz C, Santander I, Balderas G, Rios C (2013) Antioxidant, anticonvulsive and neuroprotective effects of dapsone and phenobarbital against kainic acid-induced damage in rats. Neurochem Res 38(9):1819–1827. https://doi.org/10.1007/s11064-013-1087-z
71. Zhao X, Wang S, Wu Y, You H, Lv L (2013) Acute ZnO nanoparticles exposure induces developmental toxicity, oxidative stress and DNA damage in embryo-larval zebrafish. Aquat Toxicol 136–137:49–59. https://doi.org/10.1016/j.aquatox.2013.03.019
72. Keskin Guler S, Aytac B, Durak ZE, Gokce Cokal B, Gunes N, Durak I, Yoldas T (2016) Antioxidative-oxidative balance in epilepsy patients on antiepileptic therapy: a prospective case-control study. Neurol Sci 37(5):763–767. https://doi.org/10.1007/s10072-016-2494-0
73. Wang L, Gallagher EP (2013) Role of Nrf2 antioxidant defense in mitigating cadmium-induced oxidative stress in the olfactory system of zebrafish. Toxicol Appl Pharmacol 266(2):177–186. https://doi.org/10.1016/j.taap.2012.11.010
74. Osburn WO, Kensler TW (2008) Nrf2 signaling: an adaptive response pathway for protection against environmental toxic insults. Mutat Res 659(1–2):31–39. https://doi.org/10.1016/j.mrrev.2007.11.006
75. Dai JN, Zong Y, Zhong LM, Li YM, Zhang W, Bian LG, Ai QL, Liu YD, Sun J, Lu D (2011) Gastrodin inhibits expression of inducible NO synthase, cyclooxygenase-2 and proinflammatory cytokines in cultured LPS-stimulated microglia via MAPK pathways. PloS ONE 6(7):e21891. https://doi.org/10.1371/journal.pone.0021891
76. Yang P, Han Y, Gui L, Sun J, Chen YL, Song R, Guo JZ, Xie YN, Lu D, Sun L (2013) Gastrodin attenuation of the inflammatory response in H9c2 cardiomyocytes involves inhibition of NF-kappaB and MAPKs activation via the phosphatidylinositol 3-kinase signaling. Biochem Pharmacol 85(8):1124–1133. https://doi.org/10.1016/j.bcp.2013.01.020
77. Baek NI, Choi SY, Park JK, Cho SW, Ahn EM, Jeon SG, Lee BR, Bahn JH, Kim YK, Shon IH (1999) Isolation and identification of succinic semialdehyde dehydrogenase inhibitory compound from the rhizome of *Gastrodia elata* Blume. Arch Pharm Res 22(2):219–224