Purinergic Signalling (2022) 18:307–315
https://doi.org/10.1007/s11302-022-09869-z

ORIGINAL ARTICLE

Gallic acid modulates purine metabolism and oxidative stress induced by ethanol exposure in zebrafish brain

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Received: 9 September 2021 / Accepted: 9 May 2022 / Published online: 10 June 2022

Abstract
Gallic acid (GA) is a secondary metabolite found in plants. It has the ability to cross the blood-brain barrier and, through scavenging properties, has a protective effect in a brain insult model. Alcohol metabolism generates reactive oxygen species (ROS); thus, alcohol abuse has a deleterious effect on the brain. The zebrafish is a vertebrate often used for screening toxic substances and in acute ethanol exposure models. The aim of this study was to evaluate whether GA pretreatment (24 h) prevents the changes induced by acute ethanol exposure (1 h) in the purinergic signaling pathway in the zebrafish brain via degradation of extracellular nucleotides and oxidative stress. The nucleotide cascade promoted by the nucleoside triphosphate diphosphohydrolase (NTPDase) and 5′-nucleotidase was assessed by quantifying nucleotide metabolism. The effect of GA alone at 5 and 10 mg L^{-1} did not change the nucleotide levels. Pretreatment with 10 mg L^{-1} GA prevented an ethanol-induced increase in ATP and ADP levels. No significant difference was found between the AMP levels of the two pretreatment groups. Pretreatment with 10 mg L^{-1} GA prevented ethanol-enhanced lipid peroxidation and dichlorodihydrofluorescein (DCFH) levels. The higher GA concentration was also shown to positively modulate against ethanol-induced effects on superoxide dismutase (SOD), but not on catalase (CAT). This study demonstrated that GA prevents the inhibitory effect of ethanol on NTPDase activity and oxidative stress parameters, thus consequently modulating nucleotide levels that may contribute to the possible protective effects induced by alcohol and purinergic signaling.

Keywords Purinergic system · Ethanol · Gallic acid · Oxidative stress · Zebrafish

Samira Leila Baldin She started her activities as an undergraduate student in 2015 at the Laboratory of Experimental Neurology led by Professor Eduardo Pacheco Rico, where she worked with zebrafish as a quality bioindicator for body water contaminated with residual coal extraction. Few years later, after her graduation at Biological Science, she started her Master Degree in 2018 and due to that, she changed tremendously the focus of her research from environmental toxicology to neuroscience, more specifically how alcohol consumption can impact the purinergic system. She also collaborated with other projects that gave to her the experience in biochemistry, behavior, glutamatergic, dopaminergic and cholinergic signalization.

Highlights
• Gallic acid reduces oxidative stress induced by acute ethanol in the zebrafish brain.
• Gallic acid prevents disruption of NTPDase activity promoted by ethanol.
• Ethanol alters the degradation of extracellular nucleotides in the zebrafish brain.

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Abbreviations

DCF	2′,7′-dihydrodichlorofluorescein diacetate
ATP	Adenosine 5′ triphosphate
CAT	Catalase
CNS	Central nervous system
DTNB	5,5-dithio-bis-(2-nitrobenzoic acid
NTPDase	Nucleoside triphosphate diphosphohydrolase
SOD	Superoxide dismutase
TBA-RS	Thiobarbituric acid-reactive species

Introduction

Gallic acid (GA) (3,4,5-trihydroxybenzoic acid) is a phenolic compound derived from secondary plant metabolism via the shikimic acid pathway, with the dehydrogenation of 5-dehydroshikimic acid being the predominant pathway suggested [1, 2]. This molecule has the potential for many protective activities, including anti-apoptotic [3], antimicrobial [1], and anticancer activities [4, 5]. Furthermore, GA has been shown to enter the central nervous system (CNS) by penetrating the blood-brain barrier and to have neuroprotective and antioxidant potential [6–8]. Studies have shown the effectiveness of using GA for several neurological disorders such as the 6-hydroxydopamine-induced Parkinson model [7], stroke via ischemia and reperfusion [9], and attenuation of β-amyloid-induced neurotoxicity [10]. However, there are no data on the effects of GA on the disruption of the CNS induced by alcohol toxicity.

Ethanol is a psychoactive toxic substance, the excessive consumption of which is considered worldwide to be a public health problem resulting in 2.5 million deaths each year [11]. Acute ethanol intake leads to an imbalance of oxidative stress and neurotransmission pathways in the CNS, which can be related to impairment of neurological functions such as motor response and cognition [12, 13].

From the translational neuroscience perspective, the zebrafish provides a suitable animal model for research on the mechanisms underlying alcohol toxicity [14, 15]. Several neurotransmitter systems have been identified (GABA, glutamate, acetylcholine (ACh), catecholamines, and purines), and their modulation by ethanol and its metabolites, including acetaldehyde and acetate, have been verified [16, 17]. Regarding purinergic signaling, after the release of ATP into the synaptic cleft, its extracellular levels are broken down to adenosine via enzymes called ectonucleotidases [18]. These enzymes are responsible for regulating nucleotide and nucleoside levels and their respective purine receptors and are involved in both physiological and physiopathologic processes [19, 20]. Purinergic ionotropic and metabotropic P2 receptors have been identified in zebrafish [21–23]. The enzyme cascade that hydrolyzes extracellular ATP to adenosine was studied by investigating nucleoside triphosphate diphosphohydrolase (NTPDase) and ecto-5′-nucleotidase activities and gene expression in zebrafish brain [24–26].

Behavioral and neurochemical screen studies in zebrafish offer an intriguing alternate preclinical approach to CNS drug discovery [27]. The potential neuroprotective effects of molecules such as gold nanoparticles, taurine, and plant products against ethanol-induced CNS disruption have been addressed [28, 29]. Recently, our group showed that GA positively modulates cholinergic signaling altered by acute ethanol exposure and decreases oxidative stress in the zebrafish brain [30].

Thus, considering the properties of GA and its potential neuroprotective effects, its use in investigating neurotoxicity in zebrafish is promising and relevant. However, there is little published data on the role of candidate molecules in models of toxicity induced by alcohol treatment or abuse. Accordingly, we aimed to evaluate the effects of the triphenolic compound GA on purinergic signaling via the cascade of ectonucleotidase activities in the zebrafish brain. In addition, the effect of acute EtOH exposure on neurochemical redox profile and the influence of GA treatments on neurochemical oxidative stress parameters and enzymatic antioxidant defenses were also studied.

Methods

Animals

Adult short-fin wild-type zebrafish (*Danio rerio*) of both sexes (5 months old, ~ 50:50 male/female ratio and weighing 0.400 ± 0.05 g) were obtained from the Department of Biochemistry at Federal University of Rio Grande do Sul (UFRGS). The animals were acclimated in our laboratory for at least 2 weeks in a 40-L aquarium with reverse-osmosis-filtered water equilibrated to achieve the appropriate temperature (28 ± 2 °C), pH (7.0 and 7.5), conductivity (400 to 600 µS) and ammonia < 0.02 mg L^{-1}, nitrite < 0.01 mg L^{-1}, and nitrate < 0.01 mg L^{-1}, required for this species. Fish were fed twice daily with commercial flake food (Alcon Basic®, Alcon, Brazil) supplemented with brine shrimp and subjected to a light/dark cycle of 14/10 h, respectively [31]. All protocols were conducted in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals and approved by the Ethics Committee of University of Southern Santa Catarina (UNESC) protocol number 082/2018-1.

Chemicals

Ethanol (C_2H_6O; CAS number 64-17-5) was purchased from Merck (Darmstadt, Germany). Gallic acid ($(HO)3C_6H_2CO_2H$, CAS number 149-91-7) and all other reagents used were purchased from Sigma (St. Louis, MO, USA).

Experimental design and groups

To evaluate purine metabolism, 180 animals (five brain per *n*; *n* = 6) divided in six groups were introduced to the test aquariums (35 × 16 × 18 cm, length × width × height, 10 L), containing a pretreatment of GA solution at 5 and 10 mg L^{-1} during 24 h followed by exposure to 1% ethanol or not during 1 h (Fig. 1). For the oxidative stress analysis, 180 animals (five brain per *n*; *n* = 6) were introduced to the aquariums containing the solution of GA and/or 1% ethanol (v/v) corresponding to each group. Because of the size of the aquariums, the total number of fish per experiment, and the density, our strategy was to carry out multiple rounds using 15 animals per group until completing the sample number previously described. The same time of exposure and EtOH concentration have been established according to successfully tested behavioral and cerebral ectonucleotidase activity adult zebrafish [14, 16, 32]. All fish were carefully moved from the aquariums using a net, maintaining uniform handling among the experimental groups. The water used in the experiments was obtained from a reverse osmosis apparatus and was reconstituted with Instant Ocean® marine salt. At the end of the treatment, the animals were anesthetized by immersing them in 160 mg mL^{-1} of tricaine (4 °C), suffered euthanasia by decapitation, and their brains were removed from the cranial skull by the dissection technique.

Nucleotidase activities

Membrane fraction preparation

Five zebrafish brains were pooled and homogenized in 60 vol. (v/w) of chilled Tris-citrate buffer (50 mM Tris-citrate, 2 mM EDTA, 2 mM EGTA, pH 7.4) to prepare each homogenate fraction. The brain membrane fraction was prepared as described previously [33]. In brief, the homogenates were centrifuged at 800 × g for 10 min, and the supernatant fraction was subsequently centrifuged for 25 min at 40,000 × g. The pellets of membrane preparations were frozen in liquid nitrogen to ensure the lysis of the brain vesicle membranes, thawed, resuspended, and centrifuged for 20 min at 40,000×g. The final pellets were resuspended and used for enzyme assays. All samples were maintained at 2–4 °C throughout preparation.

Analysis of ATP metabolism

Membrane fractions were incubated as previously described [34]. The reaction medium contained 50 mM Tris–HCl (pH 8.0) and 5 mM $CaCl_2$ (for NTPDase activities) in a final volume of 200 µL. The membrane preparation (30 µg protein) was added to the reaction mixture and preincubated for 10 min at 37 °C. To start the reaction, ATP was added to the medium in a final concentration of 0.1 mM at 37 °C. Aliquots of the sample were collected at different incubation times (0, 5, 10, 30, 60, 90, 120, and 180 min) and immediately placed on ice. All samples were centrifuged 14,000×g for 15 min and stored on − 80 °C until high-performance liquid chromatography (HPLC) analysis. An HPLC system equipped with an isocratic pump, a diode array detector (DAD), a degasser, and a manual injection system was used (Agilent Technologies, Santa Clara, CA, USA). Aliquots of 100 µL were applied into

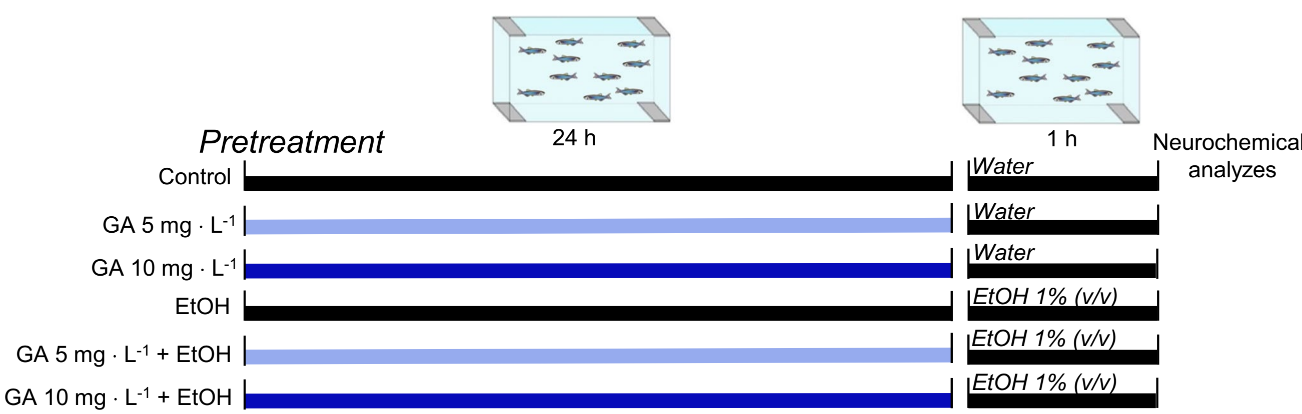

Fig. 1 Experimental protocol for exposure to ethanol and GA

the HPLC system, and chromatographic separations were performed using a reverse-phase column (150 × 4 mm, 5 μm Agilent® 100 RP-18 ec). The flow rate of the 60 mM KH_2PO_4, 5 mM tetrabutylammonium chloride, pH 6.0, in 13% methanol mobile phase was 1.2 mL/min. The absorbance was monitored at 260 nm, according to a method previously described, with few modifications [35]. The peaks of purines (ATP, ADP, and AMP) were identified by their retention times and quantified by comparison with standards. The results are expressed as micromolar of the different compounds for each different incubation times. All incubations were carried out in four independent experiments. Data were expressed as μM of nucleotide for each incubation time obtained by the area under the curve calculated for all homogenate fractions.

Oxidative stress parameters

Tissue preparation

Zebrafish brains (pool of five structures) were homogenized in 1 mL of 20 mM sodium phosphate buffer, pH 7.4, containing 140 mM potassium chloride. Homogenate fractions were centrifuged at 750 × g for 10 min at 4 °C to discard cellular debris. The pellet was discarded, and the supernatant was collected. All oxidative stress analyses were adapted from previous studies in zebrafish [36, 37].

TBA-RS levels

Lipid peroxidation by thiobarbituric acid-reactive species (TBA-RS) levels was determined according to Esterbauer and Cheeseman (1990) [38]. A calibration curve was established using 1,1,3,3-tetramethoxypropane, and each curve point was subjected to the same treatment as supernatants. TBA-RS values were expressed as nmol of TBA-RS.mg protein^{-1}.

DCFH oxidation

Reactive species production was assessed according to previously described using 2′,7′-dihydrodichlorofluorescein diacetate (DCF) [39]. The DCF fluorescence intensity parallels the amount of reactive species formed. A calibration curve was performed using standard DCF concentration range of 0.25 to 10 μM, and the levels of reactive species were expressed as nmol DCF formed mg of protein^{-1}.

Sulfhydryl (thiol) group oxidation

This assay is based on the reduction of 5,5-dithio-bis-(2-nitrobenzoic acid (DTNB) by thiols, generating a yellow derivative (TNB) whose absorbance is measured at 412 nm. The protein-bound sulfhydryl content is inversely correlated to oxidative damage to proteins. Results were expressed as nmol TNB mg protein^{-1} [40].

Antioxidant enzyme activities

A catalase (CAT; EC 1.11.1.6) activity assay was performed by measuring the absorbance decrease at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100, 10 mM potassium phosphate buffer, pH 7.0, and supernatants containing 0.1–0.3 mg protein mL^{-1} [41]. The specific activity was expressed as nmol min^{-1} mg protein^{-1}. Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined using a spectrophotometric assay based on the superoxide-dependent oxidation of epinephrine to adrenochrome at 32 °C [42]. Absorbance was measured at 480 nm. The reaction medium consisted of 50 mM glycine buffer, pH 10.2, 0.1 mM catalase, and 1 mM epinephrine. SOD-specific activity is expressed as nmol min^{-1} mg protein^{-1}.

Protein determination

Total protein content was measured using aliquots of homogenate (10 μl) following the method described by Peterson (1977) [43].

Statistical analysis

All experiments were carried out in triplicate and means ± S.E.M. of six independent experiments were presented. All data were tested for normality using a Shapiro-Wilk's test and Levene's test to examine homogeneity of variance. Because all data showed a parametric distribution, results were analyzed by two-way analysis of variance (ANOVA), followed by Tukey's multiple range test. Differences between groups were considered significant when $p < 0.05$. All analyses were performed using the GraphPad Prism 6.01 statistical program (GraphPad Software Inc., San Diego, CA, USA).

Results

Gallic acid prevents changes in nucleotidase activity induced by EtOH exposure

The nucleotide cascade promoted by NTPDase and 5′-nucleotidase was assessed by quantifying ATP hydrolysis at different incubation times (Fig. 2). Our findings confirmed the profile of nucleotide breakdown hydrolysis (0–180 min) previously described by Altenhofen et al. [34]. We observed the decrease in ATP from 82 ± 2 to 40.2 ± 3 μM (Fig. 2A) within the first 30 min, with a concomitant increase in ADP

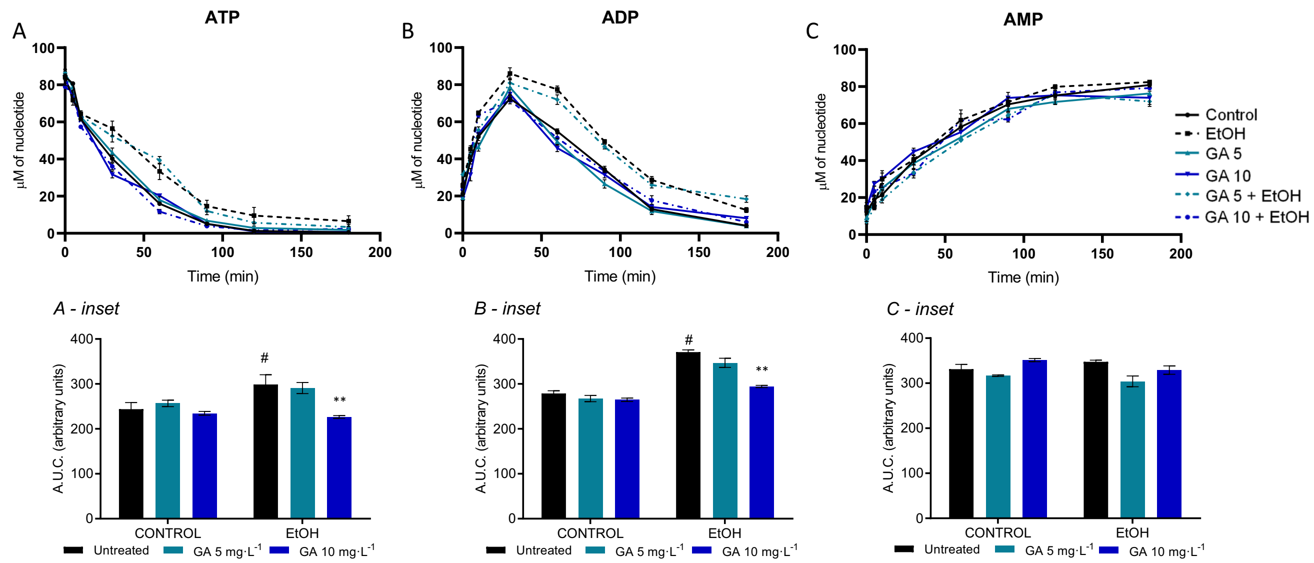

Fig. 2 Effects of GA pretreatment and acute ethanol exposure on ATP metabolism and its degradation products in zebrafish brain. **A** ATP, **B** ADP, and **C** AMP were assayed using high-performance liquid chromatography-diode array detection (HPLC-DAD). Bars represent the mean ± SEM of six independent experiments. Significant difference between the untreated group and the GA pretreatment groups (**$p < 0.01$). #Significant difference between the control group and the ethanol-treated group (two-way ANOVA followed by Tukey's test as post hoc analysis, $p < 0.05$)

from 23.3 ± 2 to 71.5 ± 5 µM (Fig. 2B). After 30 min, we observed decreases in the levels of both ATP (from 40.2 ± 3.2 to 3.3 ± 1.5 µM) and ADP (from 71.5 ± 5 to 3.8 ± 1.3 µM) with similar profiles and their entire hydrolysis. The NTPDase hydrolysis of ATP and ADP caused the constant formation of AMP, reaching the highest level after 90 min (Fig. 2C).

To verify whether ethanol and GA pretreatment altered nucleotidase activities, the areas under the curve were calculated for all groups (Fig. 2A–C, inset). A two-way analysis of variance (ANOVA) showed that the interaction of GA and ethanol had a significant difference ($F_{2,12}$ = 3.399, $p < 0.05$) (Fig. 2A, inset). The effect of GA alone at 5 and 10 mg L^{-1} did not change the profile of the ATP levels. Post hoc analyses indicated that pretreatment with 10 mg L^{-1} GA prevented (24.2%, $p < 0.01$) an ethanol-induced increase (22.5%, $p < 0.05$) in ATP levels. Ethanol and GA had a significant effect ($F_{2,12}$ = 13.86, $p < 0.05$) on ADP levels (Fig. 2B, inset). An increase in the area under the curve for ADP (33%, $p < 0.05$) was observed for the group not treated with GA and exposed to ethanol compared to the group not treated with GA and not exposed to ethanol. Post hoc analysis indicated that the ADP level of the group pretreated with 10 mg L^{-1} GA and exposed to ethanol was reduced (20.6%, $p < 0.01$) compared to that of the group only exposed to ethanol (Fig. 2B, inset). No significant difference in AMP levels was found among the groups (Fig. 2C, inset).

Gallic acid prevents ethanol-induced oxidative stress and enzymatic antioxidant activities

Previous reports have shown that acute ethanol exposure disrupts oxidative balance [28, 29]. We found that ethanol and GA treatment altered lipid peroxidation in zebrafish brain ($F_{2,25}$ = 13.54, $p < 0.05$). Figure 3 A shows an increase (50.5%, $p < 0.01$) in the levels of thiobarbituric acid reactive substances (TBA-RSs) for the group exposed to ethanol only compared to those of the untreated group. In contrast, post hoc analysis indicated that pretreatment with 10 mg L^{-1} GA prevented an ethanol-induced increase in TBA-RS (96.8%, $p < 0.05$). Two-way ANOVA showed that GA pretreatment plus ethanol exposure had a significant effect on dichlorodihydrofluorescein (DCFH) oxidation ($F_{2,25}$ = 16.74, $p < 0.001$) (Fig. 3B). Zebrafish not treated with GA showed an increase in DCFH oxidation after ethanol exposure (54.1%, $p < 0.01$). Pretreatment with 10 mg L^{-1} GA followed by ethanol exposure significantly decreased DCFH oxidation (63.4%, $p < 0.01$) compared to that after exposure to ethanol alone. Pretreatment with 10 mg L^{-1} GA alone increased DCFH oxidation compared to that for the untreated control group (32.9%, $p < 0.05$). Assessment of the protein sulfhydryl content showed there was no change among the groups evaluated ($F_{2,28}$ = 0.8442, ($p > 0.05$: Fig. 3C).

Antioxidant activities promoted by superoxide dismutase (SOD) and catalase (CAT) prevent the formation of reactive oxygen species (ROS). Pretreatment with higher concentrations of GA showed a positive modulation effect against

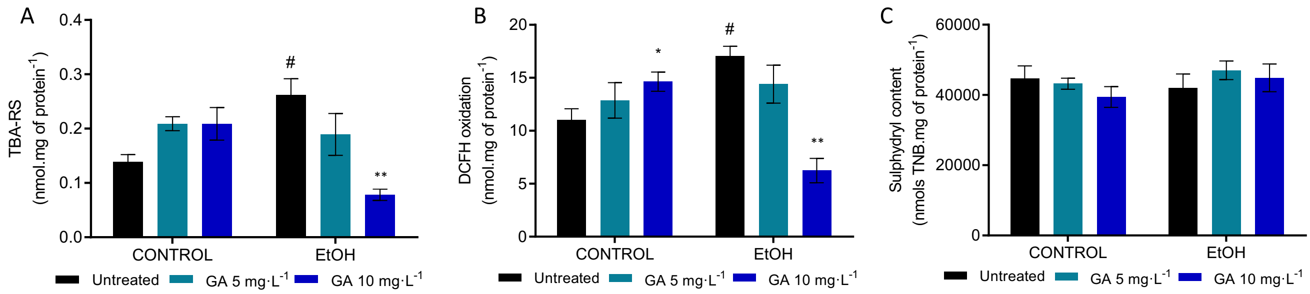

Fig. 3 Effect of GA pretreatment at concentrations of 5 and 10 mg mL^{-1} after exposure to ethanol on **A** TBA-RS levels, **B** DCFH oxidation, and **C** sulfhydryl content in zebrafish brain. Bars represent the mean ± SEM of six independent experiments. Significant difference between the untreated group and the GA pretreatment groups (*p <0.05; **p <0.01). #Significant difference between the control group and the ethanol-treated group (two-way ANOVA followed by Tukey's test as post hoc analysis, $p < 0.05$)

ethanol-induced effects on nucleotidase and oxidative stress parameters. Therefore, we evaluated the effect of 10 mg L^{-1} GA on SOD and CAT activity (Fig. 4). Acute ethanol exposure significantly decreased SOD activity (35.2%, $p <$ 0.05), but the decrease was prevented by pretreatment with 10 mg L^{-1} GA (96.6%, p <0.05) (Fig. 4A). Ethanol and GA pretreatment induced changes in CAT activity (Fig. 4B). A significant increase in CAT activity was observed with 10 mg L^{-1} GA alone (224.2%, p < 0.001) and with ethanol exposure alone (96.2%, p < 0.01).

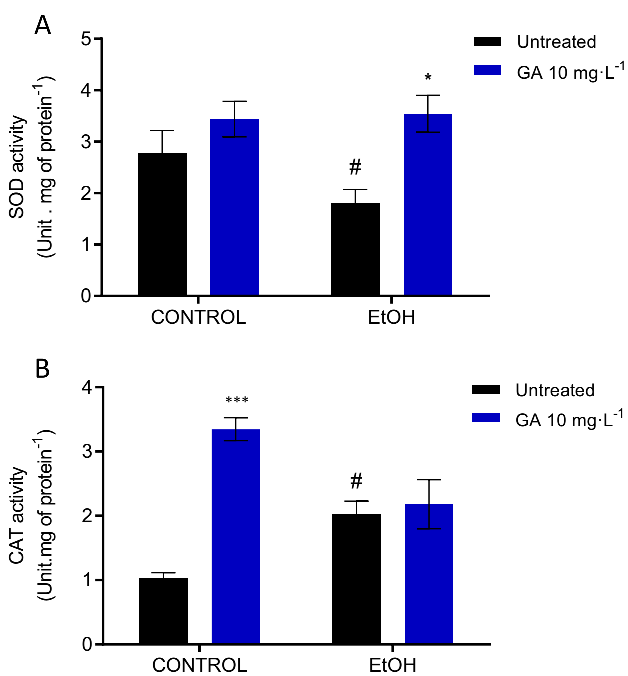

Fig. 4 Effect of GA treatment at concentrations of 5 and 10 mg mL^{-1} after exposure to ethanol on **A** SOD and **B** CAT activities in zebrafish brain. Bars represent the mean ± SEM of six independent experiments. Significant difference between the untreated group and the GA treatment groups (*p <0.05; ***p <0.001). #Significant difference between the control group and the ethanol-treated group (two-way ANOVA followed by Tukey's test as post hoc analysis, p < 0.05)

Discussion

Ethanol is a GABA agonist that assists postsynaptic inhibitory activity and, consequently, is involved in depressant effects [44, 45]. During ethanol exposure, the major inhibitory/excitatory neurotransmitter (i.e., GABA or glutamate) stimulates the restoration of balance in the CNS [46, 47]. Purinergic signaling via ectonucleotidases, P2X-ionotropic and P2Y-metabotropic receptors, and nucleoside transporters have been shown to be mediated by not only adenosine-ATP neurotransmission, but also by the homeostasis of major inhibitory/excitatory neurotransmission via neuron-glia interactions [48, 49]. Purinergic signaling plays an important role in neuronal and non-neuronal signaling and is involved in the regulation of extracellular medium and neurological disorders such as excessive alcohol consumption [50].

Studies have demonstrated the susceptibility of zebrafish to short-term exposure to ethanol. The effect of acute alcohol exposure on the behavior and various neurotransmitter systems of zebrafish makes this species an attractive model for research in neuroscience and pharmacological studies [51]. In this study, GA prevented ethanol-induced alterations by attenuating the purinergic system and oxidative balance in the zebrafish brain. Rico et al. [16] evaluated ATP, ADP, and AMP hydrolysis by separately measuring the release of inorganic phosphate. In this study, using ATP degradation analysis, we demonstrated that ethanol acutely inhibited NTPDase activity but not AMP levels. In this context, our findings lead to the conclusion that acute alcohol toxicity affects the levels of di- and triphosphate nucleotides and, consequently, the inhibitory response of NTPDases using a non-saturating concentration of ATP. Therefore, the inhibitory influence exerted by ethanol on ectonucleotidases could be an important regulatory mechanism that control external concentration of nucleotides and hence regulate P2-mediated signaling. These findings suggest that acute exposure to ethanol may lead to neurological dysfunctions by modulating purinergic ion channels and signal transduction pathways indirectly, probably by affecting the activity of NTPDases in the zebrafish brain.

To determine whether GA could prevent the inhibition of NTPDase activity by ethanol, we pretreated zebrafish brain with different GA concentrations for 1 h. The results demonstrated that only GA at 10 mg L^{-1} prevented the ethanol-induced inhibition of NTPDase. These enzymes are anchored in the plasma membrane, and their catalytic site is on the extracellular face. The surface-located E-NTPDases are glycosylated and share their general membrane topology with two transmembrane domains, which play an important role in the function and regulation of the enzymes, in addition to anchoring them in the plasma membrane [52]. In zebrafish, the deduced amino acid sequences share conserved regions for E-NTPDases, putative *N*-glycosylation, different numbers of transmembrane domains, and distinct gene expression (*entpd1-6* and *entpd8*) in the brain [25]. In this context, the preservation of plasma membrane integrity and the conformational structure of these proteins are pivotal to maintaining their catalytic activity.

The ethanol-induced alteration of NTPDase activity could be due to oxidative stress, including that caused by lipid peroxidation. At the time and concentration at which the effect of ethanol was evaluated, TBA-RS levels had increased, suggesting that lipid peroxidation occurred. Lipid peroxidation leads to the formation of ROS, which sustain this lipid peroxidation through a cascade process [53]. This change in TBA-RS levels probably contributed to the presence of ROS, because DCFH oxidation increased by ethanol. Interestingly, GA at 10 mg L^{-1}, but not at 5 mg L^{-1}, prevented the enhancement of parameters related to either lipid peroxidation or ROS production. In addition, GA is a polyphenolic compound with antioxidant properties owing to its high capacity for free radical scavenging [54, 55]. Our data suggest that the decrease in lipid peroxidation and ROS production detected after pretreatment with 10 mg L^{-1} GA followed by alcohol exposure could correlate with the inhibitory effect observed on NTPDase activity. The interaction of ethanol with biological membranes because of its lipophilic nature could result in the native conformation of proteins and membrane integrity [56]. Furthermore, the mitigation of ROS and lipid peroxidation as a result of GA pretreatment is indicative of residual homeostasis, and the stability of protein functions could explain the preventive effect of GA pretreatment on NTPDase activity. These phenomena were also observed when GA reversed the changes induced on the cholinergic system by chronic ethanol exposure via impairment of choline acetyltransferase (ChAT) enzymatic activity in zebrafish brain [30].

Ethanol is a pro-oxidative molecule that causes both direct and indirect biological effects. Acetaldehyde, the first product of ethanol catabolism, is formed by the inclusion of alcohol dehydrogenase (ADH), cytochrome P4502E1 (CYP2E1), and CAT, which mediate some ethanol activities in the brain [57]. Furthermore, the association between the induction of acetaldehyde toxicity and oxidative stress is established [45]. SOD is the first line of enzymatic defense against the intracellular production of free radicals because it catalyzes the dismutation of superoxide radicals, which produces hydrogen peroxide, a substrate for the CAT enzyme [58]. In our study, ethanol caused a decrease in SOD activity with a concomitant increase in CAT activity. These findings corroborate those of previous studies [28, 29]. These changes could contribute to the imbalance between free radical formation and lipid peroxidation. Interestingly, GA at 10 mg L^{-1} restored SOD activity to normal levels, but not CAT. These findings suggest that this triphenolic compound might have a neuroprotective role by regulating antioxidant defenses through the control of O^{2-} levels in the brain. However, GA was not effective in mitigating the inhibitory effect on CAT activity. This evidence could be attributed to the participation of this enzyme in ethanol metabolism, which it uses as a substrate for the production of hydrogen peroxide in the brain.

Collectively, the results of our study suggest that GA prevent the inhibitory effect of ethanol on NTPDase activity and oxidative stress parameters. Thus, these findings contribute a new perspective regarding the role of the purinergic system in neurobehavioral events induced by the acute ethanol consumption and the search for compounds with a possible neuroprotective effect.

Author contribution SLB: investigation, validation, data curation, writing - original draft. KPP: conceptualization, concept and design. ACSF: investigation, validation, data curation, writing - original draft. HTB: formal analysis investigation, writing - review and editing. RS: data analysis and interpretation, writing - review and editing. BCP: investigation, validation, data curation. SDP: investigation, validation, data curation. ERD: formal analysis investigation, writing - review & editing. RAM: formal analysis investigation, writing - review and editing. AGW: formal analysis investigation, writing - review and editing. EPR: funding acquisition, project administration, supervision, writing — review and editing, resources. The paper was read, revised, and approved by all the authors.

Funding This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) research Grant (Universal 429302/2018-5), Fundação de Amparo à Pesquisa do Estado de Santa Catarina (FAPESC - Universal 12/2020), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES AUXPE PROEX N. 23038.020053/2018-52).

Data availability The authors confirm that the data supporting the findings of this study are available within the article. The raw data are available from Eduardo Pacheco Rico upon reasonable request.

Declarations

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study protocol was approved by the Ethics Committee of University of Southern Santa Catarina (UNESC), Criciúma, Brazil, number 030/2019-1.

Conflicts of Interest The authors declare no competing interests.

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