Behavioral and electrophysiological study in Colossoma macropomum treated with different concentrations of Nepeta cataria oil in an immersion bath revealed a therapeutic window for anesthesia

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Behavioral and electrophysiological study in Colossoma macropomum treated with different concentrations of Nepeta cataria oil in an immersion bath revealed a therapeutic window for anesthesia.

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Abstract

The purpose of this study was to characterize the activity of essential oils from Nepeta Cataria (EON) at concentrations of 125 µ*L*.L^{-1} , 150 µ*L*.L^{-1} , 175 µ*L*.L^{-1} and 200 µ*L*.L^{-1} on the behavior of loss of the posture reflex and recovery of the posture reflex and electrocardiographic activity and recording of the opercular beat of *Colossoma macropomum* during immersion bathing for a period of 5 minutes, in order to obtain a window for safe use during anesthesia. The fish (23.38 ± 3.5g) were assigned to the following experiments: Experiment 1 (latency to loss and recovery of the posture reflex): a) 125 $\mu L.L^{-1}$ b)150 $\mu L.L^{-1}$, c) 175 $\mu L.L^{-1}$ and d) 200 $\mu L.L^{-1}$ (n=9) per group. Experiment 2 (electrocardiographic and heartbeat recordings): a) Control group; b) Vehicle control group (2 ml of alcohol per liter of water), c) 125 $\mu L.L^{-1}$ d) 150 $\mu L.L^{-1}$, e) 175 $\mu L.L^{-1}$ and f) 200 $\mu L.L^{-1}$ (n=9), per group. All the concentrations used showed efficacy in inducing loss of the posture reflex and reversibility with recovery of the posture reflex, but the electrocardiographic recordings indicated morphographic changes such as bradycardia during induction and p-wave apiculation during recovery at the highest concentrations tested. In this way, we suggest a safe use window for short-term anesthesia with EON in the concentration range of 125 $\mu L.L^{-1}$ to 150 $\mu L.L^{-1}$ for juvenile *Colossoma macropomum.*

Key words: Tambaqui, Electrocardiogram, Natural products, Anesthesia.

1 INTRODUCTION

Anesthetics are often used in fish farms and research environments that use fish as experimental models, to minimize the stress that can cause a breakdown in the organism's homeostasis during handling and transport (Gimbo *et al*. 2008; Aydin and Barbas 2020). The physical damage resulting from these accidents increases the susceptibility of fish to pathogens and infectious diseases, so sedation and anesthesia procedures can reduce undesirable occurrences in animal handling (Inoue et al. 2003; Ganesh 2020; Yang et al. 2020). Physiological stress is caused by altered homeostasis and is an attempt to provide the organism with support to respond and adapt to adverse conditions. Prolonging the stress process can cause disruptive physiological disturbances, ranging from metabolic changes to productive, reproductive and behavioral alterations (Alfonso et al. 2021; Hattori et al. 2020; Rousseu et al. 2021).

Metabolic effects, such as changes in blood glucose, blood lactate, hydromineral balance, among others, correspond to the secondary effects of stress in fish, which can lead to a drop in animal production and reproduction, as well as diseases, which are characterized as tertiary stress responses (Gesto et al. 2014; Jiang et al. 2017; Chang et al. 2020; Little et al. 2020).

Anesthetic and sedative drugs are used in aquaculture for welfare reasons, to minimize trauma, relieve pain and attenuate the physiological response to stress (Neiffer and Stamper 2009; Hilde et al. 2012; Vergneau-Grosset 2022; Harms 2000). Routinely in human and veterinary medicine, anesthesia protocols are established to ensure a smooth transition to the desired state or plane of anesthesia (sedation, immobilization, narcosis, amnesia), and are also recommended as the first step in euthanasia for most fish in the laboratory (Readman .et al. 2013, Martins et al. 2018; Matthews 2012).

A study carried out by De Souza et al. (2019) found the anesthetic effect of the *Nepeta* cataria plant (EON), popularly known as cat grass, on juvenile Tambaqui. Other studies have evaluated the action of N. cataria as an antibiotic, antiparasitic and antioxidant (Nadeem et al. 2022; Tan et al, 2019; Khan et al. 2012). Thus, this article aims to investigate the action of *Nepeta cataria* in juvenile *Colossoma macropomum* submitted to immersion baths in different concentrations, evaluating behavior and cardiac and respiratory activity, identifying the main morphographic changes in electrocardiographic recordings and indicating a concentration window for safe anesthesia.

2 MATERIAL AND METHODS

2.1 Experimental fish and acclimation period

The animals used in this study were a total of 90 juveniles of Tambaqui, Colossomo macropomum, (23.38 ± 3.5g) acquired on a commercial farm and obtained through a controlled spawning process. The fish were acclimatized in aquariums (5.0 g fish L^{-1} ; 10 fish per aquarium) for 10 days in the Experimental Vivarium of the Laboratory of Pharmacology and Toxicology of Natural Products at the Federal University of Pará. The quality parameters of the water (temperature 26.8°C; pH 7.5; dissolved oxygen >5.0 mg. L^{-1})) were monitored and maintained using a multi-parameter measuring device (HANNA™).

This study was approved by the ethics committee for animal experimentation at the Federal University of Pará (CEUA-UFPA - 3898260521 (ID 001694)).

2.2 Test substance and experimental design

The substance used in this study was the essential oil of *Nepeta cataria* (EON) (Beleza do Campo™, Barretos - SP, Brazil), purchased in commercial establishments. The composition of EON: 0.6% β-pinene, 0.2% piperitone, 77.6% nepetalactone, 7.6% dihydronepetalactone, 3.2% β-caryophyllene, 1.7% α-humulene, 1.1% farnesene and 0.6% caryophyllene oxide, according to the suppliers' information. As EON is not very soluble in water, a stock solution of EON pre-diluted in ethanol (70%) in a ratio of 1:9 was prepared, from which aliquots were taken to treat the animals. Both products were stored in amber glass bottles at 4°C until use.

After the acclimation period, the fish were randomly assigned to the following treatments: *a)* control (basal record), *b)* vehicle control (2ml of 70% alcohol in 1 liter of water); *c) fish* immersed in EON at 125 $\mu L.\ L^{-1}$ and subsequent recovery, d) fish immersed in EON at 150 $\mu L.\ L^{-1}$ and subsequent recovery, *e) fish immersed in EON at* 175 $\mu L.\ L^{-1}$ and subsequent recovery and *f) fish immersed in EON at* 200 $\mu L.\ L^{-1}$ and subsequent recovery. The immersion bath lasted 5 minutes. The latency stage was assessed, and cardiac recordings captured. After induction of anesthesia, the animals were moved to water without anesthetic and the recovery of the fish was assessed for 5 minutes.

2.3.1 Behavior analysis

Considering the contact time with EON concentrations of 125 μ$L.\ L^{-1}$, 150 μ$L.\ L^{-1}$, 175 μ$L.\ L^{-1}$and 200 μ$L.\ L^{-1}$ in an immersion bath, the latency for the loss of posture reflex behavior was assessed, which was characterized by lateral decubitus for more than 15 seconds. Afterwards, the animals were removed from contact with the EON and the latency to recover the posture reflex was assessed, which was characterized by maintaining the posture for more than 15 seconds.

2.3.2 Electrocardiogram (ECG) analysis

For cardiac monitoring, the electrodes were made from silver wire (925), with a diameter of 0.3 mm and a length of 10 mm. The reference electrode was fixed ventral in the left opercular cavity (0.2 mm) and the recording electrode was inserted 0.2 mm into the right opercular cavity, in the D-1 derivation. The electrodes were then connected to a high impedance amplifier. The recordings were used to analyze heart rate (bpm), QRS duration (ms), P-Q interval (ms), RR interval (S), S-T interval (ms) (Figures 1 A and B).

2.3.3 Opercular beat frequency

To record respiratory function through opercular beating, conjugated silver electrodes (925) with a diameter of 0.3 mm and a length of 5.0 mm were manufactured and placed on the left operculum to record activity during the immersion bath. Each recording lasted 5 minutes and responses between 265-270 s were analyzed, both during induction and recovery.

2.3.4 Analysis of records

The electrodes were connected to a digital data acquisition system using a high-impedance differential input amplifier (Grass Technologies, Model P511), adjusted with 0.3 and 300 Hz filtering, with 2000X amplification and monitored with an oscilloscope (ProteK, Model 6510). The recordings were continuously digitized at a rate of 1 KHz on a computer equipped with a data acquisition board (National Instruments, Austin, TX), and were stored on a hard disk and then processed using specialized software (LabVIEW express).

2.4 Statistical analysis

After checking that the assumptions of normality and homogeneity of variances were met, using the Kolmogorov-Sminov and Levene tests respectively, comparisons of the mean power values were made using one-way ANOVA, followed by the Tukey test. GraphPad Prism software® 8 was used for the analyses and a value of $*p<0.05$, $**p<0.01$, $***p < 0.001$, was considered statistically significant in all cases.

3 RESULTS

3.1. Behavioral data indicate reversibility of the effects of Nepeta cataria oil on fish.

Behavioral analysis showed that EON caused the animals to lose their posture reflex, which occurred in a concentration-dependent manner. The group treated with 125 $\mu L.L^{-1}$ had a mean latency of 214.4 ± 17.83 seconds, which was longer than the 150 $\mu L.L^{-1}$ EON group, which had a mean latency for loss of the posture reflex of 175.0 ± 24.77 seconds. The group treated with 175 $mg.L^{-1}$ had a mean latency of 137.7 ± 10.00 seconds, lower than the groups treated with 125 $\mu L.L^{-1}$ and 150 $mg.\ L^{-1}$, but was like the group treated with 200 $\mu L.L^{-1}$ (121.4 ± 14.35 seconds), (p= 0.225) (Figure 1 A).

The recovery of the posture reflex after a contact time of 5 minutes in an immersion bath for the group treated with $125 \mu L.L^{-1}$ had a mean of 117.7 ± 11.03 seconds, like the group treated with $150 \mu L.L^{-1}$ (125.9 ± 17.14 seconds) (p = 0.657). Recovery for the group treated with $175 \mu L.L^{-1}$ had a mean of 145.6 ± 19.28 seconds, showing a statistical difference to the $125 \mu L.L^{-1}$ and $150 \mu L.L^{-1}$ groups. The group treated with $200 \mu L.L^{-1}$showed a mean recovery of the posture reflex of 170.6 ± 11.05 seconds, a statistical difference from the other groups (Figure 1 B).

Figure 1 - Average latency (seconds) for loss of posture reflex during treatment in an immersion bath with different concentrations of EON (A). Shows the time taken for the posture reflex to recover after treatment with different concentrations of EON in an immersion bath (B). After ANOVA followed by Tukey's test (*p<0.05, **p<0.01 and ***p<0.001).

3.2. Nepeta cataria oil caused bradycardia and changes in ECG graphoelements

The normal electrocardiogram of the Tambaqui showed sinus rhythm and an average heart rate of 92.44 ± 5.981 bpm, with the P wave, the QRS complex and where T. The interval between R-R deflagrations remained regular with an average of 0.6026 ± 0.01437 seconds. The P-Q interval showed time to atrial activity and onset of ventricular activity with a mean of 87.2 ± 6.19 milliseconds. The time for the QRS complex to occur had a mean of 29.68 ± 1.55 ms. The time elapsed between the end of ventricular depolarization (S wave) and ventricular repolarization (T wave), represented by the S-T interval, had a mean of 289.2 ± 15.50 ms. Considering normal cardiac parameters, we measured the interference of increasing concentrations of EON during immersion and recovery in aquarium water (Figure 2 A and B).

Figure 2 - Normal electrocardiographic (ECG) recordings of Tambaqui, *Colossoma macropomum* (A), enlarged electrocardiogram showing heart rate (bpm), P-Q interval (ms), R-R interval (S), QRS duration (ms), S-T interval (ms) (B) (n=9).

During treatment of the groups with EON, electrocardiographic alterations could be seen which varied according to concentration (Fig. 3. A, B, C, D, E). According to the treatment, the alterations with the greatest variations in the tracings were related to the concentration of 200 $\mu L.L^{-1}$ with a decrease in heart rate and the appearance of morphographic irregularities in the recordings (Figures 3 C and D). All the deflagrations in the electrocardiogram of the Tambaqui at the concentrations studied can be identified, showing sinus rhythm, as can be seen in the magnification of the tracings shown in Figures 3 A, B, C and D.

Figure 3 - Electrocardiographic (ECG) recordings of Tambaqui, *Colossoma macropomum* (left) and their amplifications (right) during a 5-minute immersion bath with EON, with amplification occurring in the 265-270 second section of the recordings (red line), for each concentration: 125 $\mu L.L^{-1}$ (A), 150 $\mu L.L^{-1}$ (B), 175$\mu L.L^{-1}$ (C), 200 $\mu L.L^{-1}$ (D). Graph showing mean heart rates for each treatment. After ANOVA followed by Tukey's test (**p<0.01 and ***p<0.001) (n=9).

Throughout the immersion period bath treatment with EON there was a decrease in heart rate, the control group had an average of 92.44 ± 5.981 (bpm), which was higher than the other groups: 125 $\mu L.L^{-1}$ (74.00 ± 3.606 bpm), 150 $\mu L.L^{-1}$ (61.11 ± 5.66 bpm), 175 $\mu L.L^{-1}$ (48.22 ± 3.383 bpm) and 200 $\mu L.L^{-1}$ (40.22± 3.528 bpm). The treated groups showed a decrease in heart rate which was intensified by the increase in EON concentration (Figure 3 E).

The R-R interval in the control group had a mean of 0.602 ± 0.014 seconds and was lower than the means of the treated groups: 125 $\mu L.L^{-1}$ (0.814 ± 0.044 s), 150 $\mu L.L^{-1}$ (1.010 ± 0.042 s), 175 $\mu L.L^{-1}$ (1.18 ± 0.067 s) and 200 $\mu L.L^{-1}$ (1.48 ± 0.125 s). There were statistical differences between the groups treated with EON at the different concentrations (Figure 4 A).

The P-Q interval for the control group averaged 87.21 ± 6.191 ms and was similar to the groups 125 $\mu L.L^{-1}$ (81.03 ± 4.99 ms) (p= 0.449), 150 $\mu L.L^{-1}$ (78.22 ± 11.18 ms) (p= 0.119) and 175 $\mu L.L^{-1}$ (87.92 ± 6.68ms) (p= 0.999). The group treated with 200 $\mu L.L^{-1}$ had a longer mean interval (103.4 ± 8.16 ms) than the other groups (Figure 4 B).

During the process of ventricular contraction, which is represented by the QRS complex, the duration of this phenomenon is related to the time of ventricular excitability, the Tambaqui heart in sinus rhythm showed an average of 29.11 ± 1.45 ms was similar to the 125 $\mu L.L^{-1}$ group (30.00 ± 2.73 ms) (p = 0.998). The control group was larger than the groups: 150$\mu L.L^{-1}$ (39.11 ± 8.477 ms), 175 $\mu L.L^{-1}$(45.33 ± 9.91ms) and 200 $\mu L.L^{-1}$(58.227 ± 8.511ms). The group treated with 200 μL. L^{-1} was higher than the other groups (Figure 4 C).

The S-T interval indicates the time taken by the ventricle to start repolarization. The control group had a mean of 288.7 ± 15.35 ms, was similar to the 125 $\mu L.L^{-1}$ group (329.9 ± 26.77 ms) (p= 0.133) and showed a statistical difference to the 150$\mu L.L^{-1}$(367.4 ± 26.91 ms) and 175 $\mu L.L^{-1}$ (401.7 ± 42.14 ms) and 200 $\mu L.L^{-1}$ (482.8 ± 55.89 ms) groups. The group treated with 200 $\mu L.L^{-1}$ was larger than the other groups (Figure 4 D).

Figure 4 - Evaluation of the mean R-R intervals (S) (A), P-Q intervals (ms) (B), QRS duration (ms) (C) and S-T interval (ms) (D) during treatment with EON at concentrations of 125 $\mu L.L^{-1}$, 150 $\mu L.L^{-1}$, 175 $\mu L.L^{-1}$ and 200$\mu L.L^{-1}$. After ANOVA followed by Tukey's test. (*p<0.05, **p<0.01 and ***p<0.001).

During treatment at a concentration of 200 $\mu L.\ L^{-1}$ in an immersion bath, morphological changes were observed in the ECG tracings, in particular depression of the S-T segment and electrical alternation of the QRS complex. In mammals, depression of the S-T segment is observed during cardiac hypoxia and electrical alternation of the QRS complex is related to functional pathologies such as cardiac tamponade, taking into account anatomical differences. For Tambaqui in contact with 200μ$L.\ L^{-1}$ of EON, we must take into account the appearance of depression of the S-T segment associated with electrical alternation of the QRS complex in the same ECG tracing, which indicates cardiac dysfunction during immersion (Figure 5).

Figure 5 - Electrocardiographic tracing of *Colossoma macropomum* during treatment in an immersion bath with 200 μ$L.\ L^{-1}$, showing changes in depression of the S-T segment (red arrows) and electrical alternation of the QRS complex (black arrows). Recording time increased by 20 seconds.

The recoveries after treatment with different concentrations of EON were analyzed over a period of 5 minutes where cardiac activity, taking into account physiological patterns of heart rate, R-R interval, P-Q interval, S-T interval and QRS duration, for the groups that had contact with 125 μ$L.\ L^{-1}$ and 150 μ$L.\ L^{-1}$ maintained a rapid reversibility of bradycardia, but in the groups treated with 175 μ$L.\ L^{-1}$ and 200 μ$L.\ L^{-1}$bradycardia was not recovered within 5 minutes, but sinus rhythm was maintained at the concentrations (Figure 6. A, B, C and D). After immersion in 200 μ$L.\ L^{-1}$, P wave apiculation was observed (Figure 6 D).

The electrocardiogram of the control group showed an average rate of 92.44 ± 5.981 bpm, similar to the 125 μ$L.\ L^{-1}$ (97.11 ± 3.88 bpm) (p= 0.604), 150 μ$L.\ L^{-1}$(87.78 ± 3.23 bpm) (p= 0.609) groups. However, there was a difference between the groups treated with 175 μ$L.\ L^{-1}$ (68.67 ± 9.79 bpm) and 200 μ$L.\ L^{-1}$ (50.00 ± 8.83 bpm). The group treated with 200 μL. L-1 was lower than the other groups (Figure 6 E).

Figure 6 - Electrocardiographic (ECG) recordings of Tambaqui, *Colossoma macropomum* during recovery (left) and their amplifications (265 - 270 s) after immersion with EON (red line) at concentrations of 125 μ$L.\ L^{-1}$ (A), 150 μ$L.\ L^{-1}$ (B) 175 μ$L.\ L^{-1}$ (C) *and* 200 μ$L.\ L^{-1}$ (D) heart rate (bpm) (E). After ANOVA followed by Tukey's test (*p<0.05, and ***p<0.001), (n=9).

In the sinus rhythm recording of the Tambaqui, the R-R interval was kept regular and presented an average of 0.602 ± 0.014 s, similar to the groups: 125 µL. L-1 0.618 ± 0.024 s) (p = 0.991), 150µL. L^{-1} (0.625 ± 0.075 s) (p=0.966), but the control group was smaller than the 175 µL. L^{-1} (0.8250 ± 0.1195 s) and 200µL. L^{-1} (1.183 ± 0.08929 s) groups. The 200 µL. L^{-1} group was larger than the other groups (Figure 7 A).

The P-Q interval for the control group averaged 86.67 ± 6.16 ms and was similar to the groups: 125 µL. L^{-1} (81.78 ± 8.65 ms) (p= 0.456), 150 µL. L^{-1}(87.89 ± 3.219 ms) (p= 0.993) and 175 µL. L^{-1} (89.33 ± 6.65 ms) (p=0.888). However, the control group had a lower mean after contact than the 200 µL. L^{-1} group (105.3 ± 4.74 ms). The 200 µL. L^{-1} group had a higher mean than the other groups (Figure 7B).

The QRS duration after the immersion bath when compared to the heart in normal physiological functions with 29.11 ± 1.45 ms was similar to the groups: 125µL. L^{-1}(30.11± 2.52 ms) (p= 0.978), 150 µL. L^{-1} (31.56 ± 2.603 ms) (p= 0.637). However, these groups had shorter mean QRS durations than the 175 µL. L^{-1} (39.00 ± 3.60 ms) and 200µL. L^{-1} (47.11 ± 6.43 ms) groups (Figure 7C).

The Q-T interval after the immersion bath, for the control group, the heart of the Tambaqui in sinus rhythm showed an average of 288.7 ± 15.35 ms and was similar to the 125 µL. L^{-1} (284.3 ± 7.71 ms) (p= 0.996) and 150 µL. L^{-1} (290.1 ± 7.97 ms) (p= 0.999) groups. These groups had a lower mean than the 175 µL. L^{-1} group (355.8 ± 28.44 ms). The group treated with 200 µL. L^{-1} (390.2 ± 47.91 ms) had a higher mean than the other groups (Figure 7D).

Figure 7- Evaluation of the mean R-R intervals (S) (A), P-Q intervals (ms) (B), QRS duration (ms) (C) and S-T interval (ms) (D) during recovery from treatment with EON at concentrations of 125 µL. L^{-1}, 150 µL. L^{-1}, 175 µL. $L^{-1}$and 200 µL. L^{-1} .After ANOVA followed by Tukey's test.(**p<0.01 and ***p<0.001).

Analyzing the electrocardiogram in comparison to other species, the pointed P wave represents atrial overload, meaning a greater effort by the atrium to pump blood into the ventricle. The group treated with 200 μ$L.\ L^{-1}$ of EON in a 5-minute immersion bath, this morphographic characteristic of the electrocardiogram was detected in the period after the immersion bath. This electrocardiographic characteristic was maintained during the period in which the animal was recovering its posture reflex (Figure 8).

Figure 8- Electrocardiographic tracing of *Colossoma macropomum* after immersion in 200 μ$L.\ L^{-1}$, showing changes in P waves with an apiculate appearance (red arrows).

3.3. Changes in respiratory dynamics

The respiratory activity of *Colossoma macropomum* in the control group showed an average frequency of 67.11 ± 2.84 OBM, with amplitude maintained at 0.5 mV (Figure 9 A). In the 5-second amplification, the rhythm of the opercular movement can be observed (Figure 9.A right).

Recordings of the opercular beat at different concentrations during immersion in EON showed a decrease in respiratory activity at higher concentrations (Figure 9 B, C, D and E). The recordings of the respiratory activity of *Colossoma macropomum* showed that the control group had an average of 67.11 ± 2.84 OBM, which was higher than the other groups during the immersion bath: 125 μ$L.\ L^{-1}$ (40.44 ± 4.87 OBM), 150 μ$L.\ L^{-1}$(34.00 ± 2.00 OBM), 175 μL.L^{-1} (29.33 ± 1.41 OBM) and 200 μ$L.\ L^{-1}$ (26.00± 1.73). The 125 μ$L.\ L^{-1}$ group was larger than the other groups. The 150 μ$L.\ L^{-1}$immersion bath group was larger than the 175 μ$L.\ L^{-1}$ and 200μ$L.\ L^{-1}$ groups. The 175 μ$L.\ L^{-1}$ and 200 μ$L.\ L^{-1}$ immersion groups were similar (p= 0.1174) (Figure 9 F).

Figure 9 - Recordings of respiratory activity (opercular beats per minute) (OBM) in *Colossoma macropomum*, during immersion with Nepeta cataria (left), and amplification of the recording in the period 265-270s (right): Control group (A), 125 $\mu L.\ L^{-1}$ (B); 150 $\mu L.\ L^{-1}$ (C); 175 $\mu L.\ L^{-1}$(D); 200 $\mu L.\ L^{-1}$(E). Graph showing the average frequency of the groups' opercular movements during the immersion bath (F). (ANOVA followed by Tukey *p<0.05, **p<0.01; *** p<0.001; n=9).

During the recovery period from the immersion bath, the animals showed a gradual recovery of opercular motility, demonstrating the reversibility of EON (Figure 10 A, B, C and D) . The control group (67.11 ± 2.84 OBM) was similar to the group treated with 125 $\mu L.\ L^{-1}$(63.56 ± 3.71 OBM) (p= 0.219) but was higher than the 150 $\mu L.\ L^{-1}$ (54.22 ± 4.177 OBM) ; 175$\mu L.\ L^{-1}$ (48.89 ± 2.26 OBM) and 200 $\mu L.\ L^{-1}$ (42.89 ± 4.13 OBM) groups. The 150$\mu L.\ L^{-1}$ group was larger than the 175 $\mu L.\ L^{-1}$ and 200 $\mu L.\ L^{-1}$ groups. The group recovering after treatment with 175 $\mu L.\ L^{-1}$ had a higher mean opercular beat than the 200 $\mu L.\ L^{-1}$ group (Figure 10 E).

Figure 10 - Recordings of respiratory activity (opercular beats per minute) (OBM) in *Colossoma macropomum*, after immersion in the recovery period (left), and amplification of the recording in the period 265-270s (right), after contact: 125 $\mu L.L^{-1}$ (A); 150 $\mu L.L^{-1}$ (B); 175 $\mu L.L^{-1}$ (C); 200 $\mu L.L^{-1}$ (D). Graph showing the average frequency of the opercular beats of the groups after the immersion bath (E). (ANOVA followed by Tukey *p<0.05; **p<0.01; *** p<0.001; n=9).

4. DISCUSSION

According to De Souza et al. (2019), the essential oil of N. cataria caused anesthesia and myorelaxation in *Colossoma macropomum* in an immersion bath at a concentration of 175 $\mu L.L^{-1}$. which was compared to anesthesia with propofol. Our study showed an effect on cardiac activity in increasing doses of N. cataria essential oil, contributing to a better understanding of the safety of its use as an anesthetic, providing the identification of a safe therapeutic range for anesthetizing animals. Indicating cardiac effects that alter hemodynamics at the tested concentrations of EON, 125 μ$L.\ L^{-1}$, 150 μ$L.\ L^{-1}$, 175 μ$L.\ L^{-1}$ and 200 μ$L.\ L^{-1}$, based on the analysis of heart rate parameters (bpm), QRS duration (ms), P-Q interval (ms), R-R interval (S), S-T interval (ms), making it possible to define a therapeutic window for the safety of induction and recovery from anesthesia. Relating to the effect on cardiac and respiratory activity at various concentrations during an immersion bath.

In the behavior analysis, it was observed that the induction and recovery periods follow a proportional relationship between the treatment concentrations, demonstrating a dose-dependent effect. thus, at lower concentrations the time for loss of the postural reflex was greater and the recovery time was shorter, while higher doses indicate a shorter induction time and a longer recovery time. At higher concentrations, with faster induction, it does not allow the organism to adapt to the new condition and may cause a breakdown in homeostasis. This relationship is also found in other studies on anesthesia in fish using different types of drugs, such as spilanthol, citronella, menthol, lidocaine and tricaine (Barbas et al. 2016, Barbas et al. 2017, Cantanhede et al. 2021, Vieira et al. 2021, Vieira et al. 2021, Vieira et al. 2023, Fontana et al. 2021, Nordgreen et al. 2014, Savson et al. 2022). Therefore, the behavioral observations align with the results of other studies, indicating that the effects of N. cataria oil on Colossoma macropomum are dependent on the concentration used in the treatment.

The electrocardiographic data showed that during the monitoring of Colossoma macropomum, bradycardia was observed at all concentrations used in treatments, being more severe at higher concentrations, so it was possible to analyze the evolution of bradycardia in fish using ECG fingers, such as the decrease in frequency heart rate and progressive increase in the R-R interval throughout the analysis of the four treatments evaluated in this study. DE SOUZA, et al, 2019, proposed that bradycardia caused by

EON is associated with hypoventilation, which would be a direct consequence of central nervous system depression or a direct effect on cardiac tissues. We observed that the opercular beat at the concentrations used during induction showed a decrease, which indicates depression of the central nervous system with less response to hypoxia for higher concentrations of EON.

Our data indicated that hemodynamics were impaired as a result of cardiac malfunction, as animals treated at a concentration of 200 $\mu L.L^{-1}$ showed a decrease in heart rate, depression of the S-T segment and electrical alternation of the QRS complex during induction. observed, which may be related to cardiac hypoxia that may be induced by drug effects or cardiac malfunction (Schang and Pepine 1977; Liu et al. 2018; Dong et al. 2022). However, many studies have demonstrated cardiac plasticity in several fish species, the most studied being Danio rerio (Yalcin et al. 2017; Shi et al. 2018; Wilkison et al 2014; Ross et al. 2022). This demonstrates the cardiac adaptation of fish when in contact with substances that can affect cardiac functioning, and can be indicated as a biomarker.

The variation in the amplitude of the QRS complex can be seen by the alternation of the R segment, which at times the peak of the wave reaches a voltage of 0.4 mV and at other times close to 0.2 mV may be indicative of some kind of defect in atrioventricular conduction or even a case of ischemia (Pueyo et al 2008; Khayer and Haque 2004) in which the data from the respiratory analysis of the Tambaqui may be a key indication of a case of ischemia due to a triggering of hypoxia and the effects of EON on the myocardium, since all the doses tested showed a decrease in the opercular beat compared to the control group during induction and recovery, corroborating hypoxia that worsens with increasing concentration.

Electrocardiographic recordings showed ST changes, which is related to a ventricular repolarization abnormality on the electrocardiogram (Inoue et al. 2017). According to Basso et al. (2000) patients with cardiomyopathy in whom the electrocardiogram shows ST segment depression may be indicative of myocardial ischemia, producing an effect of decreasing microvascular perfusion. However, this effect was only observed in treatments using the highest concentrations of *Nepeta cataria* oil.

During the recovery period at the highest concentrations, P wave apiculation was observed, which according to Platonov (2012) morphologically, the P wave can extend beyond the context of atrial arrhythmias, making it possible for the P wave to be used as a marker of conduction defects associated with arrhythmia mechanisms, For this reason, it is worth noting that the amplitude of the P wave in the study in question is close to the amplitude of the R segment, indicating a process of atrial overload or even conduction failure, taking into account other alterations in other segments of the ECG of anesthetized Tambaqui. In this sense, a detailed study to identify the EON concentration and contact time, since maintaining the state of P wave apiculation during recovery at higher doses such as 200 $\mu L.\ L^{-1}$ can cause hemodynamic damage which worsens with contact time. However, studies involving electrocardiographic data during induction of anesthesia with agents such as citronellol, geraniol, Piper divaricatum oil, eugenol and lidocaine did not show P wave apiculation (De Araújo et al. 2023, Vilhena et al. 2022; Fujimoto et al. 2017; Vieira et al. 2023), which may be related to the effect of EON on the heart.

All EON concentrations were effective and reversible in the behavioral experiment. However, additional investigations of cardiac and respiratory function indicated excessive depression with the presence of morphographic elements that indicate changes in ventricular and atrial functioning associated with respiratory depression in treatment with higher concentrations. Therefore, EON can be used more safely at concentrations of 125 µL. L-1 to 150 µL. L-1, to provide short-term anesthesia without major risks to hemodynamics in Colossoma macropomum.

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DECLARATIONS

Ethical Approval

Certify that the proposal entitled "Behavioral and Electrocardiographic Evaluation in Tambaqui (Colossoma macropomum) after the Use of Different Concentrations of Nepeta cataria L. Oil during Immersion Bath," filed under CEUA No. 3898260521 (ID 001694), under the responsibility of Moisés Hamoy - involving the production, maintenance, and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except humans), for scientific research or teaching purposes - complies with the precepts of Law 11,794 of October 8, as well as with the regulations issued by the National Council for Animal Experimentation Control (CONCEA), and has been approved by the Ethics Committee on Animal Use at the Federal University of Pará (CEUA/UFPA) in the meeting held on June 24, 2021.

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of resources and accountability are aligned with our principles, ensuring that the results of this investigation can benefit the scientific community and society significantly.

Availability of data and materials

If requested, all material related to this study, including raw data, research instruments, and other relevant information, is available upon request. To access these resources, we ask interested parties to contact us via email at Hamoy@ufpa.br. We are committed to transparency and collaboration in scientific research, and the provision of these materials aims to facilitate replicability and verification of results by the academic community.

Declarations of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Figure – 1

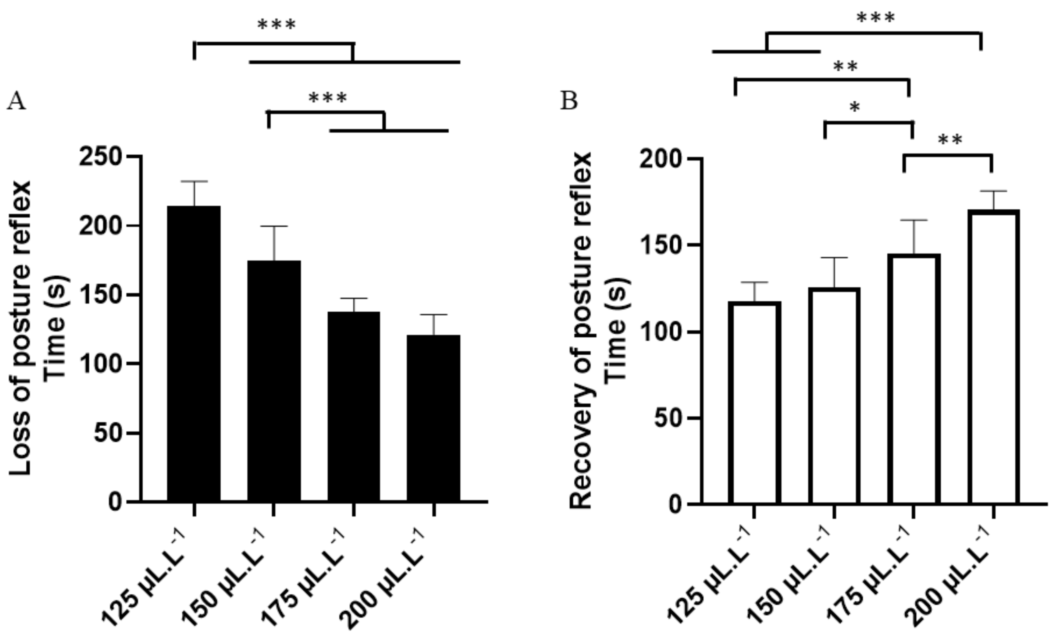

A
***
***
250
200
150
100
50
0
Loss of posture reflex
Time (s)
125 μL.L⁻¹
150 μL.L⁻¹
175 μL.L⁻¹
200 μL.L⁻¹
B
***
**
*
**
200
150
100
50
0
Recovery of posture reflex
Time (s)
125 μL.L⁻¹
150 μL.L⁻¹
175 μL.L⁻¹
200 μL.L⁻¹

Figure -2

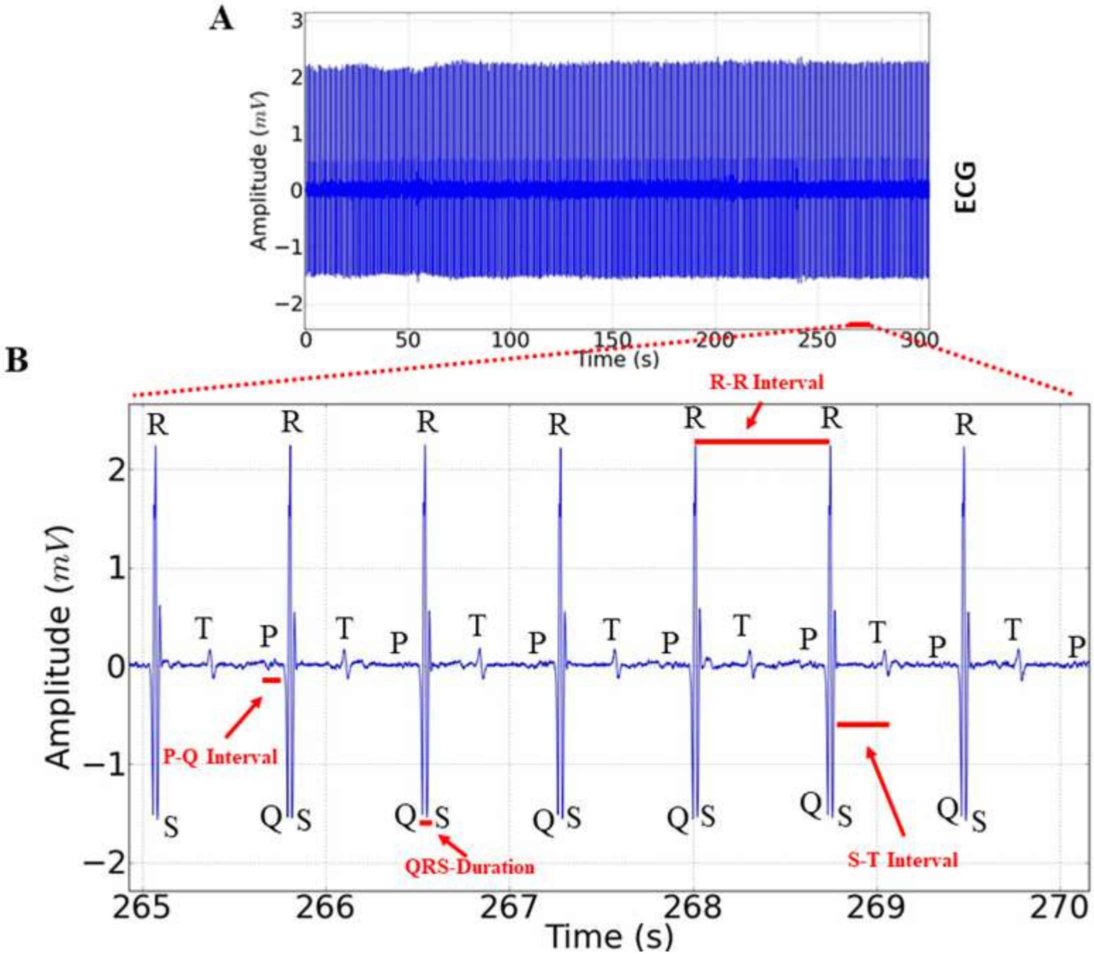

A
B
Amplitude (mV)
Time (s)
ECG
R-R Interval
P-Q Interval
QRS-Duration
S-T Interval
R
P
T
Q
S

Figure -3

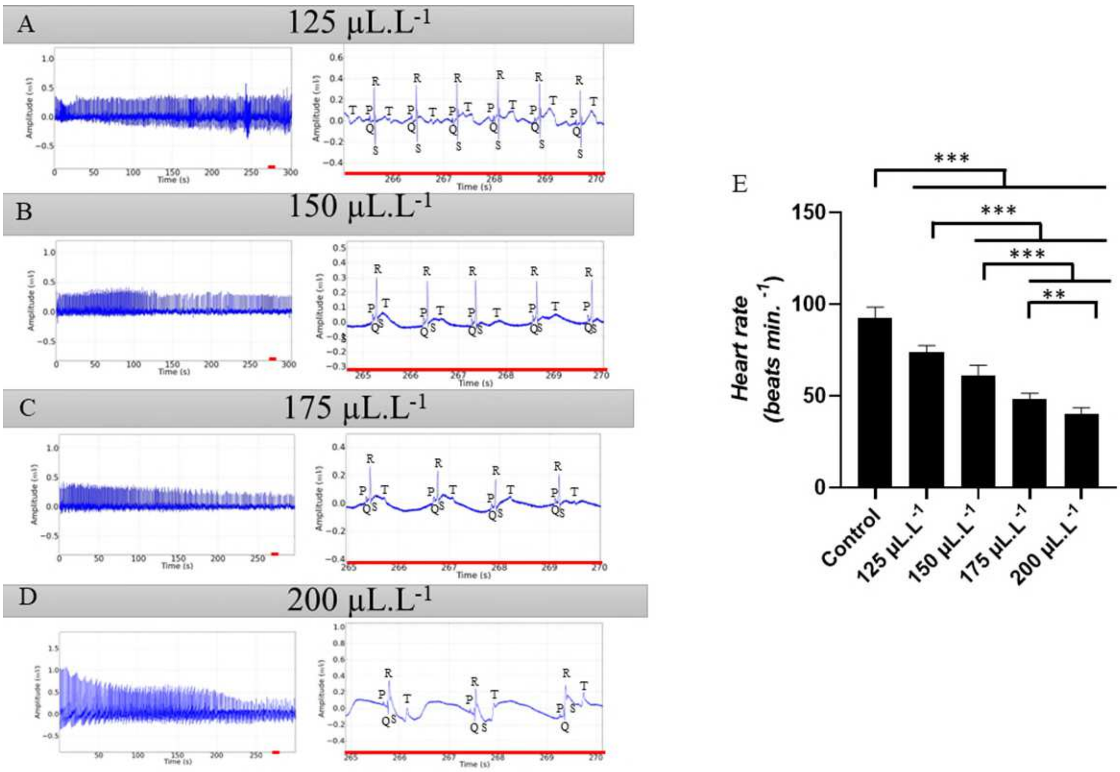

A
125 µL.L-1
B
150 µL.L-1
C
175 µL.L-1
D
200 µL.L-1
E
Heart rate (beats min. -1)
Control
125 µL.L-1
150 µL.L-1
175 µL.L-1
200 µL.L-1
***
**

Figure -4

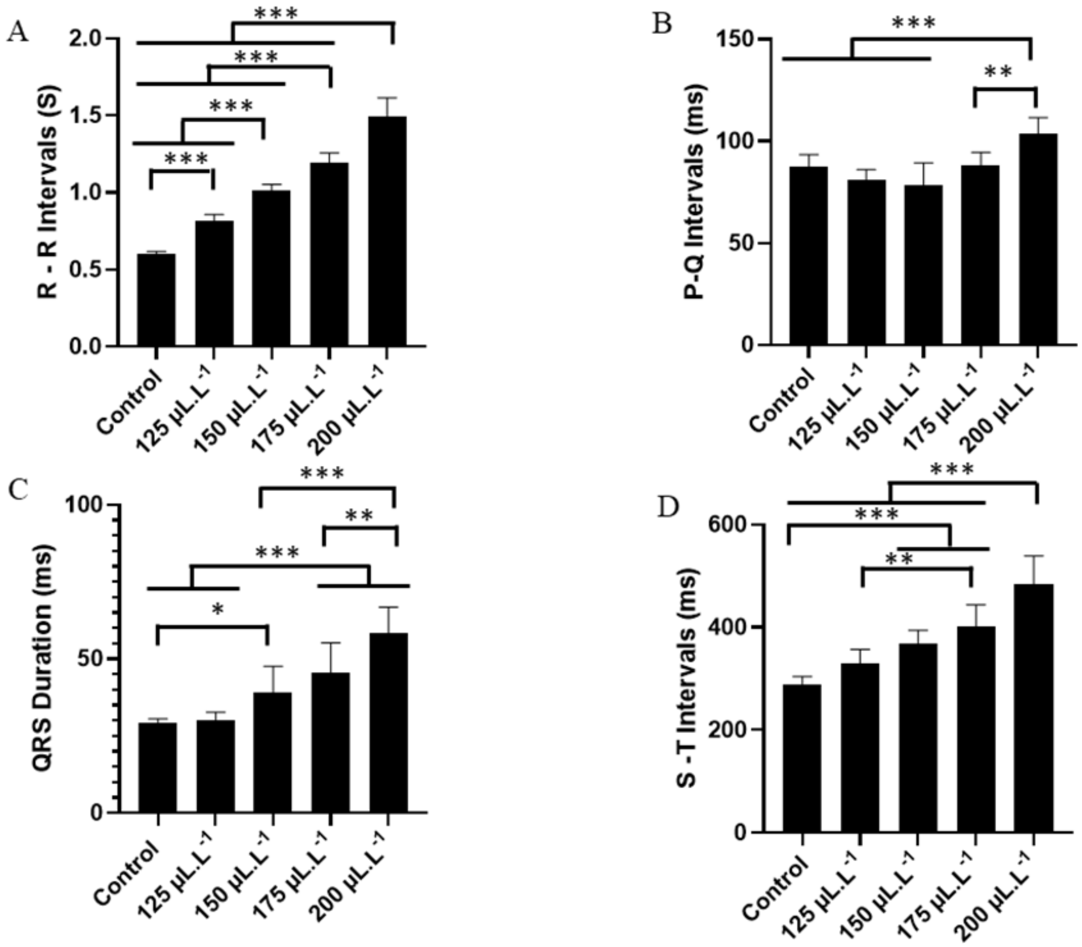

A
R - R Intervals (S)
2.0
1.5
1.0
0.5
0.0
***
***
***
***
Control
125 μL.L -1
150 μL.L -1
175 μL.L -1
200 μL.L -1
B
P-Q Intervals (ms)
150
100
50
0
***
**
C
QRS Duration (ms)
100
50
0
***
**
***
*
D
S -T Intervals (ms)
600
400
200
0
***
***
**

Figure -5

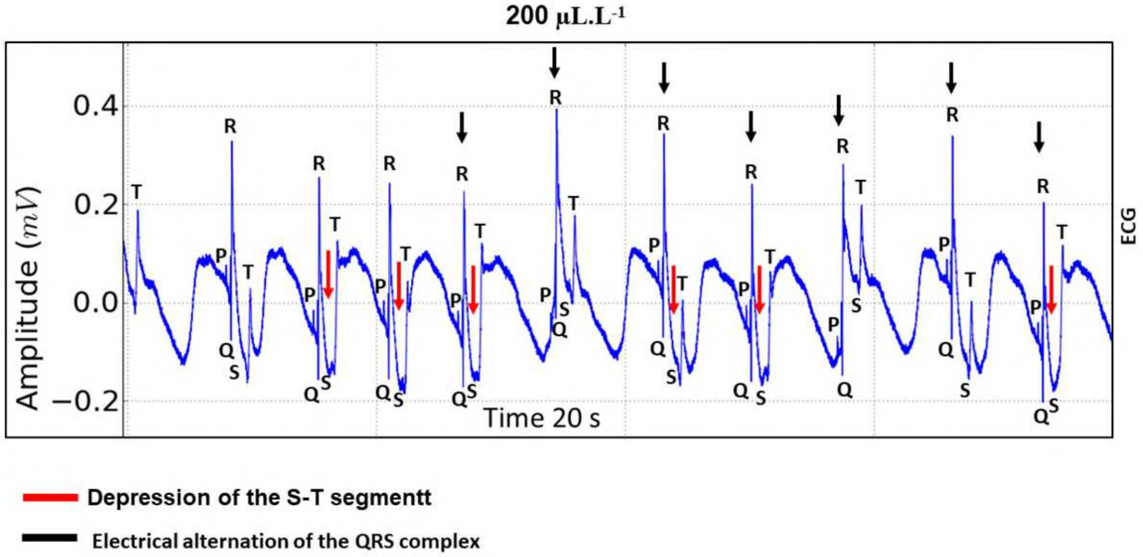
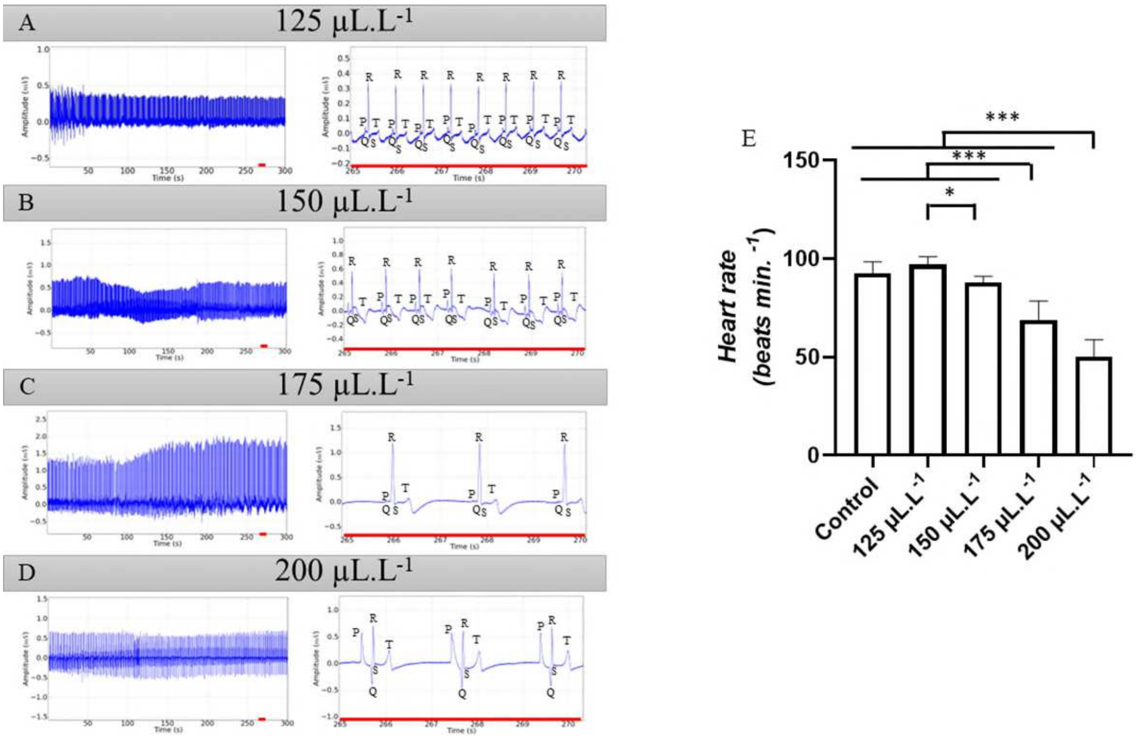

200 μL.L-1
Amplitude (mV)
0.4
0.2
0.0
−0.2
Time 20 s
ECG
Depression of the S-T segmentt
Electrical alternation of the QRS complex

Figure -6



A 125 µL.L-1
B 150 µL.L-1
C 175 µL.L-1
D 200 µL.L-1
E
Heart rate (beats min. -1)
Control
125 µL.L-1
150 µL.L-1
175 µL.L-1
200 µL.L-1

Figure -7

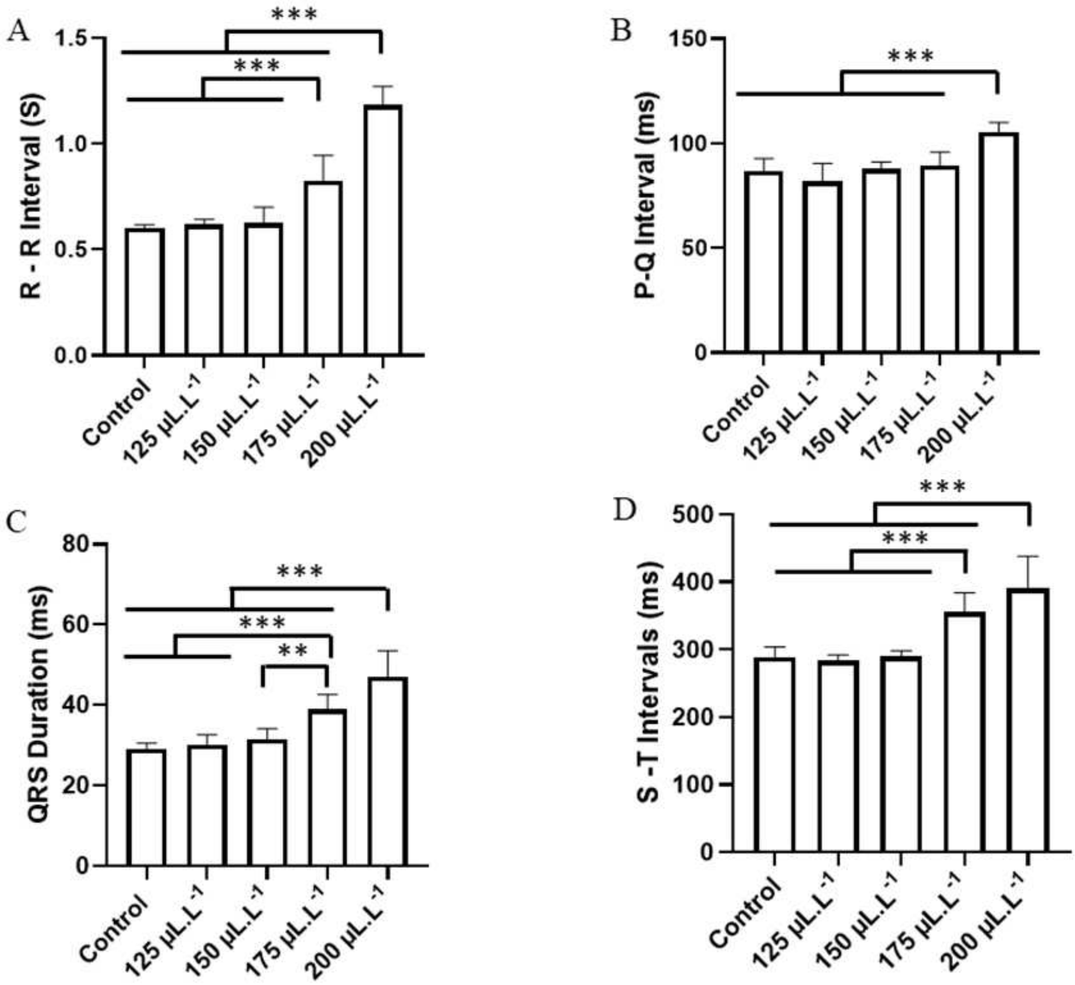
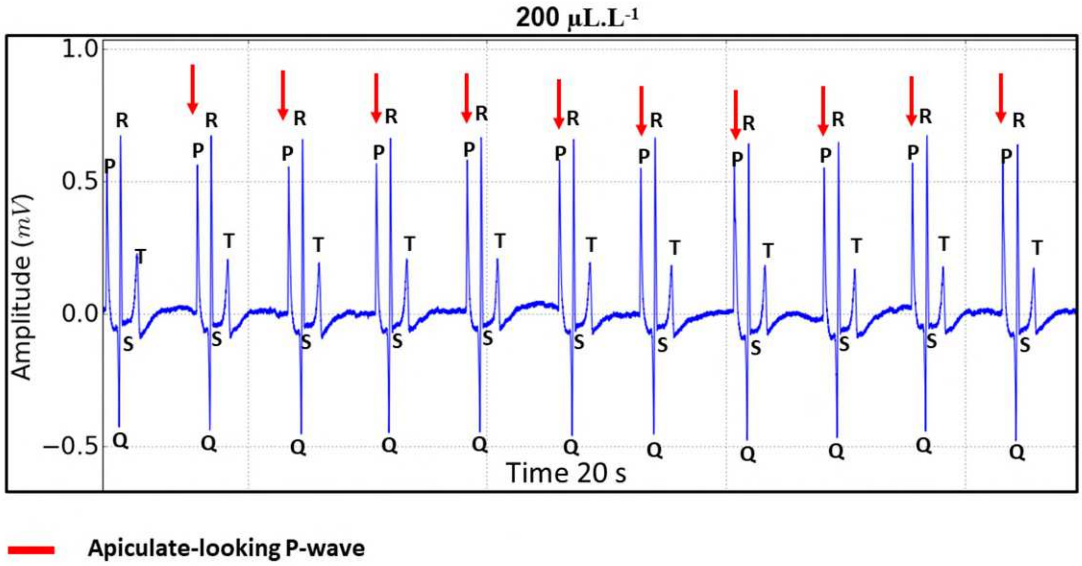

A
B
C
D
R - R Interval (S)
P-Q Interval (ms)
QRS Duration (ms)
S -T Intervals (ms)
***
**
Control
125 µL.L-1
150 µL.L-1
175 µL.L-1
200 µL.L-1

Figure -8



200 µL.L-1
1.0
0.5
0.0
−0.5
Amplitude (mV)
Time 20 s
P
R
Q
S
T
Apiculate-looking P-wave

Figure -9

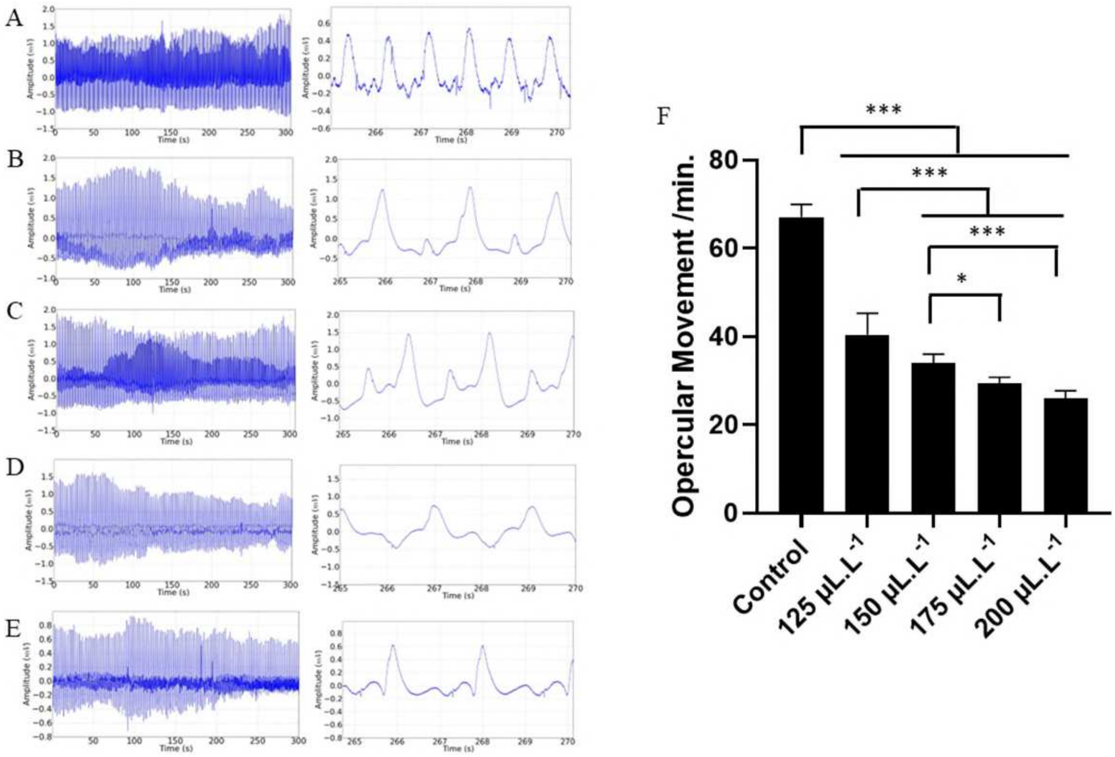
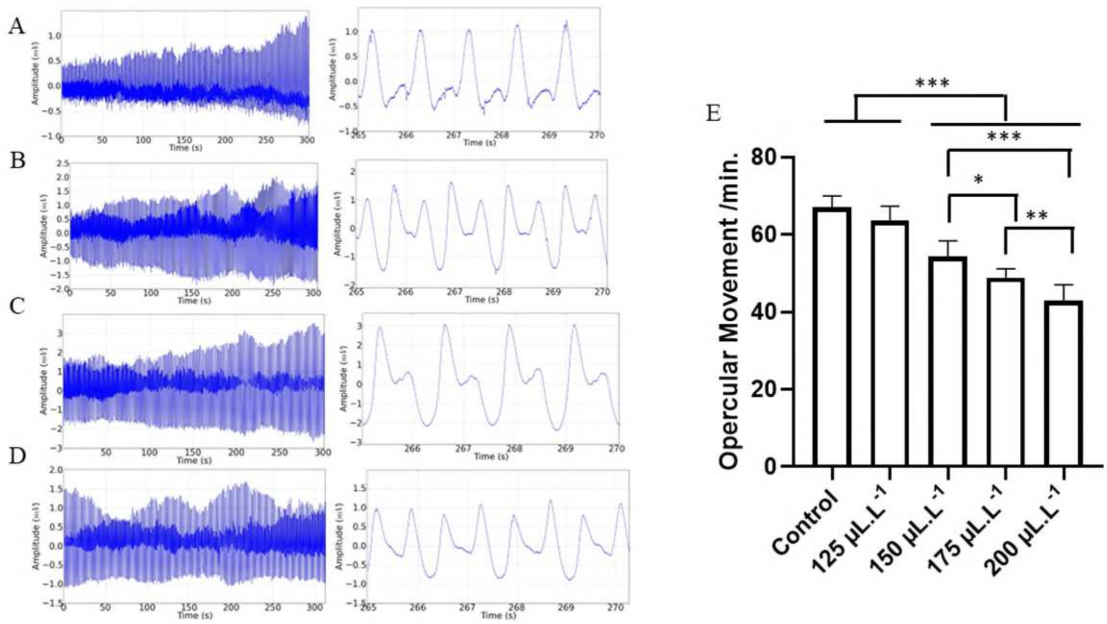

A
B
C
D
E
F
Amplitude (mV)
Time (s)
***
***
***
*
Opercular Movement /min.
80
60
40
20
0
Control
125 μL.L-1
150 μL.L-1
175 μL.L-1
200 μL.L-1

Figure -10


A
B
C
D
E
Amplitude (mV)
Time (s)
Opercular Movement /min.
Control
125 µL.L-1
150 µL.L-1
175 µL.L-1
200 µL.L-1
***
*
**