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RESEARCH PAPER

Anesthesia and anesthetic action mechanism of essential oils of *Aloysia triphylla* and *Cymbopogon flexuosus* in silver catfish (*Rhamdia quelen*)

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Abstract

Objectives To document the time for anesthesia induction and recovery using different concentrations of essential oils (EOs) of *Cymbopogon flexuosus* and *Aloysia triphylla* in silver catfish (*Rhamdia quelen*), and to determine whether the mechanism of action of either EO involves the benzodiazepine (BDZ) site of the $GABA_A$ receptor.

Study design Experimental study.

Animals A total of 144 silver catfish, length 7.5 ± 1.1 cm, weighing 3.95 ± 0.85 g.

Methods Essential oils were evaluated at concentrations of 25, 150 and 300 μL L^{-1}, and also ethanol alone (seven groups, n = 6 per group). Induction of sedation or anesthesia and recovery were assessed. In a further six groups (n = 6 per group), fish were exposed to both EOs (25, 150 or 300 μL L^{-1}) with diazepam 150 μM, and also diazepam (10 μM) alone. Flumazenil (5 or 10 μM) was added to the recovery water of fish exposed to diazepam (150 μM) or both EOs (150 and 300 μL L^{-1}) (total of 10 groups = 60 fish).

Results Both EOs induced anesthesia at concentrations of 150 and 300 μL L^{-1}, and sedation at 25 μL L^{-1}. There was no significant difference between EOs for reaching deep anesthesia; there was a significantly longer recovery time for the EO of *C. flexuosus*. The addition of diazepam (150 μM) resulted in faster induction of anesthesia with both EOs, with no significant change in recovery times. Flumazenil (10 μM) reversed the diazepam-induced anesthesia, but not the anesthesia induced by EOs.

Conclusions and clinical relevance The EO of *C. flexuosus* induced effective sedation (25 μL L^{-1}) and anesthesia (150 and 300 μL L^{-1}) without short-term mortality. The modulation of the BDZ site of the $GABA_A$ receptor in the anesthetic action mechanism of both EOs was not demonstrated.

Keywords diazepam, fish, flumazenil, gamma-aminobutyric acid, sedation.

Introduction

Aquaculture practices such as biometry, the collection of blood and other materials for analysis, hormonal implants and transportation, often stress fish, affecting their subsequent performance and resulting in disorders contributing to disease and mortality (Barton 2000). Synthetic drugs, such as tricaine methanesulfonate (MS-222), quinaldine, etomidate, metomidate, benzocaine, barbiturates

and propofol, have been used in various aquatic species to minimize the stress induced by these procedures (Amend et al. 1982; Mattson & Ripple 1989; Gomes et al. 2001; Ross & Ross 2008; Gressler et al. 2012).

Studies investigating alternatives to these synthetic substances have identified sedative and anesthetic effects of plant essential oils (EOs) or compounds isolated from EOs, often with similar activity (Palic et al. 2006; Gonçalves et al. 2008; Gressler et al. 2014).

The plant *Aloysia triphylla* (Verbenaceae) is an aromatic shrub with an EO that is primarily a mixture of α-citral (geranial) and β-citral (neral). Anesthetic activities of this EO were reported in white shrimp (*Litopenaeus vannamei*) (Parodi et al. 2012) and silver catfish (*Rhamdia quelen*) (Gressler et al. 2012; Parodi et al. 2014). The plant *Cymbopogon flexuosus* (Poaceae) is an aromatic grass, and also has an EO with citral as the predominant component (Taskinen et al. 1983).

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter in the central nervous system. Modulation of the GABAergic system through the benzodiazepine (BDZ) site of the $GABA_A$ as an anesthetic mechanism of action of EOs of *Lippia alba* and *Ocimum gratissimum* has recently been suggested in silver catfish (Heldwein et al. 2012; Silva et al. 2012). Expressions of this system have been identified in several species of vertebrates, from fish to mammals, with GABAergic functional evidence (immunocytochemistry) being described in the brain of zebrafish, *Danio rerio* (Kim et al. 2004; Delgado & Schmachtenberg 2008).

Benzodiazepines are positive modulators of the BDZ site of the $GABA_A$ receptors, resulting in sedative, hypnotic, muscle relaxant and anxiolytic effects in mammals (Foster & Kemp 2006). Flumazenil (RO 15-1788) is an imidazo-benzodiazepine that antagonizes the central effects of benzodiazepines by competition for the BDZ site (Darragh et al. 1982).

As both EOs have the same main compound (citral), it is possible that the EO of *C. flexuosus* induces anesthesia and that the mechanism of action of both EOs are related to the BDZ site of the $GABA_A$. This study aimed to evaluate the anesthetic efficacy of the EO of *C. flexuosus* in silver catfish and whether this EO induces anesthesia, and to compare the time for anesthesia induction and recovery for different concentrations with the EO of *A. triphylla*. The study also aimed to evaluate any potentiation offered by the inclusion of diazepam and to determine the involvement of the EOs in the BDZ site of the $GABA_A$ by observing responses to treatment with flumazenil. The hypotheses of this study were that the EO of *C. flexuosus* would induce anesthesia and that flumazenil would confirm that the action of both EOs is through the BDZ site of the $GABA_A$ in silver catfish.

Material and methods

Plant material

The *A. triphylla* was cultivated and identified according to Parodi et al. (2012). The *C. flexuosus* was cultivated in the city of Tiradentes do Sul, Rio Grande do Sul state (RS), Brazil. The species was identified by agronomist engineer Luiz Volnei of the Regional University of Northeastern Rio Grande do Sul (UNIJUI), and a voucher specimen was deposited in the Herbarium of Forest Sciences Department (HDCF no. 6748) at the Federal University of Santa Maria (UFSM).

Drugs, extraction and analysis of EOs

Flumazenil (Flumazenil injectable solution 0.1 mg mL^{-1}; Cristália Produtos Químicos e Farmacêuticos Ltda, SP, Brazil) and diazepam (Compaz injectable solution 5 mg mL^{-1}; Cristália Produtos Químicos e Farmacêuticos Ltda) were obtained from local trade sources (LDT Produtos Farmacêuticos e Hospitalares Ltda, RS, Brazil). The EO of *A. triphylla* was obtained from fresh leaves and the extraction and chromatographic analysis of the constituents of EOs were performed as described by Parodi et al. (2012). The EO of *C. flexuosus* was obtained from fresh leaves by hydrodistillation for 2 hours, according to the European Pharmacopoeia (European Directorate for the Quality of Medicines 2007), and stored in amber bottles at −4 °C. The gas chromatography-mass spectrometry (GC-MS) analysis was performed on an Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer with a nonpolar HP5-MS fused silica capillary column (5% phenyl – 95% methylsiloxane, 30 m × 0.25 mm internal diameter × 0.25 μm film thickness) and electron ionization MS of 70 eV (Agilent Technologies Brasil Ltda, SP, Brazil). The operating conditions were as follows: carrier gas, He, at a flow rate of 1 mL $minute^{-1}$; split inlet, 1:100; injector and detector temperatures, 250 °C; temperature program, 40 °C for 4 minutes and 40–320 °C

