

Proconvulsant and anticonvulsant effects of *Citrus aurantium* L in zebrafish

Abstract

Bitter orange leaf (*Citrus aurantium*, CA) has anticonvulsant and anxiolytic properties. We examined the effect of a hydroethanolic CA extract (CAe) on seizure behavior. Adult Zebrafish (*Danio rerio*) were pre-incubated with different CAe concentrations, while untreated fish served as controls. The convulsant activity was assessed by exposing fish to pentylenetetrazole (3 mg/mL) and recording the seizure latency. Proconvulsant effects are observed at low CAe concentrations (0.005-0.063 mg/mL), followed by an apparent plateau, and at concentrations above 0.175 mg/mL, anticonvulsant effects. The proconvulsant effects might be due to the interaction of glutamate receptors (GluR) because CA increases [³H]glutamate binding at low concentrations (0.001-0.01mg/mL), interacting with NMDA and mGluR-II receptors. An aqueous extract, CAa, has similar anticonvulsant properties to those of the leaf infusion. Understanding the mechanisms underlying the proconvulsant effects of low CAe doses could identify strategies to reduce or eliminate them, ultimately enhancing its therapeutic potential.

Keywords: *Citrus aurantium*, pentylenetetrazole, zebrafish, proconvulsant, anticonvulsant

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Abbreviations: ASM, antiseizure medications; CA, *Citrus aurantium* L; CAe, ethanolic extract; CAa, CA aqueous extract; PTZ, pentylenetetrazole

Introduction

Epilepsy, one of the most common neurological diseases, affects people of all ages, races, social classes, and geographical locations.¹ Furthermore, adverse events associated with antiseizure medications (ASM) are frequent, with up to 60% of people with epilepsy reporting issues like cognitive impairment, incoordination, sedation, and mood disturbances.² Moreover, 30% of patients with this lifelong condition experience seizures despite using various antiseizure medications, leading to their identification as having “drug-resistant epilepsy”.³ Taken together, these observations highlight the need for research into new and improved therapeutic agents and approaches that can enhance the effects of currently available ASM.

Natural products and ethnobotanical resources are valuable sources of potential therapeutic agents. Among these ethnobotanical resources, *Citrus aurantium* (CA) L, commonly known as bitter orange, has multiple pharmacological and therapeutic properties.⁴⁻⁷ *C. aurantium* formulations have anticonvulsant and anxiolytic properties in zebrafish exposed to pentylenetetrazole (PTZ), a convulsant agent commonly used to model seizures.^{8,9} Nonetheless, the CA infusion exhibits anticonvulsant activity at high concentrations (15 mg/mL). We examined the effects of macerated aqueous and hydroalcoholic CA extracts to enhance their pharmacological profiles.

Materials and methods

Animals: Adult wild-type zebrafish (*Danio rerio*), 3–6 months old and weighing 0.25 ± 0.04 g, were obtained from Caribe Fisheries Inc. (Lajas, Puerto Rico). All fish were kept in water at 25–27 °C under standard natural light cycles. The experimental procedures were conducted between 9:00 a.m. and 12:00 p.m. We used 9–12 animals/group in several experiments. All procedures were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) of the University of Puerto Rico, Medical Sciences Campus, Protocol No. 3180110.

Preparation of the *C. aurantium* extract: Intact leaves were collected from local trees in the Eastern and Northern regions of Puerto Rico. A voucher specimen was deposited at the herbarium of the Botanical Garden of the University of Puerto Rico (voucher 008253). To remove dust and debris, we gently cleaned the leaves with a moist paper towel and blended them with 1:5 (w/v) distilled water for 30 seconds. After this, the volume was brought to 1:10 (w/v) with either 70% ethanol or distilled water. The mixture was further blended for an additional 30 seconds, then stirred magnetically for 1 hour at room temperature. The mixture was decanted, filtered, and stored at 4–5 °C until used.

***C. aurantium* response curve:** Randomly selected untreated zebrafish were placed in a small chamber containing 15 mL of the extract concentration in aquarium water for 1 hour. The control group was incubated with aquarium water. The fish were then transferred to a clear tank containing PTZ (3 mg/mL). Seizure latency was defined as the time between initial PTZ exposure and the onset of loss of coordination and postural control following the characteristic “wild jump.” We recorded all trials and quantified changes in seizure latency to assess extract effectiveness.

Statistical analyses: The results are presented as mean $\pm$ SEM for 9–12 animals/parameter. After confirming the normal distribution and homogeneity of the data, the differences between the groups were subjected to analysis of variance (one-way ANOVA) and two-way ANOVA. A one-sample t-test with a two-tailed P-value was used to assess statistical significance. Values with $p \leq 0.05$ were considered significant. Significance was assessed using GraphPad (<https://www.graphpad.com>).

Results

The effects of three different CA extracts on seizure latency are shown in Figure 1. The ethanolic extract shows proconvulsant effects at low concentrations (0.005-0.063 mg/mL), followed by an apparent plateau, and clear anticonvulsant effects at concentrations above 0.175 mg/mL. In contrast, the aqueous infusion (modified from Rosa-Falero et al)⁹ Proconvulsant effects are observed below 7.0 mg/mL, while anticonvulsant effects are observed at higher concentrations. The

CA aqueous extract (insert) shows anticonvulsant effects at higher concentrations except for one concentration (0.064 mg/mL).

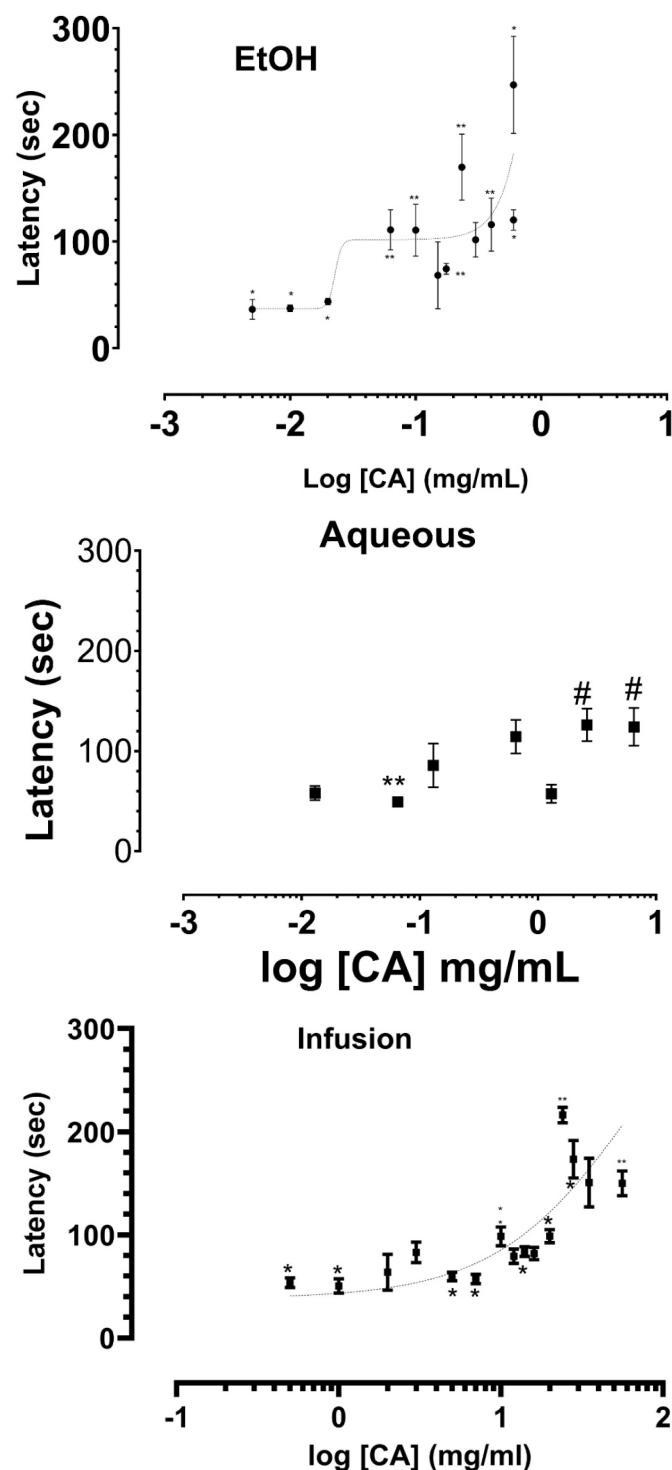


Figure 1 Effects of different CA extracts on seizure latency. The ethanolic extract (CAe), shows proconvulsant effects at low concentrations (0.005–0.063 mg/mL), followed by an apparent plateau, and clear anticonvulsant effects at concentrations above 0.175 mg/mL. In contrast, the CA aqueous extract shows anticonvulsant effects, which are observed at higher concentrations (except for one concentration (0.064 mg/mL)). The effects of a CA aqueous infusion (modified from Rosa-Falero et al.)⁹ show proconvulsant effects below 7.0 mg/mL, while anticonvulsant effects are observed at higher concentrations. # $p \leq 0.05$, ** $p \leq 0.01$, * $p \leq 0.001$ vs control; One-sample t-test. Results are

shown as average $\pm$ SEM of at least three independent experiments, n: 9–12 per concentration.

Discussion

Citrus aurantium L. (Rutaceae), known as bitter orange, possesses multiple therapeutic properties, including anticancer, antianxiety, ant obesity, antibacterial, antioxidant, pesticidal, and antidiabetic activities.^{4–7} The essential oil of *C. aurantium* exhibits marked pharmacological effects and substantial variation in chemical composition across growing locations and seasonal fluctuations.^{5,10,11}

A leaf infusion of *Citrus aurantium* (CA) has anticonvulsant and anxiolytic properties.^{9,10} The anticonvulsant effects appear to be mediated by NMDA and mGluR-II receptors but not GABA receptors.⁹ The proconvulsant effects at low CAe concentrations may be due to interactions with glutamate receptors because at low concentrations (0.001–0.01 mg/mL) CA infusion increases [³H]glutamate binding and interacts with NMDA and mGluR-II receptors.⁹

As expected, maceration of CA leaves yields more potent extracts, as shown by the leftward displacement of the dose-response curves. The anticonvulsant effects of the aqueous extract CAa have similar anticonvulsant properties to those of the leaf infusion; however, proconvulsant effects are observed at low doses.

Our results show that different formulations of the same leaves can have opposing pharmacological effects. If one extrapolates these observations to humans, the use of CA extracts in seizure-prone patients should be guarded. Isolation of the different molecules responsible for the proconvulsant/anticonvulsant effects could provide an understanding of possible mechanisms of different CA doses, leading to reducing or eliminating their effects, ultimately enhancing their therapeutic potential.

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Conflict of interest

The authors declare that there is no conflict of interest.

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