Cedrus Atlas (Cedrus atlantica) essential oil induces anesthesia in Amazonian fish: a compartmental, electrophysiological and metabolic study

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Abstract

The study aimed to evaluate the anesthetic and physiological effects of *Cedrus atlantica* essential oil (CAEO) in *Colossoma macropomum* (tambaqui), an important Amazonian fish species in South American aquaculture. Fish were exposed to different concentrations of CAEO (10–80 $\mu L \cdot L^{-1}$) to determine induction and recovery times. Behavioral and compartmental responses were monitored, along with cardiorespiratory and metabolic parameters, including heart rate, ventilation frequency, and blood glucose. Electrophysiological activity was assessed through electroencephalography (EEG) and electromyography (EMG). Chemical composition of CAEO was analyzed by gas chromatography–mass spectrometry (GC–MS). CAEO promoted rapid and reversible anesthesia, with dose-dependent induction and recovery times. Electrophysiological recordings revealed decreased cortical wave amplitude and frequency, indicating central nervous system depression. Cardiorespiratory and metabolic analyses demonstrated reduced ventilation rate and glycemic stability, suggesting mild systemic stress. Limonene (42.3%) and β-himachalene (35.7%) were identified as major constituents, contributing to the observed anesthetic profile. *Cedrus atlantica* essential oil acts as an effective natural anesthetic for *C. macropomum*, reducing neural activity and stress-related responses while ensuring safe recovery. These findings highlight its potential as an eco-friendly and sustainable anesthetic alternative for fish handling and transport in tropical aquaculture.

Introduction

Colossoma macropomum (Tambaqui) is a freshwater fish native to the Amazon, with significant value in Brazilian aquaculture. It is primarily consumed in the northern and central-western regions of Brazil and is the most widely cultivated native fish species in the country (Associação Brasileira da Piscicultura, 2023). The use of anesthetics in aquaculture is crucial for weighing, marking, transportation, and handling procedures. If performed incorrectly, these activities can cause physical injuries to fish, reducing their market value. Consequently, research on fish anesthesia has increased in recent years, aiming to improve animal welfare and ensure high-quality commercial products (Roth & Skåra, 2021; Bowker et al., 2018).

Anesthetics can be classified as synthetic, such as tricaine methanesulfonate (MS-222) and benzocaine, or natural, such as eugenol (Zeng et al., 2024; Inoue & Moraes, 2007; Roth & Skåra, 2021). Studies indicate that the use of natural anesthetics, particularly essential oils, has advantages over synthetic alternatives, as they are biodegradable and exhibit lower toxicity (Vergneau-Grosset & Benedetti, 2022). Consequently, the search for new natural anesthetic sources is of great economic importance in aquaculture, as well as from a scientific perspective, considering fish as experimental models. The effects of anesthetics can vary depending on species-specific intrinsic characteristics and environmental conditions. The primary methods for assessing anesthesia levels in fish include monitoring postural reflex maintenance and gill respiration rates (Bianchi et al., 2014; Ross & Ross, 2008). In addition to behavioral evaluation, electrophysiology, such as electrocardiography (ECG), can be

used to analyze the cardiac safety or toxicity of a given anesthetic concentration in fish (Reis et al., 2024; Santos et al., 2020; Nascimento et al., 2024; Araújo et al., 2023).

On the other hand, *Cedrus atlantica* essential oil (CAEO), also known as Atlas Cedar essential oil, has been recognized as a promising natural compound due to its rich chemical composition and pharmacological properties. These include antioxidant, antimicrobial, anti-inflammatory, and analgesic effects (El Hachlafi et al., 2022; Emer et al., 2018). Recent studies emphasize the significance of himachalene sesquiterpenes (*β-himachalene, α-himachalene, and γ-himachalene*) as the primary bioactive compounds responsible for the oil's biological activities, highlighting structural and molecular mechanisms involved in its pharmacological effects (Ez-Zriouli et al., 2021; Faris et al., 2023). Studies by Emer et al. (2018) suggest that himachalenes can act as multifunctional modulators, interacting with neural receptors and ion channels, such as GABA-A and potassium channels, to induce muscle relaxation and analgesia.

This study presents a multidisciplinary and innovative approach to evaluating the anesthetic potential of *Cedrus atlantica* essential oil (CAEO) in *Colossoma macropomum*, integrating behavioral, electrophysiological, and metabolic analyses to provide a comprehensive understanding of its effects. Unlike previous research that primarily focused on induction time or survival rates, this work explores both neural and systemic mechanisms underlying anesthesia through real-time electrocorticographic, electrocardiographic (ECG), and electromyographic (EMG) monitoring. By correlating central nervous system activity with physiological and biochemical indicators, this study advances the understanding of natural anesthetic agents in fish and emphasizes the potential of *C. atlantica* oil as an eco-friendly and sustainable alternative for aquaculture management, promoting animal welfare and operational safety. Based on this rationale, we investigated the effects of CAEO in juvenile *C. macropomum* at concentrations of 200, 250, 300, 350, and 400 $\mu L \cdot L^{-1}$. The study focused on assessing the safety and reversibility of anesthesia induced by CAEO through short-term immersion baths, analyzing behavioral, electrophysiological, and glycemic parameters to determine a safe and effective therapeutic window for use in *C. macropomum* handling procedures.

Methods

Experimental Animals

This work was approved by the ethics committee for teaching and animal experimentation at UFPA and registered under number CEUA 2325250724 (ID 002671).

A total of 198 male and female Colossoma macropomum specimens were used in the study. The fish were kept in 250-L aquariums under controlled conditions, with temperatures between 25 and 28°C, a 12-hour light/dark photoperiod, and a pH of 7.6, in the Laboratory of Pharmacology and Toxicology of Natural Products of the Institute for Biological Sciences of the Federal University of Pará (ICB/UFPA). Oxygenation was provided by a 3,000-liter-per-hour pump with a distribution and filtration system for five

aquariums. Feeding was provided twice daily with a 32% protein diet. Daily siphoning was performed to remove uneaten food and feces, and 30% of the water volume was replaced with water from the same source. The fish underwent a 20-day acclimation period.

Organoleptic Properties and Chromatographic Analysis

The essential oil of *Cedrus atlantica* was extracted by steam distillation and its composition was analyzed by high-performance gas chromatography (GC-7890B-MSD-5977 A) under the following conditions: Column: DB1, 60 m × 0.25 mm × 0.25 µm; Column temperature: 70°C (0 min), increasing at 3°C/min to 250°C; Injector temperature: 250°C; Split ratio: 1/50; Detector temperature (FID): 260°C; Injection volume: 1 µL (1% in chloroform). Chromatography was performed at the Department of Chemistry and Food Engineering of the Technological Center of the Federal University of Santa Catarina, Brazil. The organoleptic characteristics of *Cedrus atlantica* essential oil include a yellow-orange liquid with a camphoraceous, cresolic, and milky odor. The phytoconstituents identified in the essential oil are listed in Table 1, with β-himachalene (32.34%), α-himachalene (16.04%) and γ-himachalene (11.06%) being the predominant components (Fig. 1).

Table 1
Compounds Identified by High-Efficiency Gas Chromatography (GC) with Their Respective Retention Times and Percent Area in *Cedrus atlantica* Essential Oil.

TR	Identification	%	Molecular formula
12.31	4-Acetyl-1-methylcyclohexene	0.80	$C_9 H_{14}O$
23.07	4,5-dehydro-isolongifolene	1.21	$C_{15} H_{22}$
23.62	α-Gurjunene	0.88	$C_{15} H_{24}$
23.70	Longifolene	0.73	$C_{15} H_{24}$
24.61	Himachala-2,4-diene	0.54	$C_{15} H_{24}$
25.59	α-Himachalene	16.04	$C_{15} H_{24}$
26.05	β-Vatirenene	0.55	$C_{15} H_{22}$
26.42	y-Muurolene	1.13	$C_{15} H_{22}$
26.75	γ-Himachalene	11.06	$C_{15} H_{24}$
26.84	α-Curcumene	2.51	$C_{15} H_{22}$
27.78	β- Himachalene	32.34	$C_{15} H_{24}$
28.14	β-Curcumene	2.39	$C_{15} H_{24}$
28.55	δ-Cadinene	4.78	$C_{15} H_{24}$
28.71	4,5,9,10-dehydro-isolongifolene	2,34	$C_{15}H_{20}$
29.06	8,9-dehydro-Neoisolongifolene	0.71	$C_{15} H_{20}$
29.28	α-Calacorene	1.79	$C_{15} H_{20}$
32.10	Calarene epoxide	0.97	$C_{15} H_{24}O$
32.50	Cubenol	1.00	$C_{15} H_{26}O$
33.19	Himachalol	0.66	$C_{15} H_{26}O$
34,90	8-Cedren-13-ol	0.80	$C_{17}H_{26}O_2$
35.02	α-Bisabolone	2.37	$C_{15} H_{24}O_2$
35.36	Trans-y-Atlantone	1.07	$C_{15} H_{22}O$

TR	Identification	%	Molecular formula
35.76	Cis-α-Atlantone	0.79	$C_{15}H_{22}O$
37.83	Trans-α- Atlantone	4.10	$C_{15}H_{22}O$
	Minor compounds(< 0.53%)	5.61	
	Unidentified compounds	2.83	

Figure 1 **here**

Table 1

Experimental Design

Juvenile *Colossoma macropomum* (Tambaqui) with an average weight of 18.15 ± 3.1 g were randomly distributed among the following treatment groups: a) Control group; b) Vehicle group (fish subjected to an immersion bath with 3 mL of 70% ethanol diluted in 1 liter of aquarium water); c) Positive control group (fish treated with eugenol 60 $\mu L.L^{-1}$); d) Fish treated with CAEO at 200 $\mu L.L^{-1}$; e) 250 $\mu L.L^{-1}$; f) 300 $\mu L.L^{-1}$; g) 350 $\mu L.L^{-1}$; and h) 400 $\mu L.L^{-1}$.

All groups underwent anesthetic induction for 10 minutes, followed by observation of anesthetic recovery for 10 minutes in water without CAEO. Each experimental group consisted of n = 9 fish per treatment.

Experiment 1 - Evaluation of Behavioral Characteristics of Anesthetic Induction and Recovery

Considering the immersion bath treatment time with CAEO, the following groups were analyzed: a) Positive control (eugenol 60 $\mu L.L^{-1}$); b) CAEO 200 $\mu L.L^{-1}$; c) 250 $\mu L.L^{-1}$; d) 300 $\mu L.L^{-1}$; e) 350 $\mu L.L^{-1}$; and f) 400 $\mu L.L^{-1}$. Each treatment group consisted of n = 9 fish, totaling 54 animals. The latency time for postural reflex loss, characterized by lateral recumbency, was evaluated during anesthetic induction. After exposure to CAEO, the fish were transferred to water without the anesthetic, and the latency time for postural reflex recovery was assessed (Fig. 2A)

Experiment 2 - Electromyography and Electrocardiographic Activity Recording During Anesthetic Induction and Recovery

To analyze muscle and cardiac function, the experimental groups were divided as follows: a) Control group; b) Vehicle group; c) Positive control group; d) CAEO-treated group at 200 $\mu L.L^{-1}$; e) 250 $\mu L.L^{-1}$; f) 300 $\mu L.L^{-1}$; g) 350 $\mu L.L^{-1}$; and h) 400 $\mu L.L^{-1}$. Each group consisted of n = 9 fish, totaling 72 animals.

For muscle activity acquisition, electrodes were fabricated using 925 silver, with a diameter of 0.5 mm and a length of 15 mm. The electrodes were paired at a distance of 4 mm and insulated with liquid insulator (Quimatic). The electrode positioning for dorsal muscle recording was 0.5 cm below the dorsal fin. During the recordings, the muscle contraction power was evaluated during both anesthetic induction and recovery (mV^2/Hz) (Fig. 2B).

For ECG recordings, electrodes were also fabricated using 925 silver, with a diameter of 0.3 mm and a length of 12 mm, subsequently insulated with Quimatic. These electrodes were non-paired. The reference electrode was placed to indicate the cardiac vector (negative pole at the base of the heart and positive pole at the heart apex), fixed on the ventral portion of the left operculum, 0.2 mm before the end of the opercular cavity. The recording electrode was inserted 2.0 mm before the end of the right opercular opening, capturing signals near lead D1 derivation (Fig. 2B).

Figure 2 **here**

The electrodes were connected to a digital data acquisition system through a differential amplifier with high input impedance (Grass Technologies, Model P511), configured with a 0.3 to 300 Hz filter, an amplification factor of 2000X, and monitored using an oscilloscope (ProteK, Model 6510). The recordings were continuously digitized at a sampling rate of 1 kHz using a computer equipped with a data acquisition board (National Instruments, Austin, TX) and stored on a hard drive for subsequent processing using specialized software (LabVIEW Express). This setup allowed for the analysis of muscle contraction power (mV^2/Hz) in EMG recordings, as well as various ECG parameters, including heart rate (bpm), QRS complex amplitude (mV), QRS complex duration (ms), R-R interval (ms), P-Q interval (ms), and S-T interval (ms).

Experiment 3 - Blood Glucose Analysis

Considering the exposure time to CAEO treatments, the following experimental groups were analyzed: a) Control; b) Vehicle; c) Positive control; d) 200 $\mu L.L^{-1}$; e) 250 $\mu L.L^{-1}$; f) 300 $\mu L.L^{-1}$; g) 350 $\mu L.L^{-1}$; and h) 400 $\mu L.L^{-1}$. Each treatment group consisted of n = 9 fish, totaling 72 animals. Blood glucose levels were assessed thirty minutes after exposure to CAEO. Blood samples were collected from the caudal vein of Colossoma macropomum, and glucose levels were measured using a single drop of blood analyzed with an Accu-Chek Active glucometer (mg/dL).

Statistical Analysis

After verifying the assumptions of normality and homogeneity of variances using the Kolmogorov-Smirnov and Levene tests, respectively, comparisons of mean power values were conducted using one-way ANOVA, followed by Tukey's post-hoc test. The statistical analyses were performed using GraphPad Prism 8. In all cases, a significance level of $p < 0.05$ was considered statistically significant.

Results

Behavioral analysis showed that CAEO induced postural reflex loss in fish, with shorter latency in groups treated with higher concentrations. The group treated with 200 $\mu L.L^{-1}$ had a mean latency for postural reflex loss of 243.4 ± 13.61 s, which was higher than the other groups. The group treated with 250 $\mu L.L^{-1}$ had a mean latency of 191.3 ± 20.01 s, while those treated with 300 $\mu L.L^{-1}$ (162.8 ± 17.07 s), 350 $\mu L.L^{-1}$ (137.9 ± 9.49 s), and 400 $\mu L.L^{-1}$ (113.0 ± 17.69 s) exhibited progressively shorter latencies. These results demonstrate that increasing the treatment concentration reduces the time required for the onset of behavioral changes. The positive control group showed a response like that of the group treated with 400 $\mu L.L^{-1}$ ($p = 0.9522$) (Fig. 3A).

Postural reflex recovery in the group treated with 200 $\mu L.L^{-1}$ of CAEO had a latency of 101.6 ± 13.88 s, which was shorter than in the other groups: 250 $\mu L.L^{-1}$ (131.4 ± 14.97 s), 300 $\mu L.L^{-1}$ (157.3 ± 11.45 s), 350 $\mu L.L^{-1}$ (204.8 ± 23.53 s), and 400 $\mu L.L^{-1}$ (250.8 ± 23.32 s). All groups exhibited a concentration-dependent latency for recovery, meaning that higher concentrations resulted in a longer recovery time for postural reflex. The reversibility of the effect occurred more slowly in groups that received higher concentrations. The positive control group showed a response like that of the group treated with 350 $\mu L.L^{-1}$ ($p = 0.7379$) (Fig. 3B).

Figure 3 **here**

The dorsal muscle activity of *Colossoma macropomum* showed mean amplitudes of 1.2 mV in the control and vehicle groups (Fig. 4A and B). The power distribution during muscle contraction was presented in the spectrogram of frequencies up to 40 Hz, where red coloration indicates higher signal intensity (Fig. 4A and B).

In the positive control group, muscle silence was observed as a result of muscle relaxation induced by eugenol in the somatic activity of the fish (Fig. 4C). Muscle silence, characterized by myorelaxation, was also observed in all groups treated with CAEO (Fig. 4D, E, F, G, and H), showing similar effects between the positive control and the CAEO-treated groups ($p = 0.999$) (Fig. 4I).

Figure 4 **here**

Although higher concentrations of CAEO caused postural reflex loss with reduced muscle activity in a shorter latency, this resulted in a longer recovery time for postural reflex. However, CAEO components demonstrated pharmacological reversibility, which is an essential characteristic of drugs with anesthetic potential (Fig. 5A, B, C, D, E, and F). Normal muscle contraction in the control group had a mean value of 14.05 ± 3.28 mV^2/Hz, which was similar to the vehicle group ($p = 0.8915$), the positive control group ($p = 0.9901$), and the groups treated with 200 $\mu L.L^{-1}$ ($p = 0.9921$), 250 $\mu L.L^{-1}$ ($p = 0.999$), 300 $\mu L.L^{-1}$ ($p = 0.996$), 350 $\mu L.L^{-1}$ ($p = 0.6237$), and 400 $\mu L.L^{-1}$ ($p = 0.2662$) (Fig. 5G).

Figure 5 **here**

The normal electrocardiogram of *Colossoma macropomum* exhibited a sinus rhythm with an average heart rate of 98.44 ± 8.293 bpm. The identified waveform components included the P wave, QRS

complex, and T wave (Fig. 5A). Cardiac activity in the control group was similar to that of the vehicle group (p = 0.9999) (Fig. 6B).

Using a 10-second amplification, the intervals were evaluated during the CAEO immersion bath treatment, revealing its effects on cardiac activity during anesthetic induction (Fig. 6C, D, E, F, G, and H).

During the immersion bath treatment with 200 $\mu L.L^{-1}$, the animals exhibited a 49.20% reduction in heart rate compared to the control group (Fig. 6D). In the group treated with 250 $\mu L.L^{-1}$, the heart rate decreased by 58.91% (Fig. 6E), while those treated with 300 $\mu L.L^{-1}$ showed a 58.46% reduction (Fig. 6F). The group treated with 350 $\mu L.L^{-1}$ had a 56.65% decrease (Fig. 6G), and those exposed to 400 $\mu L.L^{-1}$ experienced a 61.62% reduction (Fig. 6H). The positive control group had a 50.56% decrease (Fig. 6C).

Cardiac activity decreased progressively with increasing CAEO concentrations. The control group had an average heart rate of 94.44 ± 8.29 bpm, similar to the vehicle group (p = 0.999) and significantly higher than all other groups. The positive control group (49.78 ± 4.52 bpm) was similar to the groups treated with 200 $\mu L.L^{-1}$ (p = 0.999) and 350 $\mu L.L^{-1}$ (p = 0.0846). The group treated with 200 $\mu L.L^{-1}$ (50.00 ± 3.31 bpm) was comparable to the 350 $\mu L.L^{-1}$ group (p = 0.673). The 250 $\mu L.L^{-1}$ group (40.89 ± 1.94 bpm) had similar heart rates to the 300 $\mu L.L^{-1}$ group (p = 0.999), the 350 $\mu L.L^{-1}$ group (p = 0.9837), and the 400 $\mu L.L^{-1}$ group (p = 0.955) (Fig. 6I).

The mean QRS complex amplitude in the control group was 2.18 ± 0.343 mV, similar to that observed in the vehicle group, positive control group, and all CAEO-treated groups ($F(7, 64) = 0.1514$; p = 0.993) (Fig. 6J).

The mean R-R interval in the control group was 612.2 ± 50.21 ms, similar to that of the vehicle group (p = 0.999) but lower than in the positive control and CAEO-treated groups. The group treated with 200 $\mu L.L^{-1}$ had a mean R-R interval of 1203 ± 76.97 ms, which was similar to the positive control group (p = 0.9465). The group treated with 250 $\mu L.L^{-1}$ was comparable to those treated with 300 $\mu L.L^{-1}$ (p = 0.999) and 350 $\mu L.L^{-1}$ (p = 0.7714). The 300 $\mu L.L^{-1}$ group (1466 ± 64.25 ms) was similar to the 350 $\mu L.L^{-1}$ group (p = 0.9273) (Fig. 6K).

The mean P-Q interval in the control group was 69.44 ± 10.48 ms, similar to that of the vehicle group (p = 0.933). The group treated with 200 $\mu L.L^{-1}$ (96.89 ± 5.08 ms) was comparable to the positive control group (p = 0.748).The group treated with 250 $\mu L.L^{-1}$ (114.8 ± 6.18 ms) showed similarities to the 300 $\mu L.L^{-1}$ group (p = 0.9953) and the 350 $\mu L.L^{-1}$ group (p = 0.8943). The 300 $\mu L.L^{-1}$ group (117.6 ± 5.98 ms) was also like the 350 $\mu L.L^{-1}$ group (p = 0.999) (Fig. 6L).

The mean QRS complex duration in the control group was 18.87 ± 2.64 ms, similar to that of the vehicle group (p = 0.999) and lower than in all treated groups. The positive control group, with a mean duration of 26.22 ± 3.89 ms, was similar to the groups treated with 200 $\mu L.L^{-1}$ (p = 0.997) and 250 $\mu L.L^{-1}$ (p = 0.522).The group treated with 250 $\mu L.L^{-1}$ (28.80 ± 3.06 ms) was comparable to the groups treated with 300 $\mu L.L^{-1}$ (p = 0.369), 350 $\mu L.L^{-1}$ (p = 0.2065), and 400 $\mu L.L^{-1}$ (p = 0.175) (Fig. 6M).

For the control group, the mean S-T interval was 214.9 ± 17.24 ms, similar to the vehicle group (p = 0.999) and lower than in all other groups. The positive control group (289.9 ± 7.02 ms) was comparable to the group treated with 200 $\mu L.L^{-1}$ (p = 0.999). The group treated with 250 $\mu L.L^{-1}$ (310.1 ± 24.01 ms) was similar to the groups treated with 300 $\mu L.L^{-1}$ and 350 $\mu L.L^{-1}$ (p = 0.999). The 400 $\mu L.L^{-1}$ group (356.1 ± 21.95 ms) had the highest values among all groups (Fig. 6N).

Figure 6 **here**

During the recovery from anesthesia induced by CAEO, reversibility of the electrocardiographic alterations was observed (Figs. 7A, B, C, D, E, and F). However, the recovery process was slower in the groups treated with higher concentrations. The cardiac activity recovery in the group treated with 200 $\mu L.L^{-1}$ was 88.04% relative to the control group (Fig. 7B). Fish treated with 250 $\mu L.L^{-1}$ showed a 83.74% recovery of cardiac function compared to the control group (Fig. 7C). For the group treated with 300 $\mu L.L^{-1}$, recovery reached 62.98% (Fig. 7D), while the 350 $\mu L.L^{-1}$ group showed 64.33% (Fig. 7E). The 400 $\mu L.L^{-1}$ group exhibited 61.17% of the control group's cardiac activity (Fig. 7F). Treated fish exhibited slow reversibility, while maintaining sinus rhythm. The positive control group showed a 74.94% recovery compared to the control (Fig. 7A).

During the recovery period, the control group had a mean heart rate of 98.44 ± 8.29 bpm, which was similar to the vehicle group (p = 0.999) and higher than all treated groups. The 200 $\mu L.L^{-1}$ group (86.67 ± 3.16 bpm) was similar to the 250 $\mu L.L^{-1}$ group (p = 0.624). The 300 $\mu L.L^{-1}$ group (62.00 ± 3.00 bpm) was comparable to the 350 $\mu L.L^{-1}$ and 400 $\mu L.L^{-1}$ groups (p = 0.8865) (Fig. 7G).

During the recovery period, the QRS complex amplitude in the control group had a mean value of 2.18 ± 0.34 mV, which was like all treated groups during anesthesia recovery ($F(7, 64) = 0.276$, p = 0.961) (Fig. 7H).

During the recovery period, the mean R-R interval in the control group was 612.2 ± 50.21 ms, which was like the vehicle group (p = 0.999) and the 200 $\mu L.L^{-1}$ group (p = 0.0747). The 200 $\mu L.L^{-1}$ group (687.6 ± 28.40 ms) was comparable to the 250 $\mu L.L^{-1}$ group (p = 0.7243). The 300 $\mu L.L^{-1}$ group (976.6 ± 45.74 ms) was similar to the 350 $\mu L.L^{-1}$ and 400 $\mu L.L^{-1}$ groups (p = 0.1209). The positive control group (818 ± 62.22 ms) had higher values compared to the vehicle and control groups (Fig. 7I).

During the recovery period, the P-Q interval in the control group was 69.44 ± 10.48 ms, which was similar to the vehicle group (p = 0.9528), positive control (p = 0.999), 200 $\mu L.L^{-1}$ (p = 0.6073), 250 $\mu L.L^{-1}$ (p = 0.9990), and 300 $\mu L.L^{-1}$ (p = 0.9999). The group treated with 200 $\mu L.L^{-1}$ (62.11 ± 5.349 ms) was similar to the groups treated with 250 $\mu L.L^{-1}$ and 300 $\mu L.L^{-1}$ (p = 0.254).The 350 $\mu L.L^{-1}$ group (85.11 ± 8.06 ms) was comparable to the 400 $\mu L.L^{-1}$ group (p = 0.1736) (Fig. 7J).

During the recovery period, the QRS complex duration in the control group had a mean value of 18.67 ± 2.646 ms, which was similar to the vehicle group, positive control, and groups treated with 200 $\mu L.L^{-1}$,

250 μL.L⁻¹, 300 μL.L⁻¹, and 350 μL.L⁻¹ (p = 0.4049).The group treated with 400 μL.L⁻¹ had higher values compared to the other groups (Fig. 7K).

During the recovery period, the S-T interval in the control group was 214.9 ± 17.24 ms, which was similar to the vehicle group (p = 0.999) and to the groups treated with 200 μL.L⁻¹ (p = 0.9994) and 250 μL.L⁻¹ (p = 0.555).The vehicle group (216.3 ± 11.59 ms) was similar to the groups treated with 200 μL.L⁻¹ (p = 0.9999), 250 μL.L⁻¹ (p = 0.6842), and 300 μL.L⁻¹ (p = 0.072).The positive control group (241.8 ± 19.80 ms) was similar to the groups treated with 200 μL.L⁻¹ (p = 0.0534), 250 μL.L⁻¹ (p = 0.6551), and 300 μL.L⁻¹ (p = 0.9998). The groups treated with 200 μL.L⁻¹, 250 μL.L⁻¹, and 300 μL.L⁻¹ were similar to each other (p = 0.1610) (Fig. 7L).

Figure 7 **here**

In the evaluation of plasma glucose, the control group (69.33 ± 8.98 mg/dL) showed similar values to the vehicle group (p = 0.999), positive control group (p = 0.999), and the group treated with 200 μL.L⁻¹ (p = 0.382), 250 μL.L⁻¹ (p = 0.800), 300 μL.L⁻¹ (p = 0.877), and 350 μL.L⁻¹ (p = 0.177). The group treated with 400 μL.L⁻¹ was superior to the control, vehicle, and positive control groups, but similar to the other treated groups (p = 0.0733) (Fig. 8).

Figure 8 **here**

Discussion

The chromatographic analysis of CAEO confirmed β-himachalene (39.35%), α-himachalene (15.00%), and γ-himachalene (9.71%) as major constituents, compounds previously described as relevant to biological activities related to neuronal modulation and anti-inflammatory responses (El Hachlafi et al., 2022; Faris et al., 2023). The high lipophilicity of these sesquiterpenes favors their incorporation into cell membranes and interaction with transmembrane proteins, including ion channels and receptors, thereby modulating neuronal excitability and synaptic transmission (Ananchenko et al., 2022; Wang; Heinbockel, 2018).

These chemical characteristics were reflected in the effects observed in *Colossoma macropomum.* CAEO promoted anesthetic induction in a concentration-dependent manner, with significantly shorter latencies at higher doses, evidencing a clear dose–response relationship. This finding is consistent with results obtained with other essential oils, such as *Ocimum basilicum* (Ventura et al., 2021) and *Nepeta cataria* (Santos et al., 2024), which also demonstrated progressive reductions in induction time as concentrations increased. In the group treated with 400 μL·L⁻¹, induction latency was comparable to the positive control with eugenol, confirming that CAEO can achieve efficacy like this widely established anesthetic (Inoue; Moraes, 2007; Zeng et al., 2024).

Complementarily, postural reflex recovery also exhibited a concentration-dependent profile. At lower doses, such as 200 μL·L⁻¹, fish recovered quickly, whereas higher concentrations prolonged recovery,

although still in a completely reversible manner. This pattern suggests that CAEO can be adjusted according to the duration and complexity of the procedure, a particularly useful characteristic in aquaculture practice. Similar recovery trends have been reported with *Ocimum basilicum* oil (Ventura et al., 2021) and with essential oils containing geraniol and citronellol (Araújo et al., 2023).

The prolonged recovery observed at higher concentrations may be associated with the redistribution and slower metabolism of lipophilic constituents such as himachalene compounds (Faris et al., 2023). Although this characteristic can be advantageous for interventions requiring sustained anesthesia, it also demands caution to avoid excessively long recovery periods. Importantly, Ez-Zriouli et al. (2023) reported LD_{50} values indicative of low acute toxicity for CAEO in rodents, supporting a favorable safety profile. However, extrapolation of these data to fish should be approached with caution, and further pharmacokinetic and long-term exposure studies in aquatic organisms are required.

Behavioral findings were corroborated by electrophysiological recordings. Electromyography demonstrated a significant reduction in muscle activity during CAEO exposure, characterized by consistent relaxation comparable to eugenol. This effect is consistent with the action of lipophilic compounds on GABA-A receptors and potassium channels, mechanisms involved in reducing neuronal excitability and inducing anesthetic states (Tanwar et al., 2019; Wang; Heinbockel, 2018).

From a cardiovascular perspective, electrocardiographic recordings showed progressive reductions in heart rate with increasing CAEO concentrations, in magnitudes similar to those observed with eugenol. Prolongation of R-R and P-Q intervals, along with increased QRS duration, indicated a depressive effect on cardiac conduction, consistent with ion channel modulation described in anesthesia studies using *Ocimum basilicum* oil (Ventura et al., 2021) and *Piper divaricatum* oil (Vilhena et al., 2022). Despite these alterations, all animals exhibited complete restoration of sinus rhythm, with no evidence of impaired myocardial contractility, reinforcing the safety of CAEO under the tested conditions.

Finally, glycemic analysis revealed stability in most groups, except for a transient increase at 400 $\mu L \cdot L^{-1}$. This effect is consistent with stress responses previously described in anesthetized fish, resulting from catecholamine release and increased energy demand during recovery (Souza et al., 2019; Hohlenwerger et al., 2016). The reversibility of this alteration reinforces the metabolic safety of CAEO, particularly at low and intermediate concentrations.

In summary, CAEO demonstrated anesthetic potential in *Colossoma macropomum*, producing behavioral, muscular, cardiac, and metabolic changes in a dose-dependent manner, with effects comparable to eugenol. The observed safety profile, combined with the flexibility of adjusting doses according to the duration and complexity of the procedure, highlights CAEO as a natural and sustainable alternative to synthetic anesthetics in aquaculture.

Conclusion

This study demonstrated that *Cedrus atlantica* essential oil exerts anesthetic effects on *Colossoma macropomum*, with the depth of anesthesia dependent on the concentration used. Therefore, we recommend concentrations of 200 to 250 $mL.L^{-1}$ for superficial anesthesia, and concentrations of 300 to 400 $mL.L^{-1}$ for deeper anesthesia. However, all concentrations tested provided good muscle relaxation for handling the animals. Although higher concentrations prolonged recovery, they were compatible with the positive control eugenol. Higher concentrations promoted an increase in plasma glucose; all observed effects were reversible, with no evidence of cardiac or muscle function impairment.

Abbreviations

MS-222, Tricaine methanesulfonate; ECG, Electrocardiography; CAEO, *Cedrus atlantica* essential oil; EMG, Electromyogram; ICCB/UFPA, Institute for Biological Sciences of the Federal University of Pará; CG, Gas Chromatography; FAPESPA, Amazon Foundation for Studies and Research Support of the State of Pará; Brazilian CAPES, Coordination for the Improvement of Higher Education Personnel; CEUA/UFPA, Ethics Committee on Animal Research and Experimentation of the Federal University of Pará.

Declarations

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CRediT Authorship Contribution Statement

Moisés Hamoy: Conceptualization**; Júlia Silva, Daniella Rocha Bittencourt:** Writing- Original draft preparation; **Letícia Cerqueira Duarte, Luiz Fernando Duarte De Andrade Junior, Emile Cardoso, Juliana Lobo, Sarah Câmara, Luana Vasconcelos, Stephany Alves, Marcos Canindé, Moisés Hamoy**: Methodology **Júlia Silva**: Writing- Reviewing and Editing; **Moisés Hamoy**: Data curation, **Tayssa Reis, Clarissa Paz**: Supervision, **Moisés Hamoy**: Software, **Júlia Silva, Tayssa Reis, Axell Lins , Moisés Hamoy**: Validation.

Ethics Declarations

All procedures involving animals were approved by the Animal Use Ethics Committee of the Federal University of Pará (CEUA/UFPA, Protocol No. 2325250724 (ID 002671)). Experimental research on vertebrates was conducted in accordance with institutional, national, and international ethical guidelines for animal research and complies with the principles of the Basel Declaration (https://animalresearchtomorrow.org/en).

DAS statement request

All data generated or analyzed during this study are available in the public repository at the following link: https://drive.google.com/file/d/1mIV-H2h8p6lh6bBwmBbXJoAQPAkAvbIQ/view?usp=sharing. The datasets support the findings of this study and include the original electrophysiological and behavioral data.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work the author(s) used ChatGPT in order to Orthographic revision. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Figures

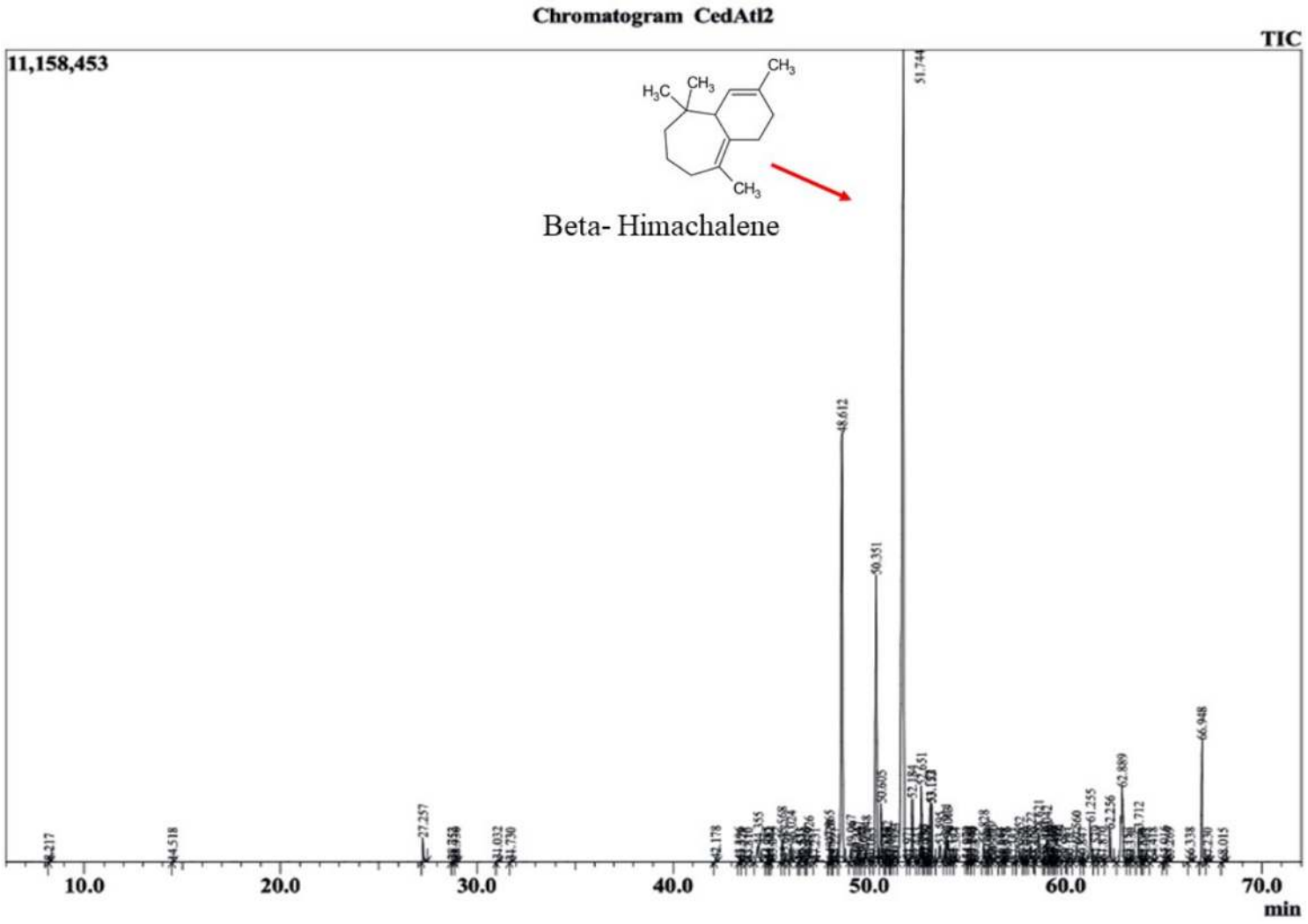

Figure 1

Chromatographic profile of *Cedrus atlantica* essential oil, highlighting β-himachalene as the major compound detected by gas chromatography analysis.

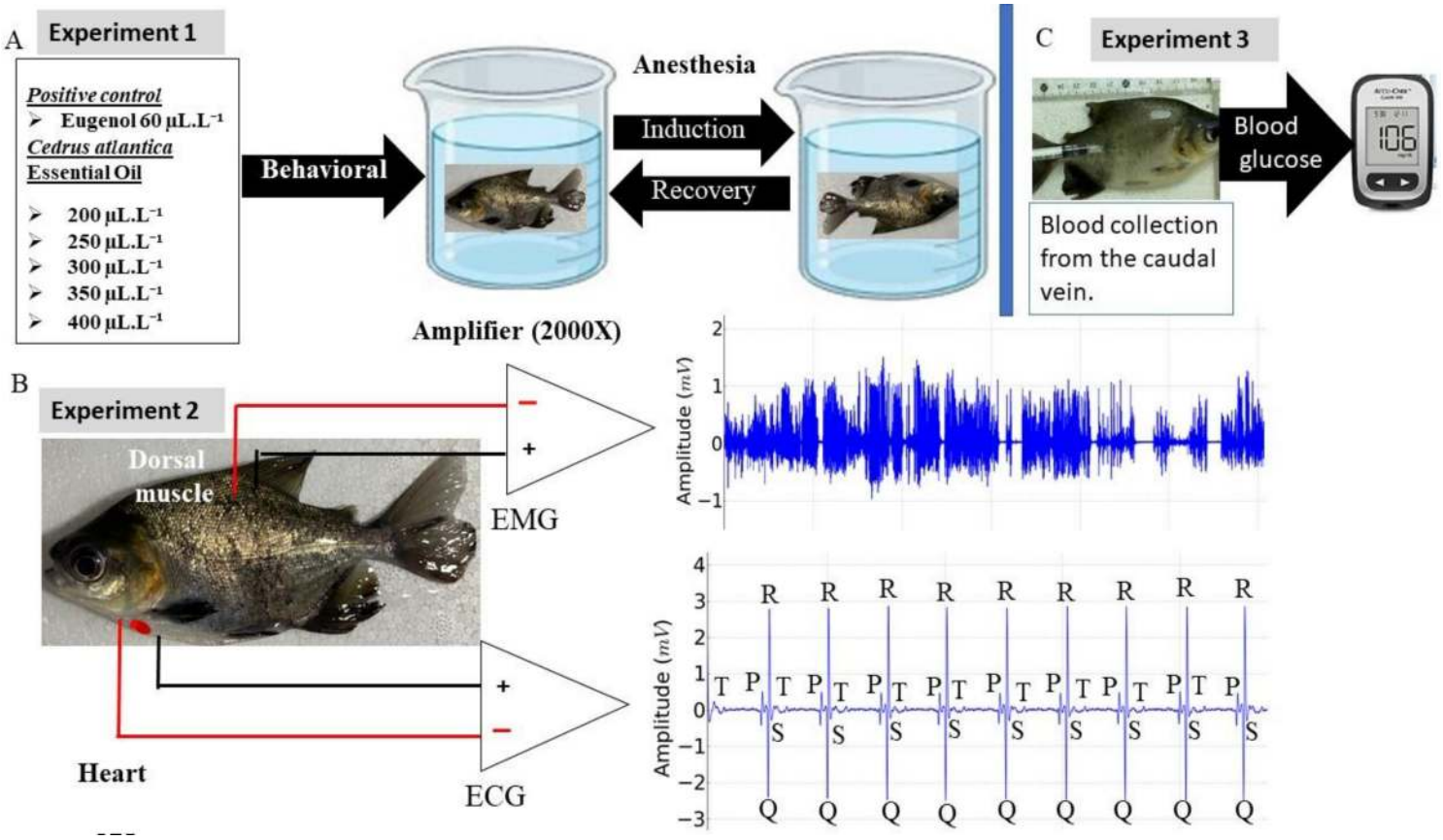

Figure 2

Experimental design illustrating (A) behavioral evaluation of anesthetic induction and recovery in *Colossoma macropomum*immersed in *Cedrus atlantica* essential oil baths, and (B) electrophysiological recordings of cardiac (ECG) and skeletal muscle (EMG) activity in juvenile tambaqui.

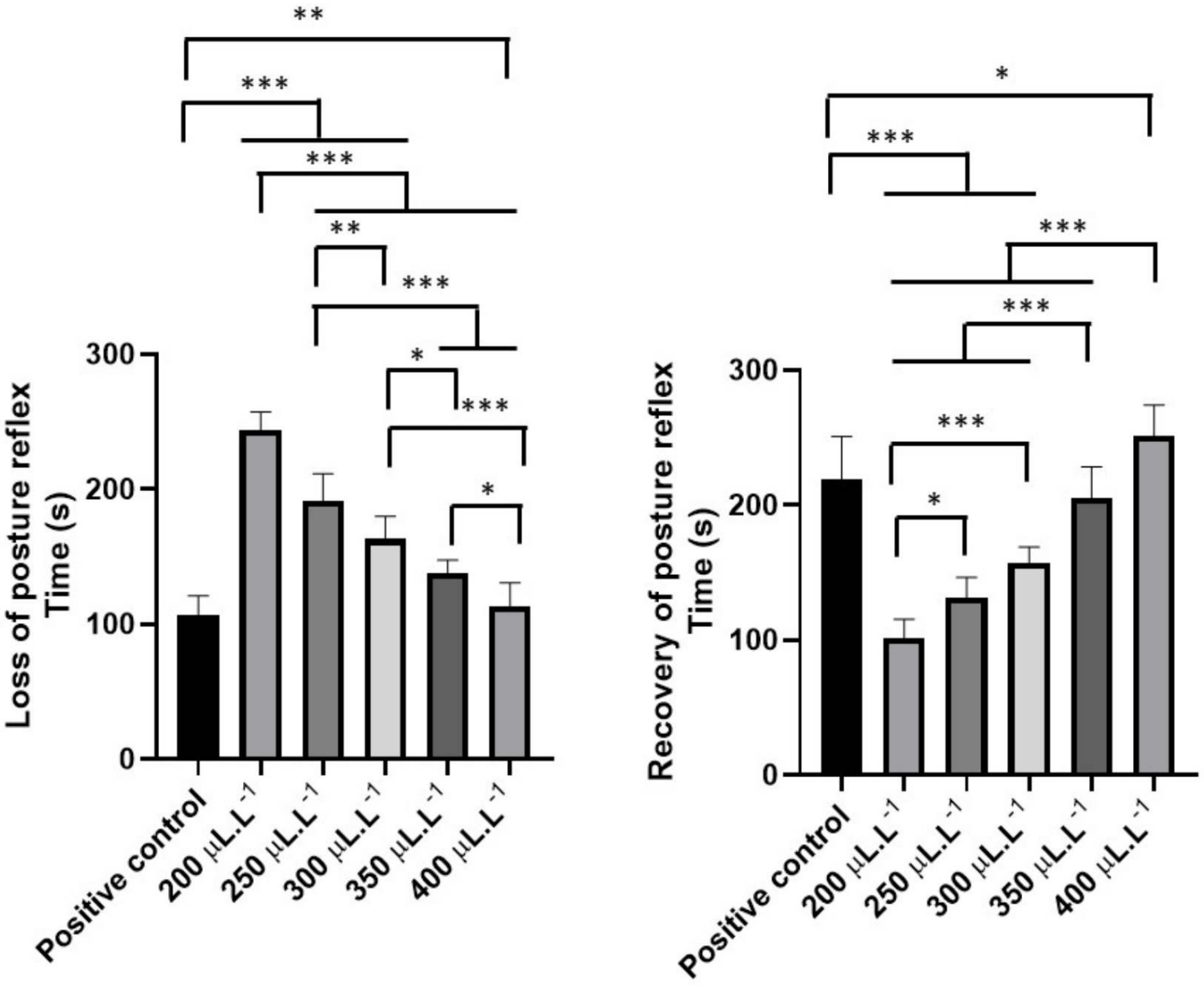

Figure 3

Mean latency (seconds) for (A) loss of postural reflex during anesthetic induction and (B) recovery of postural reflex after treatment with different concentrations of CAEO. (ANOVA followed by Tukey's test; $p < 0.05$).

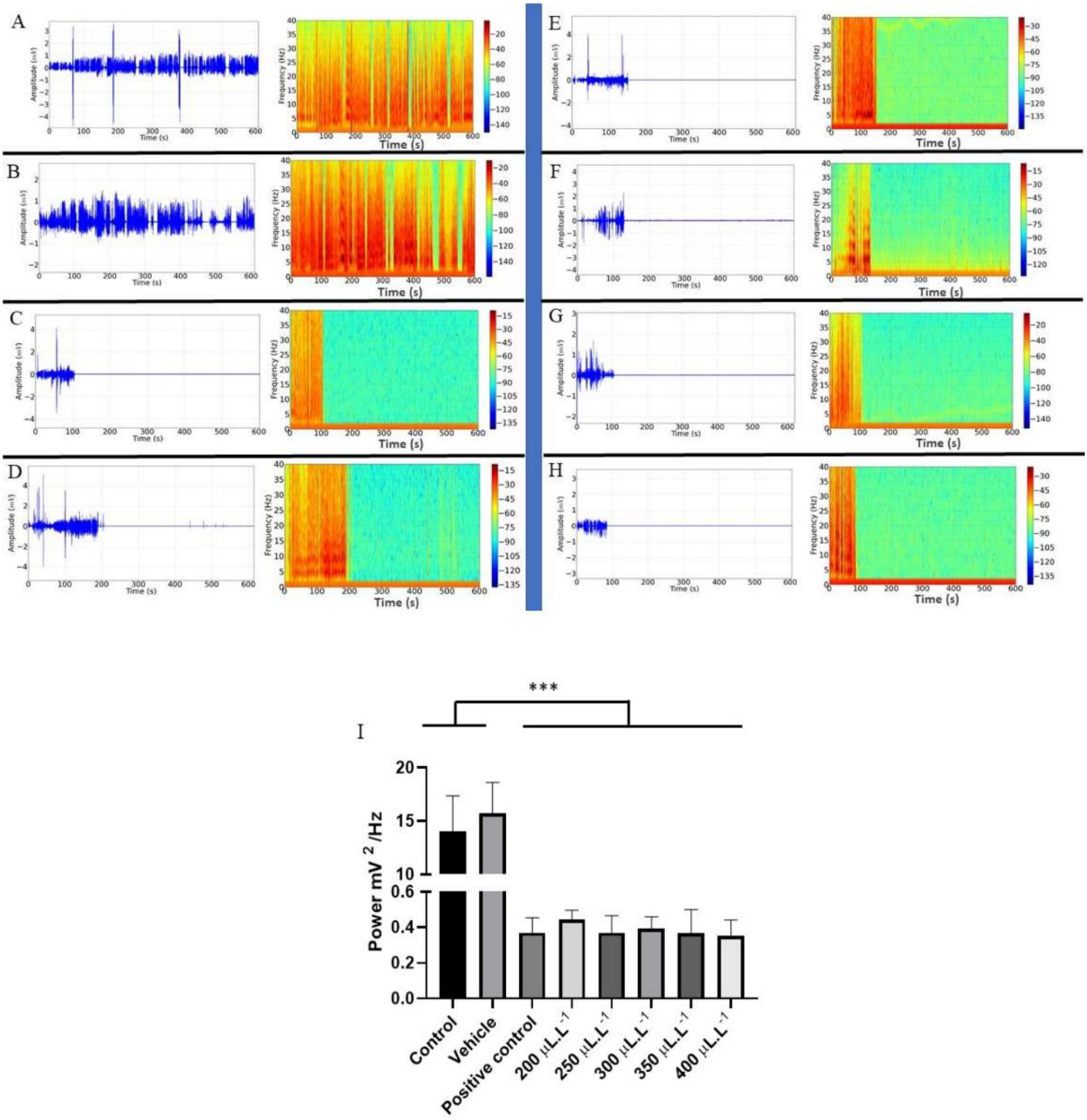

Figure 4

Dorsal muscle contraction activity of *Colossoma macropomum* during immersion baths with CAEO. Electromyographic recordings over 600 seconds (left) and frequency spectrograms up to 40 Hz (right) are shown for control, vehicle, positive control, and CAEO-treated groups (200–400 µL·L⁻¹). Graph shows muscle contraction power during anesthetic induction. (ANOVA followed by Tukey's test; $p < 0.05$; $n = 9$).

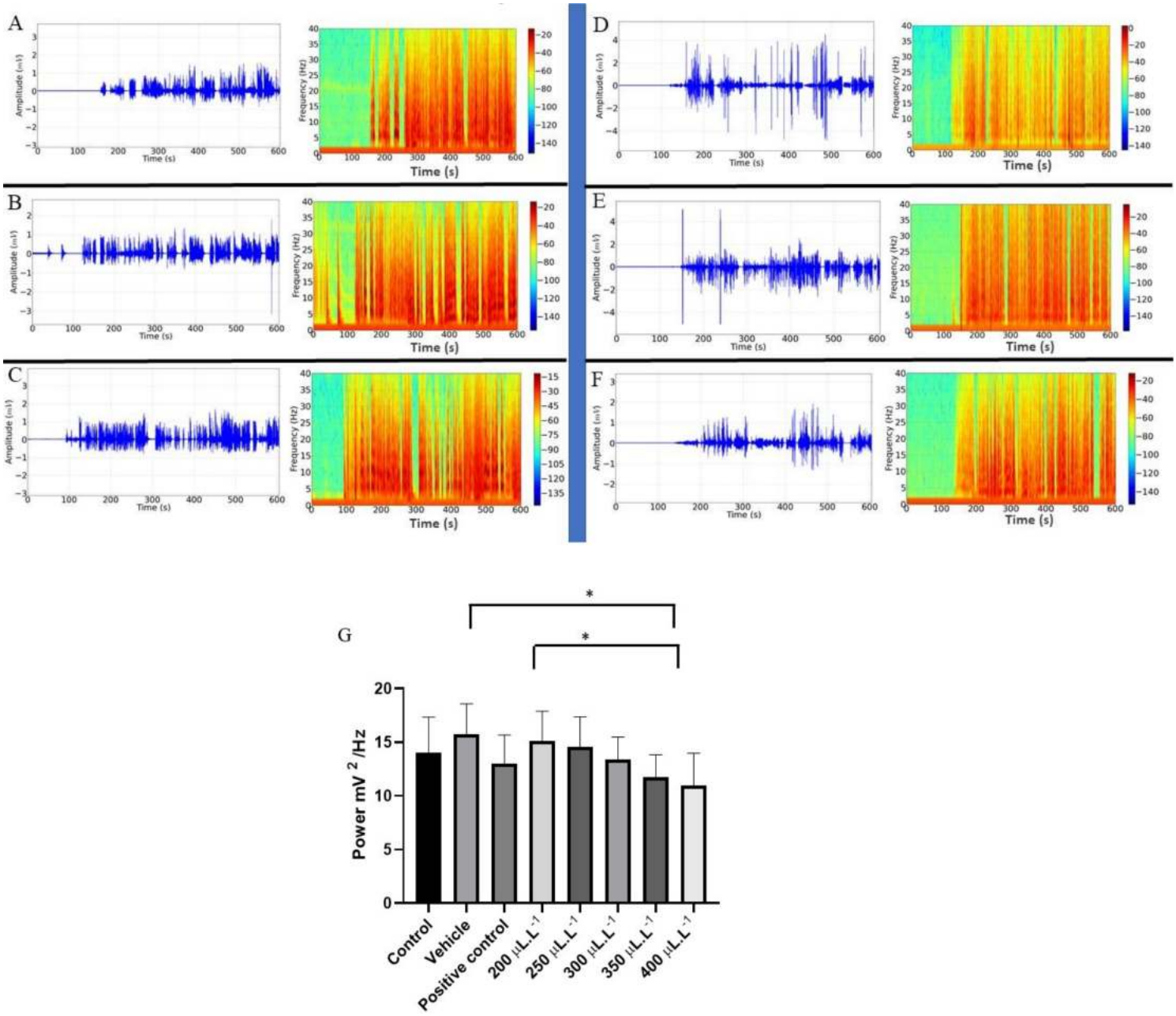

Figure 5

Dorsal muscle contraction activity of *Colossoma macropomum* after immersion in CAEO. EMG recordings over 600 seconds (left) and frequency spectrograms (right) for positive control and CAEO-treated groups (200–400 µL·L⁻¹). Graph shows muscle contraction power during anesthetic recovery. (ANOVA followed by Tukey's test; $p < 0.05$; $n = 9$).

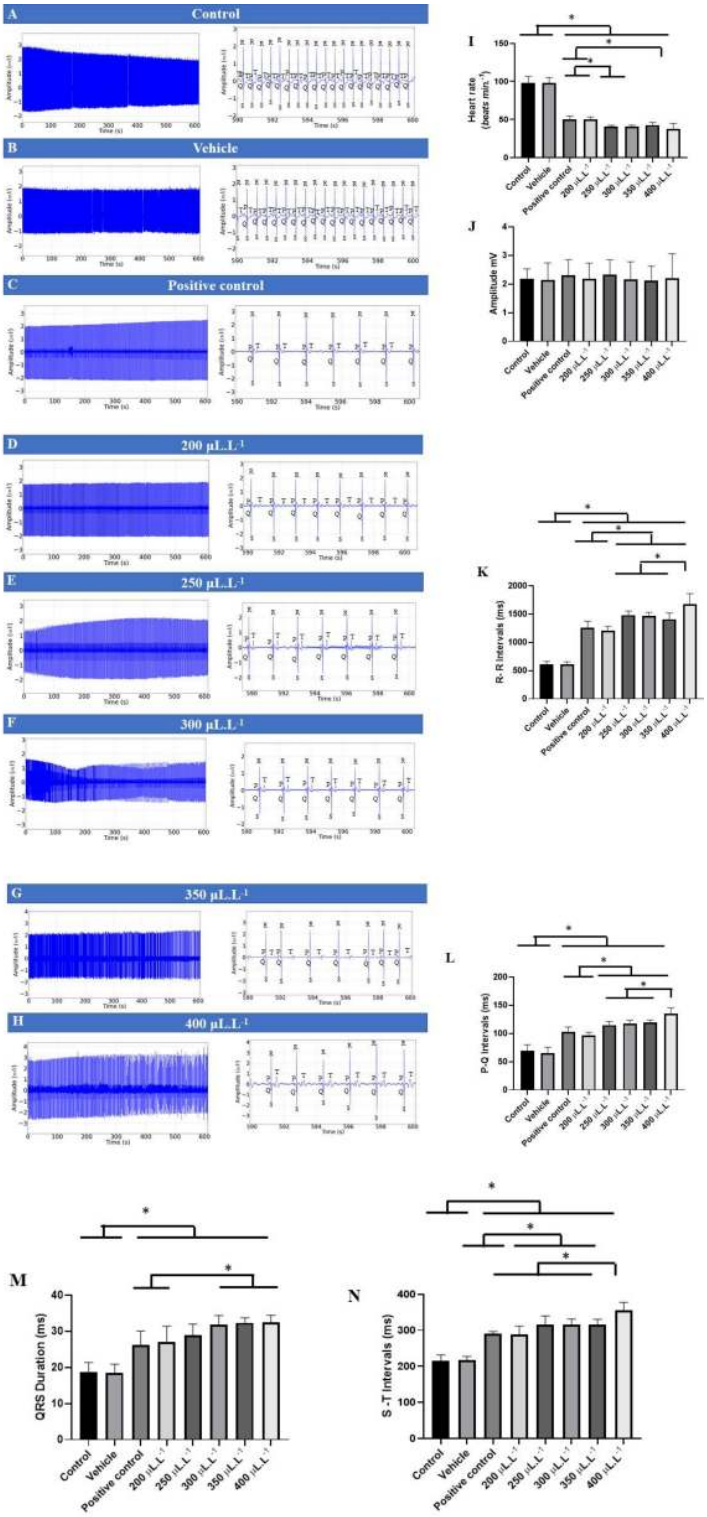

Figure 6

Electrocardiographic recordings showing cardiac activity of *Colossoma macropomum* during 600-second immersion baths with CAEO. Representative traces (left), 10-second amplified waveforms (right), and graphs for heart rate, QRS amplitude, P–Q interval, R–R interval, QRS duration, and S–T interval. (ANOVA followed by Tukey's test; $p < 0.05$; $n = 9$).

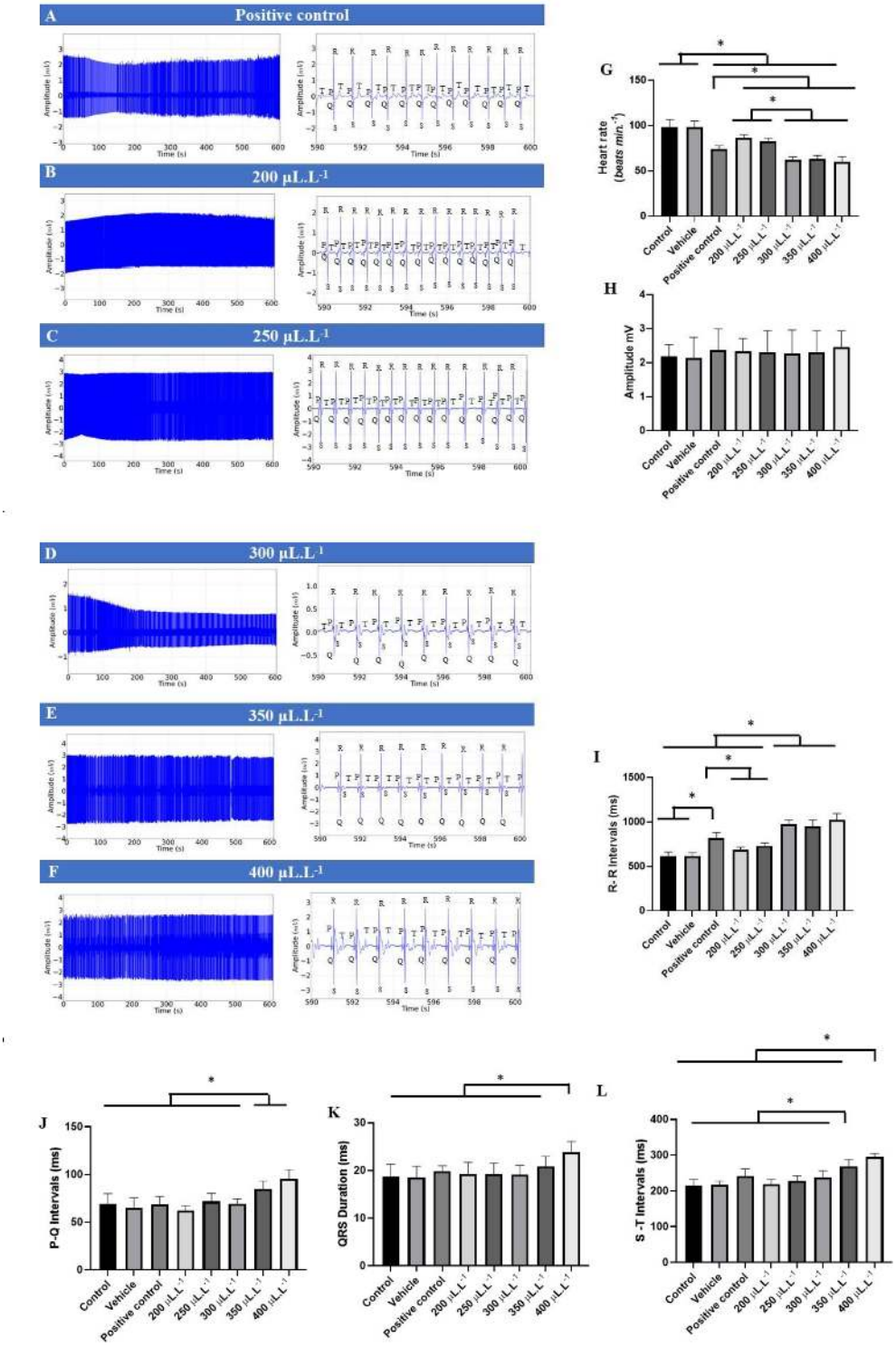

Figure 7

ECG recordings of *Colossoma macropomum* during recovery after exposure to CAEO. Representative traces (left), 10-second amplifications (right), and graphs showing recovery of heart rate, QRS amplitude, R–R interval, P–Q interval, QRS duration, and S–T interval. (ANOVA followed by Tukey's test; $p < 0.05$; $n = 9$).

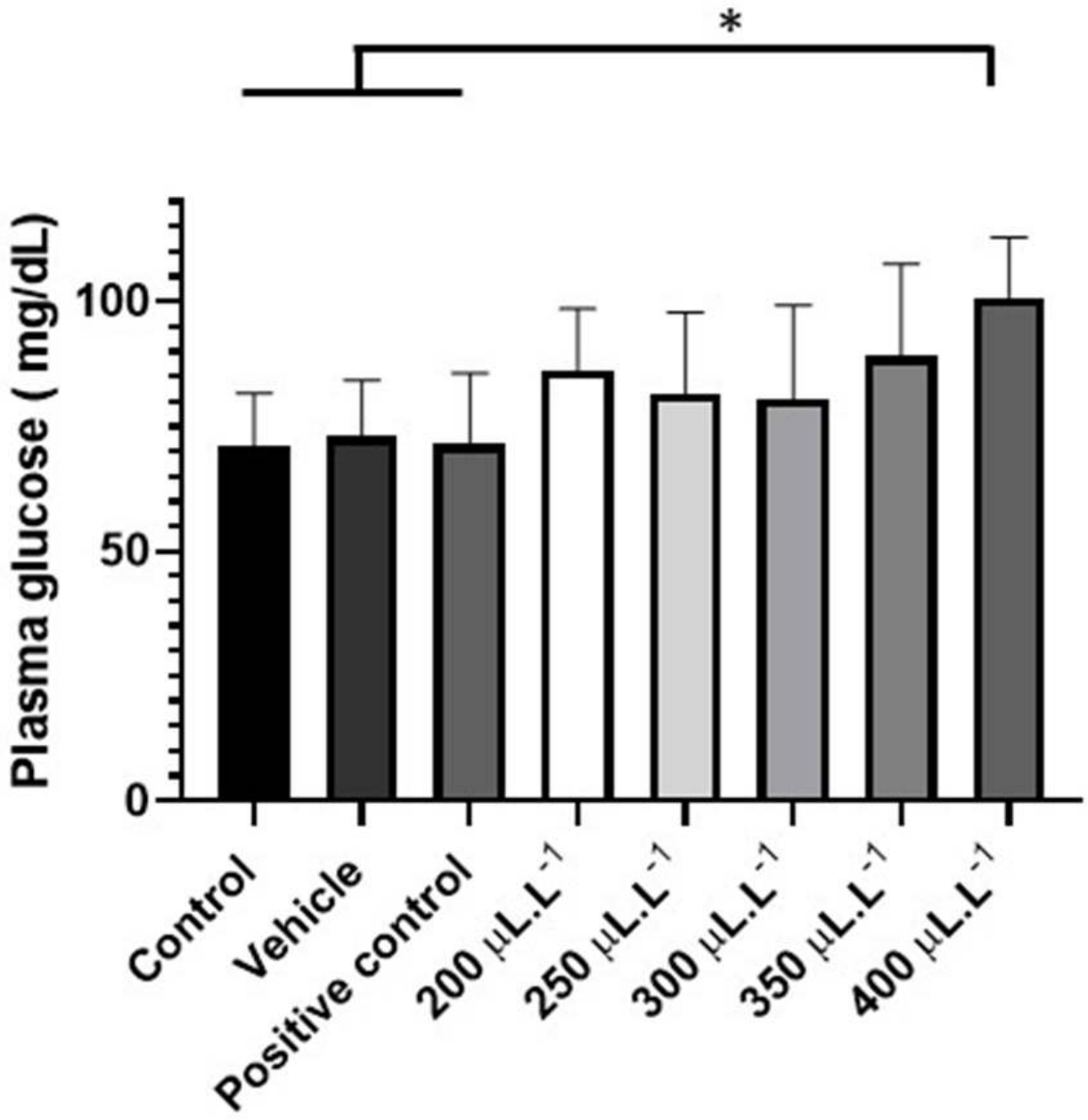

Figure 8

Plasma glucose levels in juvenile *Colossoma macropomum* 30 minutes after exposure to different concentrations of *Cedrus atlantica* essential oil (CAEO) in immersion baths. (ANOVA followed by Tukey's test; $p < 0.05$; $n = 9$).