Coriandrum sativum Extract Prevents Alarm Substance-Induced Fear- and Anxiety-Like Responses in Adult Zebrafish

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Abstract

Anxiety disorders appear to involve distinct neurobiological mechanisms and several medications are available against this mental health problem. However, pharmacological therapeutic approaches display undesirable side effects for patients, particularly when long-term therapy is required. Some evidences have suggested that *Coriandrum sativum* extract (CSE) provide sedative and anxiolytic effects. We investigate if CSE could attenuate anxiety-like behaviors induced by novelty and alarm substance exposures in zebrafish. Adult zebrafish were injected with vehicle, clonazepam, or CSE (25, 50 or 100 mg/kg) and submitted to novel tank test. At the end, saline or alarm substance was added and anxiety-like responses were recorded. Twenty-four hours after, fish were submitted to the light/dark test. Novelty associated with alarm substance exposure decreased distance traveled and total time mobile in novel tank, and CSE (at 50 and 100 mg/kg) prevented these alterations similarly to clonazepam. Alarm substance reduced the time spent in white compartment ($p=0.0193$ as compared with vehicle group). Clonazepam and CSE prevented this anxiogenic effect of alarm substance. CSE presents anxiolytic effects against alarm substance-induced locomotor and anxiogenic responses similarly to clonazepam. These data corroborate with the use of this plant in traditional medicine and provides a putative new pharmacological intervention for anxiety disorders.

Keywords: anxiety, zebrafish, alarm substance, *Coriandrum sativum*

Introduction

ANXIETY DISORDERS ARE among the most prevalent and disabling mental health conditions worldwide.¹ They are considered risk factors for the development of additional mental health disorders and they are associated with an increased risk of suicide in adulthood.² Current pharmacological anxiety treatments include the use of selective serotonin reuptake inhibitors, benzodiazepines, alpha/delta calcium channel blockers, azapirones, and beta blockers.³ Although clinical trials have showed the efficacy of the mentioned drugs, they have displayed a long list of undesirable side

effects for patients, including sedation, muscle relaxation, amnesia, tolerance, and dependence, particularly when long-term therapy was required.⁴ These unwanted adverse effects have propelled scientists to search new chemical entities, mainly those from traditional medicine.⁵

In this context, several plants from different parts of the world, such as *Passiflora coerulea*, *Matricaria recutita*, *Valeriana officinalis*, *Salvia guaranitica*, *Tilia europaea*, and *Tilia tormentosa*, have been employed to discover new chemicals with putative sedative or anxiolytic effects.^{6,7}

Another plant with reported anxiolytic and sedative effects in folk medicine is *Coriandrum sativum* (Apiaceae).

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Popularly known as “coentro” in Brazil and coriander in other places, *C. sativum* has been used as a culinary and medicinal plant over the years.^{8,9} Their leaves and seeds are widely used by folk medicine for treatment of digestive disorders, appetite loss, diabetes, and other diseases.^{8,10} Regarding its biological activity, this plant has hypoglycemic, anti-inflammatory, antioxidant, and antimicrobial effects.^{8,11,12}

Moreover, other additional effects, such as hypolipidemic, antimutagenic, antihypertensive, diuretic, carminative, antispasmodic, and relaxant, were observed.^{13–20} In Iranian traditional medicine, the coriander aerial parts have been used for relief of anxiety and insomnia because of its sedative and hypnotic effects.^{16,21,22} The traditional dose of seed powder is from 1 to 5 g, three times per day. This translates to a 14–71 mg/kg dose, three times per day.^{21,23} Importantly, coriander decoction to compose polyherbal traditional remedies from Ksar Lakbir district (NW Morocco) are used as neurosedative and to calm.²⁴

On the brain, *C. sativum* extracts (CSEs) have been reported to exhibit sedative and anxiolytic effects. Mahendra and Bisht⁹ showed that hydroalcoholic extract of coriander (100 and 200 mg/kg) produced anxiolytic effects similar to diazepam in different animal models of anxiety in mice. In addition, Emamghoreishi *et al.*¹⁶ and Latha *et al.*²⁵ demonstrated that aqueous extract of coriander (100 and 200 mg/kg, respectively) induced an anxiolytic activity in mice by increasing both the time spent and the percentage of entries in the open arms of elevated plus maze.

Considering the traditional use of *C. sativum* and its sedative effects reported in rodents, as also the possibility of having high amount of quercetin in coriander extracts, it has been hypothesized that CSE might also possess anxiolytic properties. Thus, the present study aimed to investigate the putative antianxiety and antistress actions of *C. sativum* hydroalcoholic leaf extract in zebrafish. For inducing anxiety and stress responses we exposed zebrafish to two distinct paradigms: novel tank test and alarm substance exposure. Novel tank test is widely used as a tool to induce anxiety responses associated to a novel environment exposure.^{26,27}

Materials and Methods

Plant collection

C. sativum L. was collected in Aquidabã (10° 16' 53" S 37° 01' 07" W), Sergipe, Brazil, between June and August. A voucher specimen number ASE 35.092 was deposited at Herbarium of the Department of Botany, Federal University of Sergipe (UFS), Brazil.

Extract preparation

C. sativum fresh leaves (282 g) were macerated with 2 L of ethanol (70%) at room temperature. Crude hydroalcoholic extract was concentrated up to dryness in a rotary evaporator (Fisatom®, model 802, Brazil) at 40°C. The product formed was frozen at –20°C for 24 h and lyophilized (Labconco®, FreeZone 4.5 model). Total yield was 3.898%.

High-performance liquid chromatography analysis

Chromatographic analysis was performed using a Shimadzu HPLC system (Shimadzu, Columbia, MD) composed of two pumps with an online degasser, an autoinjector, and a

C18 column (4.6 × 250 mm, 5 μm) coupled to a DAD-UV/Vis detector. Sample volume of 20 μL (1 mg/mL) was dissolved in HPLC grade methanol and subjected to gradient elution with mobile phases consisting of water and methanol from 5% to 100% during 60 min in a wavelength range from 220 to 400 nm. Shimadzu LC Solution software (Kyoto, Japan) was used for data acquisition. Identification of the compounds was performed with the coinjection of standard secondary metabolites, according with their retention time (RT) and UV spectra. Gallic acid (C₇H₆O₅), caffeic acid (C₉H₈O₄), luteolin (C₁₅H₁₀O₆), quercetin 3-β-D-glucoside (C₂₁H₂₀O₁₂), and rutin (C₂₇H₃₀O₁₆), were obtained from Sigma-Aldrich® and diluted with methanol (see chromatogram in Fig. 1). To calculate the concentration of each secondary metabolite, calibration curves for each compound were performed with five different concentrations ranging from 1 to 50 μg/mL. Analyses were performed in triplicate (n = 3).

Animals and treatments

A total of 105 male adult zebrafish (*Danio rerio*) (short fin wild-type strain; 4–6 months old) were used in the present work. They were obtained from a local commercial supplier (Aquazoo, SE, Brazil) and were acclimated for at least 2 weeks prior the experiments. Animals were maintained in “home tanks,” which consisted in 20 L aerated tanks (three animals/L) filled with distilled water under mechanical and chemical filtration. Water temperature, pH, and conductivity were adjusted to 26°C ± 2°C, 7.0–8.0, and 500 μS, respectively.

Ceiling-mounted fluorescent white lamps provided room illumination (600 lux). Light/dark cycle was 12/12 h (lights on at 7:00 am). Only animals weighing between 0.25 and 0.35 g were used. All animals were fed four times a day with ALCON BASIC™ flake food (ALCON, Brazil; twice a day) and *Artemia* sp. nauplii (twice a day). All procedures were performed according to the Brazilian's Law for Care and Use of Laboratory Animals (Law 11794/2008) and previously approved by the Animal Research Ethics Committee from UFS/Brazil (protocol number 37/2015).

Alarm substance extraction. Alarm substance was extracted as described previously.^{28–30} One set of zebrafish (five animals that were not used for behavioral analysis) were cold anesthetized and quickly euthanized by decapitation. Alarm substance was extracted performing fifteen superficial cuts in the skin of animals and then washed with saline solution (10 mL/animal). During the extraction and further use, alarm substance solution was kept on ice, at 4°C.

Drug administration. Another set of animals (n = 100 animals) were anesthetized by tricaine immersion (160 μg/mL)³¹ and further injected i.p. with CSE (25, 50 or 100 mg/kg), or clonazepam (0.05 mg/kg) or vehicle (20 animals/group). The maximal volume of injection was 10 μL/g of body mass.³² Immersion in tricaine lasted 1–2 min, until fish were completely immobile and decreased opercular opening. Vehicle was composed of 40% propylene glycol, 10% ethyl alcohol, 5% sodium benzoate, and 1.5% benzyl alcohol.^{33,34} Drugs were administered 30 min before the behavioral evaluations.⁹

FIG. 1. Chromatogram of a fresh leaf aqueous alcoholic extract (70% ethanol) from *Coriandrum sativum* after lyophilization.

Behavioral procedures and alarm substance exposure

All behavioral tests were performed between 9:00 a.m. and 4:00 p.m. Behavioral analysis was conducted as depicted in Figure 2. Animals treated with vehicle, clonazepam, or CSE were submitted to novel tank test for 6 min. At the end of the novel tank test, animals were exposed to alarm substance. Without removing animals from the novel tank apparatus, alarm substance (3.5 mL of skin washout/L) or saline solution were carefully delivered in the tank through a syringe connected to a polypropylene tube ($n = 10$ animals for each group).

Swimming behavior was recorded for additional 6 min. After this period, fish were removed from the novel tank apparatus and placed, in pairs, in 1.5-L tanks until the beginning of the light/dark test. This strategy was used for keeping all experimental groups for the same time period of 24 h until the beginning of light/dark test. All behavioral tests were conducted under white illumination (800 lux of intensity). Behavioral analysis was performed in four independent experiments (25 animals/experiment). Animals were randomly distributed in each experiment as well as in experimental group by using an online tool for randomization (www.randomizer.org).

Novel tank test. Novel tank consisted of a 1.5-L trapezoidal tank (23.9 cm along the bottom $\times$ 28.9 cm at the top $\times$ 15.1 cm high $\times$ 15.9 cm along the diagonal side) similar to those previously described by Rosemberg *et al.*³⁵ Tank was filled with 1.5 L home-tank water and was divided in three equally virtual horizontal areas (named bottom, middle, and top). The following behavioral variables were evaluated:

total time mobile (s), distance travelled (m), total time spent (s) in bottom, middle, and top areas. A video camera located ~ 40 cm ahead the tank was used to monitor the activity of fish. ANY-Maze[®] software was used to track the swimming pattern of fish. Immobility was previously defined as the absence of movement or fish moving slower than 1 cm/s.

Light/dark test. The light/dark test was performed accordingly to Maximino *et al.*³³ with modifications. Apparatus consisted of a tank (15 $\times$ 10 $\times$ 45 cm, height $\times$ depth $\times$ length) equally divided into two distinct compartments (one black and other white covered). Opaque plastic self-adhesive films were used for covering walls and floor of both compartments. Apparatus was filled with 1.5 L home-tank water. Animals were individually placed in the lit area of the tank and were allowed to explore the apparatus for 15 min. The following behavioral parameters were recorded: number of entries and time spent in each compartment, and number of risk assessment behaviors. Risk assessment behaviors were defined as a partial entry in the white compartment followed by a fast return to the dark one.³⁶

Statistical analysis

Data were expressed as mean $\pm$ standard deviation. All data were first tested for normality by D'Agostino–Pearson omnibus normality test. Basal novel tank data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test. Behavioral data from light/dark test and alarm substance exposure were analyzed by two-way ANOVA followed by Sidak's *post hoc* test. For each data, Cohen's *d* was shown as

FIG. 2. Diagram illustrating the experimental design. After acquisition, animals were acclimated for 2 weeks before the beginning of the experiments. Animals were injected with vehicle, clonazepam, or CSE and were submitted to novel tank test for evaluating the basal locomotor and exploratory activities. At the end of novel tank test, saline or alarm substance were added to the tank and the locomotor and anxiety-like responses were recorded during 6 min. After this period, animals returned in pairs to the home-tank. Twenty-four hours after, fish were submitted to the light/dark test. CSE, *Coriandrum sativum* extract.

Cumming estimation plot. The raw data were plotted on the upper axes of figures; each mean difference was plotted on the lower axes of figures as a bootstrap sampling distribution. Mean differences are depicted as dots; 95% confidence intervals are indicated by the ends of the vertical error bars. $p \leq 0.05$ was considered significant.

Results

HPLC characterization of CSE

Chromatographic profile of the CSE was shown in Figure 1. Fractions P1 (RT: 9.16 min), P2 (RT: 12.58 min), P3 (RT: 21.09 min), P4 (RT: 26.49 min), and P5 (RT: 30.37 min), were

FIG. 3. Baseline locomotor profile of fish treated with vehicle, clonazepam or CSE; $n = 20$ animals/group. (A) Total distance travelled (m) in apparatus during 6 min of trial; (B) Total mobile time (s) during 6 min. Data expressed as mean $\pm$ standard deviation (one-way ANOVA followed by Tukey's *post hoc* test).

FIG. 4. Baseline exploratory profiles into two horizontal portions of the apparatus: (A) Total distance travelled in top area (m); (B) Total distance travelled in bottom area (m); (C) Total time spent in top area (s); (D) Total time spent in bottom area (s) of fish treated with vehicle, clonazepam, or CSE. $n=20$ animals/group. Data expressed as mean $\pm$ standard deviation (one-way ANOVA followed by Tukey's *post hoc* test).

identified as gallic acid, caffeic acid, luteolin, quercetin 3- β -D-glucoside, and rutin, respectively. Quantitative analysis was obtained by using calibration curves of each secondary metabolite. All curves showed a correlation coefficient of $r \geq 0.999$. Concentrations of P1, P2, P3, P4, and P5 were 1.88, 2.32, 5.61, 43.56, and 3.21 $\mu\text{g/mL}$, respectively.

Effect of CSE on alarm substance-induced alterations in locomotor and exploratory activities

Baseline locomotor and exploratory activities did not differ among experimental groups (Figs. 3–5). Animals spent more time in the bottom area. Clonazepam or CSE administration did not alter distance traveled or time spent in homebase area when compared with control group, suggesting a classical homebase formation (Fig. 5). In control groups, alarm substance exposure reduced distance traveled and total time mobile (Fig. 6A $F[1,90]=7.638$ and B $F[1,90]=3.997$; $p=0.0257$ and 0.0343 , respectively). To distance parameter, the unpaired Cohen's d between vehicle-saline and vehicle-alarm is -1.27 [95.0% CI $-2.55, -0.242$]. The two-sided p value of the Mann-Whitney test is 0.0257 ; the unpaired Cohen's d between clonazepam-saline and clonazepam-alarm is -0.512 [95.0% CI $-1.45, 0.454$]. The two-sided p value of the Mann-Whitney test is 0.273 ; the unpaired Cohen's d between 25 mg/kg-saline and 25 mg/kg-alarm is -1.5 [95.0% CI $-2.6, -0.368$]. The two-sided p value of the Mann-Whitney test is 0.00911 ; the unpaired Cohen's d between 50 mg/kg-saline and 50 mg/kg-alarm is 0.0705 [95.0% CI $-0.864, 1.0$]. The two-sided p value of the Mann-Whitney test is

0.734 ; the unpaired Cohen's d between 100 mg/kg-saline and 100 mg/kg-alarm is 0.0891 [95.0% CI $-0.862, 1.0$]. The two-sided p value of the Mann-Whitney test is 0.734 .

Only treatment with 25 mg/kg of CSE ($p=0.0322$) did not prevent the alarm substance-induced behavioral changes. In contrast, 50 and 100 mg/kg of CSE prevented the reduction in distance travelled and total time mobile induced by alarm substance (Fig. 6A, B, respectively). There was no difference between groups regarding distance traveled and time spent in top and bottom areas (Fig. 7). Although distance traveled in top area was not different among groups, alarm substance exposure appears to reduce suddenly the distance traveled in vehicle group ($p=0.2621$) (Fig. 7A).

Effect of CSE on anxiety-like behaviors

Alarm substance exposure reduced the time spent in white compartment when compared with fish from saline group ($p=0.0193$). Treatment with clonazepam and CSE in all tested concentrations prevented this reduction (Fig. 8A; $F[1,90]=5.514$). The unpaired Cohen's d between vehicle-saline and vehicle-alarm is -1.35 [95.0% CI $-2.53, -0.2$]. The two-sided p value of the Mann-Whitney test is 0.0173 ; the unpaired Cohen's d between clonazepam-saline and clonazepam-alarm is 0.704 [95.0% CI $-0.236, 1.78$]. The two-sided p value of the Mann-Whitney test is 0.186 ; the unpaired Cohen's d between 25 mg/kg-saline and 25 mg/kg-alarm is -2.06 [95.0% CI $-2.98, -1.03$]. The two-sided p value of the Mann-Whitney test is 0.00131 ; the unpaired

FIG. 5. Locomotor profiles in homebase area of fish treated with vehicle, clonazepam or CSE. (A) Total distance traveled and (B) total time spent in the home base area. $n=20$ animals/group. Data expressed as mean $\pm$ standard deviation (one-way ANOVA followed by Tukey's *post hoc* test). Data expressed as mean $\pm$ standard deviation.

FIG. 6. Locomotor and exploratory profiles of vehicle, clonazepam-, or CSE-treated groups after alarm substance exposure. $n = 10$ animals/group. (A) Total distance traveled and (B) total time mobile in the apparatus. $*p \leq 0.05$ differs significantly when compared with the respective control group (two-way ANOVA followed by Sidak's *post hoc* test). Data were expressed as mean $\pm$ standard deviation.

FIG. 7. Locomotor and exploratory profiles of vehicle, clonazepam-, or CSE-treated groups after alarm substance exposure. $n=10$ animals per group. (A) Total distance traveled in the top area; (B) total distance traveled in the top bottom; (C) total time spent in the top area; and (D) total time spent in the top area of the apparatus. Data are expressed as mean $\pm$ standard deviation and were analyzed by two-way ANOVA followed by Sidak's *post hoc* test.

Cohen's *d* between 50 mg/kg-saline and 50 mg/kg-alarm is -0.253 [95.0% CI $-1.22, 0.736$]. The two-sided *p* value of the Mann-Whitney test is 0.623; the unpaired Cohen's *d* between 100 mg/kg-saline and 100 mg/kg-alarm is -0.498 [95.0% CI $-1.39, 0.489$]. The two-sided *p* value of the Mann-Whitney test is 0.345.

Moreover, alarm substance induced a significant increase in the risk assessment behaviors in animals treated with vehicle ($p < 0.0001$) and clonazepam ($p = 0.0173$). There was no difference in risk assessment behaviors in CSE-treated animals (Fig. 8C; $F[1,90] = 5.514$). The unpaired Cohen's *d* between vehicle-saline and vehicle-alarm is 6.6 [95.0% CI 4.81, 8.81]. The two-sided *p* value of the Mann-Whitney test is 0.000176; the unpaired Cohen's *d* between clonazepam-saline and clonazepam-alarm is 1.43 [95.0% CI 0.667, 2.31]. The two-sided *p* value of the Mann-Whitney test is 0.009; the unpaired Cohen's *d* between 25 mg/kg-saline and 25 mg/kg-alarm is 0.688 [95.0% CI $-0.293, 1.72$]. The two-sided *p* value of the Mann-Whitney test is 0.0818; the unpaired Cohen's *d* between 50 mg/kg-saline and 50 mg/kg-alarm is 0.751 [95.0% CI

$-0.211, 1.46$]. The two-sided *p* value of the Mann-Whitney test is 0.172; The unpaired Cohen's *d* between 100 mg/kg-saline and 100 mg/kg-alarm is 0.944 [95.0% CI $-0.158, 1.85$]. The two-sided *p* value of the Mann-Whitney test is 0.0885. The number of crossing was not altered in any groups (Fig. 8B).

Discussion

To evaluate the locomotor and exploratory activities in fish treated with CSE, we tested it at concentrations ranging from 25 to 100 mg/kg. After treatments, animals performed novel tank test before (baseline) and after (post stress) saline or alarm substance exposure. We observed that CSE-treated animals did not present alterations in baseline behavior. In the present study motor and nonmotor patterns associated with anxiety-like behaviors after stressful paradigm were analyzed, to investigate the putative CSE anxiolytic effect. In poststress session, total distance traveled and total time mobile decreases after alarm substance administration when compared with saline administration in vehicle-treated animals,

whereas treatment with clonazepam and CSE (50 and 100 mg/kg) did not alter this parameter. We also found that acute exposure to alarm substance inhibited transitions to the top of the tank. These data corroborate with previous findings in which alarm substance exposure disrupts rapid habituation response of zebrafish in the novel tank test³⁷ and suggest that alarm substance is effective in inducing fear responses in zebrafish.²⁸ Thus, CSE-treated animals presented an anxiolytic response similar to clonazepam-treated animals into stressful paradigm used in the present study.

Situations like threat or danger cause fear and/or anxiety that may elicit innate defensive behaviors. Pharmacological studies show that alarm substance released from injured skin cells may elicit defensive behaviors characterized by increased speed of movement, erratic movements, freezing bouts, and agglomeration in the bottom zone of the tank.^{27,28,30,38,39} Therefore, decrease in movement observed in the poststress session in novel tank may be related to alarm substance exposure added to new environment interaction, considering that first novel tank session provides a stressor stimulus.

In fact, besides alarm substance exposure, we explore the novel tank test to propitiate stressful condition, because it is known as a novel tank that is being utilized as a tool to reveal the animal's interaction with a novel environment.^{26,27} Several studies justified this test as a model of anxiety based on the animal's natural instinct to seek protection in an unfamiliar environment presented exploration, freezing, or immobility and top entries,^{26,29,40} and it is a behavioral tool that is used to measure novelty-associated behavioral stress responses.⁴¹

To confirm the anxiolytic action of CSE demonstrated in novel tank test, we evaluated the light/dark test. It has been used as an anxiety test and anxiolytic drugs have been found to increase the time spent in the light zone, whereas anxiogenic drugs decreased it.^{33,34,42} We demonstrated for the first time that novel tank associated with alarm substance administration produces an anxiogenic effect that persists during 24 h. Importantly, CSE and clonazepam administration provide a control of this long-term behavior alteration. Our results demonstrated that CSE prevents anxiety-like phenotype produced by novelty response associated to alarm substance exposure, as well as exploratory and locomotor influences produced by acute alarm substance administration.

C. sativum, popularly known as “coentro” or coriander, is traditionally used for gastrointestinal and cardiovascular disorders.⁴³ Additionally, some evidence supports this plant use in convulsions, anxiety, insomnia, because of its sedative property.^{16,17,44} Also, in the Indian traditional medicine, it is used in the disorders of digestive, respiratory, and urinary systems, as it has diaphoretic and diuretic effects.⁹

Several compounds have been isolated from coriander oil, such linalool, α -pirene, γ -terpinene, geranyl acetate, camphor,

and geraniol,^{14,15} although many biologically active compounds are also soluble in water.⁴⁵ CSE was characterized for LC-MS screening, which revealed the presence of a high amount of quercetin. There are several studies, which have shown that quercetin produces a variety of anxiolytic-like behavioral effects.^{46–48} Nevertheless, it has been reported that

FIG. 8. Anxiety-like behavioral profile of vehicle, clonazepam, or CSE-treated groups after alarm substance exposure. (A) Total time spent in the white compartment of black/white apparatus; (B) number of risk assessment behaviors; and (C) number of crossings between white and black compartment. $n = 10$ animals per group. $*p \leq 0.05$ and $***p \leq 0.0001$ differs significantly when compared with respective to control group (two-way ANOVA followed by Sidak's *post hoc* test).

quercetin present good potential to reduce stress, decreased plasma cortisol levels, and improve memory performance.^{49,50} Moreover, quercetin could prevent the impairment of the enzymes that regulate the purinergic and cholinergic extracellular signaling as adenosine deaminase and acetylcholinesterase improving the memory and anxiety-like behavior induced by streptozotocin-induced diabetic rats.⁵¹ Taken together, these results may suggest that quercetin might be responsible for improving behavioral dysfunction during anxiety episodes. Although quercetin is the major compound in CSE, its anxiolytic effect is probably related to polyphenol mixture, considering the potential of these compounds for modulation of the anxiety-like phenotype.^{52,53}

In the present work, treatment with clonazepam prevented the alarm substance-induced decrease in time spent in white compartment. Another study demonstrated a similar result for clonazepam (0.3 mg/L) in light/dark test.⁴² Studies verified that treatment with anxiolytic drugs as fluoxetine, clonazepam, buspirone, and ethanol reduced fear- and anxiety-like behavior.^{34,40,42} Doses of CSE administrated in this work did not alter the number of crossing responses in the light/dark test, suggesting a potential anxiolytic effect without producing effects on locomotion. Indeed, vehicle- and clonazepam-treated animals increased the number of risk assessment episodes, whereas CSE prevent this effect in the light/dark test. This parameter indicates a potential anxiolytic activity to CSE. Due to this, CSE represents a new potential candidate for treatment of anxiety disorders without producing effects on locomotion.

Conclusion

Our findings suggest that long-term anxiety behavior may be achieved using a combination of novelty response in novel tank and alarm substance exposure in zebrafish. In this approach, CSE promoted anxiolytic effect. Thus, this work assists in the efficacy confirmation of this plant used in traditional medicine and can provide a new pharmacological intervention in anxiety disorders.

Disclosure Statement

No competing financial interests exist.

Funding Information

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de nível Superior (CAPES), FAPERGS/PRONEX edital no. 16/2551-0000499-4, CNPq – Edital Doenças Neurodegenerativas no. 467676/2014-3.

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