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Physiological responses of *Rhamdia quelen* (Siluriformes: Heptapteridae) to anesthesia with essential oils from two different chemotypes of *Lippia alba*

Carine de Freitas Souza[1], Matheus Dellaméa Baldissera[2], Joseânia Salbego[1], Jane Mello Lopes[3], Rodrigo de Almeida Vaucher[4], Rosa Helena Veras Mourão[5], Braulio O. Caron[6], Berta Maria Heinzmann[7], Lenise Vargas Flôres da Silva[8] and Bernardo Baldisserotto[1]

The aim of this study was to evaluate if *Lippia alba* has different chemotypes according to the chemical composition of the essential oil (EO) considering collection site, and if the EO may have different effects on blood and plasma parameters in silver catfish, *Rhamdia quelen,* during and immediately after anesthesia. The citral (EO-C) and linalool (EO-L) chemotypes were identified, and both presented similar anesthetic effects for silver catfish. Fish were exposed to two concentrations of each EO, which induced slow and fast anesthesia (100 and 300 μL L^{-1}, respectively). Blood ions did not change at any time of anesthesia induction and recovery and, therefore, the electrolyte balance was not altered. Blood gases oscillated through all exposure and recovery times, but there was an increase in pO_2 after 10 min recovery in fish anesthetized with EO-C. Glucose increased in fish exposed to both EOs when compared with the control group. Overall, exposure to both EOs (except 100 μL L^{-1} EO-L at most times) reduced plasma cortisol levels compared to the control and/or ethanol groups. However, as plasma creatinine levels in fish anesthetized with EO-C were higher than control fish, the use of EO-L is preferable.

Keywords: Blood gas, Cortisol, Glucose, Plasma ions, Silver Catfish.

O objetivo deste estudo foi avaliar se *Lippia alba* apresenta diferentes quimiotipos de acordo com a composição química do óleo essencial (OE), considerando local de coleta e se o OE causa diferentes efeitos nos parâmetros sanguíneos e plasmáticos em jundiá, *Rhamdia quelen,* durante e imediatamente após a anestesia. Os quimiotipos citral (OE-C) e linalol (OE-L) foram identificados e ambos apresentaram efeito anestésico semelhante para jundiá. Os peixes foram expostos a duas concentrações de cada OE, que induziram anestesia lenta e rápida (100 e 300 mL L^{-1}, respectivamente). Íons sanguíneos não se alteraram em nenhum tempo e consequentemente, o equilíbrio eletrolítico não foi alterado. Os gases sanguíneos oscilaram durante todo tempo de exposição e recuperação, mas houve aumento na pO_2 após 10 min de recuperação em peixes anestesiados com OE-C. Níveis sanguíneos de glicose aumentaram nos peixes expostos a ambos OEs quando comparados com o grupo controle. De um modo geral, a exposição a ambos OEs (exceto 100 μL L^{-1} OE-L na maioria dos tempos) reduziu o cortisol plasmático comparado aos grupos controle e etanol. No entanto, como os níveis de creatinina plasmática em peixes anestesiados com OE-C foram maiores que nos peixes controle, é preferível o uso do OE-L.

Palavras-chave: Cortisol, Gases sanguíneos, Glicose, Íons plasmáticos, Jundiá.

[1]Departamento de Fisiologia e Farmacologia, Universidade Federal de Santa Maria - UFSM, Av. Roraima, 1000, Camobi, 97105-900 Santa Maria, RS, Brazil. (CFS) carinedefs@yahoo.com.br, (JS) josalbego2004@yahoo.com.br, (BB) bbaldisserotto@hotmail.com (corresponding author)
[2]Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria - UFSM, Av. Roraima, 1000, Camobi, 97105-900 Santa Maria, RS, Brazil. matheusd.biomed@yahoo.com.br
[3]Universidade Federal do Maranhão, Campus de Chapadinha, 65500-000 Chapadinha, MA, Brazil. janemellolopes@hotmail.com
[4]Laboratório de Cultura Celular, Centro Universitário Franciscano, Rua dos Andradas, 1614, 97010-032 Santa Maria, RS, Brazil. rodvaucher@hotmail.com
[5]Programa de Pós-Graduação em Recursos Naturais da Amazônia, Laboratório de Bioprospecção e Biologia Experimental, Universidade Federal do Oeste do Pará-UFOPA, Rua Vera Paz, s/n, 68035-110 Santarém, PA, Brazil. mouraorhv@yahoo.com.br
[6]Departamento de Agronomia e Ciências Ambientais, Universidade Federal de Santa Maria, Centro de Educação Superior Norte do Rio Grande do Sul, Linha 7 de Setembro, s/n, 98400-000 Frederico Westphalen, RS, Brazil. otomarcaron@yahoo.com.br
[7]Departamento de Farmácia Industrial, Universidade Federal de Santa Maria - UFSM, Av. Roraima, 1000, Camobi, 97105-900 Santa Maria, RS, Brazil. berta.heinzmann@gmail.com
[8]Instituto de Ciências e Tecnologia das Águas, Universidade Federal do Oeste do Pará (UFOPA), Rua Vera Paz, s/n, 68035-110 Santarém, Pará, Brazil. lvfrsi@yahoo.com.br

Introduction

In aquaculture, anesthetics (synthetics or plant extratives) are widely employed: from light sedation, to reduce stress during handling and non-invasive procedures, to full anesthesia to avoid pain during surgery and larger interventions (Small, 2003; Roubach *et al*., 2005; Ross, Ross, 2008; Kiessling *et al*., 2009; Neiffer, Stamper, 2009; Silva *et al*., 2013a; Roohi, Imanpoor, 2015).

The anesthetic efficacy of several essential oils (EOs), such as *Hyptis mutabilis* (Silva *et al*., 2013a), *Ocotea acutifolia* (Silva *et al*., 2013b)*, Hesperozygis ringens* (Silva *et al*., 2013b; Toni *et al*., 2014), *Aloysia triphylla* (Parodi *et al*., 2014) and *Ocimum gratissimum* (Silva *et al*., 2015), has been demonstrated in fish. The EO of *Lippia alba* has been highlighted in the last decade through studies reporting its potential as antioxidant, anesthetic and sedative for fish (Cunha *et al*., 2010; Becker *et al*., 2012; Heldwein *et al*., 2014; Toni *et al*., 2014; Hohlenwerger *et al*., 2016).

Lippia alba occurs in all regions of Brazil (Zétola *et al*., 2002; Oliveira *et al*., 2006; Neto *et al*., 2009; Cunha *et al*., 2010; Teles *et al*., 2012; Vale *et al*., 2012; Soares *et al*., 2016). Due to its genetic variation, wide geographical distribution and exposure to different soil and weather conditions, and distinct seasons of collection, *L. alba* can produce EOs with different chemical composition, which expresses the occurrence of distinct chemotypes (Pascual *et al*., 2001; Hennebelle *et al*., 2008; Maffei *et al*., 2011; Teles *et al*., 2012). There are numerous chemotypes of *L. alba* in Brazil, such as: citral, linalool, ß-caryophyllene, tagetenone, limonene, carvone, myrcene, γ-terpinene, camphor-1,8-cineole and estragole, which produce different pharmacological effects (Oliveira *et al*., 2006; Hennebelle *et al*., 2008; Vale *et al*. 2012; Viccini *et al*., 2014). Thus, the distinct composition of same EO may result in different physiological and pharmacological effects during anesthesia.

Hematological and biochemical parameters of fish are valuable markers, since they can be used as indicators of physiological conditions, as well as in the control of diseases and stress manipulation (Aldrin *et al*., 1982; Tavares-Dias *et al*., 2008; Araújo *et al*., 2009). Plasma cortisol is one of the most used indicators to evaluate stress in fish (Wendelaar Bonga, 1997) and the two major actions of this hormone are the control of the ionoregulatory balance and energy metabolism (Liew *et al*., 2015). The electrolytic imbalance can be observed by changes in plasma or blood ions (McDonald, Milligan, 1997; Wendelaar Bonga, 1997; Takahashi *et al*., 2006). Glucose levels are also widely used as indicator of stress, hyperglycaemia being reported for several teleosts in this situation (Barton, Iwama, 1991). Stress also has an effect on other blood biochemical parameters such as levels of enzymes and substances with important metabolic functions, such as urea and creatinine, which indicate the overall health of the fish (Cnaani *et al*., 2004).

Physiological effects of the EO of *L. alba* cultivated in southern Brazil as anesthetic and sedative for silver catfish, *Rhamdia quelen*, was verified by many authors (Cunha *et al*., 2010; Heldwein *et al*., 2014; Toni *et al*., 2014; Salbego *et al.*, 2014), but only the linalool chemotype. Therefore, it is of interest to investigate the anesthetic and physiological effects of EO obtained from other chemotypes of *L. alba*. Considering that a different chemotype of *L. alba* (myrcene-citral) cultivated in northern Brazil was identified by Oliveira *et al.* (2006), the aim of this study was to investigate a possible geographic effect in the EO composition (*L. alba* cultivated in northern and southern Brazil) and, if these EOs have different compositions, to evaluate their sedative and anesthetic effects in silver catfish, as well as their physiological effects on blood and plasma parameters.

Materials and Methods

Animals. One hundred sixty-eight juveniles silver catfish (*Rhamdia quelen*; 51.17 ± 1.69 g and 20.21 ± 1.40 cm) were obtained from a local fish farm and brought to the Fish Physiology Laboratory at the Universidade Federal de Santa Maria (UFSM). The species was identified at the Ichthyology Laboratory (Universidade Federal do Rio Grande do Sul) and a voucher specimen was deposited in this laboratory at number UFRGS 19612. The fish were maintained for one week in 250 L tanks (50 fish/tank) with continuous aeration; temperature 21 ± 2 °C; pH 6.5-7.5 and dissolved oxygen above 5.5 mg L^{-1}. The animals were fed once a day with commercial feed and kept fasted for a period of 24 h prior to the experiments. The experimental protocol was approved by the Committee on Animal Experimentation - UFSM, under the registration number 074/2014.

Essential oils extraction and analysis. The specimens of *Lippia alba* linalool chemotype were cultivated at the Centro de Educação Superior do Norte (CESNORS-UFSM) - Frederico Westphalen, Rio Grande do Sul State, southern Brazil (27°23’48”S, 53°25’45”W), soil classified as Oxisol typical clayey. The climate is Cfa (humid subtropical) with average annual temperature of 19.1°C and rainfall of 1892 mm. Those from the citral chemotype were cultivated in Santarém, Pará state, northern Brazil (02º26’35”S, 054º54’54”W), soil classified as ultisol yellow Hapludox + yellow latosol Hapludox, but in the culture it was used black soil and cattle manure (3:1). The climate is Am (humid tropical) with average annual temperature of 25.9°C and rainfall of 2,150 mm.

Botanical identification of *L. alba* linalool chemotype was made by Gilberto Dolejal Zanetti (Department of Industrial Pharmacy, UFSM) and a voucher specimen (SMDB 10050) was deposited in the herbarium of the Department of Biology (UFSM). The *L. alba* citral chemotype was identified by Dr. Fatima Salimena (Universidade Federal de Juiz de Fora) and a voucher was registered in the herbarium of this institution under number CESJ 65276.

The essential oils were obtained by hydrodistillation of fresh leaves for 3h in a Clevenger apparatus (European Pharmacopoeia, 2007) and stored at -4°C until utilization. The analysis of the EOs was performed by gas chromatography-mass spectrometry-total ion chromatogram using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 mass selective detector and employing a HP5-MS column (5% phenyl, 95% methylsiloxane, 30 m x 0.25 mm i.d. x 0.25 mm) as described by Silva *et al*. (2012). The constituents were identified by comparison of the Kovats retention index and their mass spectra with data from the mass spectral library (NIST, 2002) and the literature (Adams, 2001).

Experiment 1. Anesthetic induction and recovery times. Anesthesia induction and recovery were tested at concentrations of 25, 50, 100, 200, 300 μL L^{-1} for both EOs. Eight fish were used for each concentration tested, and each juvenile was used only once. Sedation was characterized by the decreased reactivity to external stimuli, and anesthesia by total loss of equilibrium and cessation of locomotion, according to Small (2003). The EOs were previously diluted in 95% ethanol (1:10). Ethanol at the highest concentration used does not have any anesthetic effect in silver catfish (Cunha *et al.*, 2010). After induction, fish were transferred to anesthetic-free aquaria to measure anesthesia recovery time. The fish were considered to be recovered when they returned to normal swimming and reacted to external stimuli.

Experiment 2. Exposure to anesthetics for physiological evaluation. Silver catfish were individually placed in an 8 L aquarium containing one of the EOs at 100 μL L^{-1} for up to 5 min or 300 μL L^{-1} for up to 2 min. These concentrations led to sedation and deep anesthesia, respectively (Cunha *et al*., 2010). Afterwards, fish from all groups were transferred individually to 8 L aquaria with anesthetic-free water for up to 10 min for recovery. There were also groups subjected to 26700 μL L^{-1} ethanol (the concentration used to dilute the highest EO concentration) and water only (control), which were handled as outlined above.

Blood analysis. Blood was collected from the caudal vein of silver catfish in less than 30 s with heparinized syringes at 1, 2 and 5 min of exposure (groups exposed to the concentration of 300 μL L^{-1} were not assessed at 5 min) and 5 and 10 min of recovery (total of 30 fish per treatment, n = 6 for each EO, concentration and collection time, each fish was sampled only once). Control and ethanol exposed fish were held tightly for blood collection. An aliquot of this blood was used to measure Na^+, K^+, Ca_2^+, glucose, pH, partial pressures of O_2 (pO_2) and CO_2 (pCO_2) using the i-STAT® portable clinical analyzer with CG8+ cartridges (Abbott Laboratories, Chicago, IL, USA). The sample temperature was corrected to match the experimental water temperature (Roth, Rotabakk, 2012). The use of i-STAT® and calculations for blood gases have been described for several fish species (Jacobs *et al*., 1993; Pidetcha *et al*., 2000; Harrenstien *et al*., 2005; Kristensen *et al*., 2010; Barbas *et al.*, 2016).

Plasma analysis. The remaining blood collected was centrifuged (800 *x g* for 10 min) and the plasma was used for analysis of creatinine and urea using an automated Vitros 250 (Ortho - Clinical Diagnostics) and Johnson & Johnson dry chemistry kits. All tests were carried out in duplicate.

Plasma cortisol was also determined in duplicate using an enzyme-linked immunosorbent assay (ELISA) kit (Diagnostics Biochem Canada Inc., Canada). This analysis was previously validated (Souza *et al*., 2015). Absorbance was measured in a spectrophotometer at 450 nm, and intra- and inter-assay coefficients of variation were 6.3% and 5.2%, respectively.

Statistical analysis. Data are reported as mean ± SE. The homogeneity of variances among groups was determined with the Levene test. All treatment groups were compared by two-way analysis of variance (time x concentration) and Tukey's test; or, when homogeneity of variances was not obtained, by the Scheirer-Ray-Hare extension of the Kruskal-Wallis test and the Nemenyi test. Analyses were performed using the STATISTICA software package, version 5.1 (StatSoft, Tulsa, OK, USA), and the minimum significance level was set at $p < 0.05$.

Results

Chemical composition of the essential oils. A total of 65 compounds were identified in the EO of *L. alba* collected in southern Brazil (EO-L) and 67 compounds in the EO of *L. alba* collected in northern Brazil (EO-C) (Tab. 1). The major component in the EO-L was β-linalool (50.56%), while the major compounds in the EO-C were E-citral (29.84%) and Z-citral (24.41%).

Anesthetic induction and recovery times. Both EO-C concentrations induced sedation faster than EO-L, but anesthesia was faster only at the lowest EO-C concentration. Fish anesthetized with EO-C took longer to recover than those anesthetized with EO-L (Fig. 1). Ethanol did not show any sedative or anesthetic effect.

Blood analysis. Blood pH, Na^+, K^+ and Ca^{2+} did not differ between any of the treatments and overall means were: pH (7.32 ± 0.04), Na^+ (134.90 ± 0.89 mmol L^{-1}), K^+ (2.89 ± 0.12 mmol L^{-1}), Ca^{2+}(1.18 ± 0.06 mmol L^{-1}).

Overall, blood pO_2 levels of silver catfish anesthetized with both EOs and exposed to ethanol were lower than control fish, increasing after 10 min recovery (Figs. 2a-b). In contrast, an increase in pCO_2 was observed for fish anesthetized with EO-C and no significant change was observed for silver catfish anesthetized with EO-L. At the end of 10 min of recovery fish anesthetized with EO-C still maintained blood PCO_2 levels higher than control fish (Figs. 2c-d).

Tab. 1. Chemical composition of the essential oils of *Lippia alba* collected from southern (linalool chemotype - EO-L) and northern (citral chemotype - EO-C) Brazil. RI calc= calculated Kovats retention index; RI ref= reference Kovats retention index; (Adams, 2001; NIST, 2002).

RT (min)	Constituent	Relative percentage (%)		RI calc	RI ref
		EO-L	EO-C		
11.334	sabinene	1.05	0.47	972	968[1,2]
13.597	limonene	0.63	6.15	1026	1029[2]
13.698	1,8-cineole	7.01	-	1029	1031[1,2]
14.535	E-β-ocimene	1.10	0.35	1049	1050[1]
14.882	γ-terpinene	-	3.16	1058	1060[1]
16.623	β-linalool	50.56	0.73	1100	1099[2]
21.599	Z-geraniol	0.49	3.57	1229	1230[1]
22.037	Z-citral	-	24.41	1241	1238[1,2]
22.587	E-geraniol	0.17	5.32	1256	1253[1]
23.131	E-citral	1.51	29.84	1270	1267[1,2]
27.375	β-elemene	2.66	0.30	1391	1391[1,2]
28.291	E-caryophyllene	4.56	0.99	1418	1419[1]
28.758	γ-elemene	1.27	0.08	1433	1437[1]
30.174	γ-muurolene	5.23	2.46	1476	1480[1]
30.815	bicyclogermacrene	0.22	3.72	1495	1500[1]
32.47	elemol	0.13	3.15	1549	1550[1]
32.717	germacrene B	2.37	0.23	1557	1561[1]
33.532	caryophyllene oxide	1.12	0.80	1584	1583[2]
	% Identified	80.08	85.73		

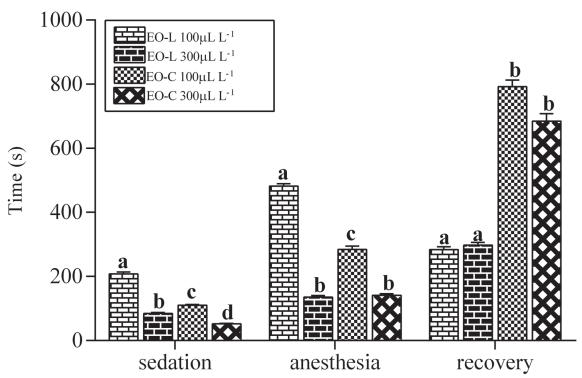

Fig. 1. Time required for silver catfish (*Rhamdia quelen*) anesthesia induction and recovery (n=8 for each concentration tested) using different concentrations of essential oils from the linalool (EO-L) and citral (EO-C) chemotypes of *Lippia alba*. Stages are defined according to Small (2003). Values are mean ± SEM. Different letters indicate difference between concentrations and EOs in the same anesthetic stage. Based on two-way ANOVA followed by the Tukey *post hoc* test ($p < 0.05$).

Blood glucose levels increased in control and ethanol groups after 5 min, compared to initial values, returning to initial values at the end of the recovery period in the control group. Exposure to both EOs (except 300 µL L^{-1} EO-L) did not avoid this increase of blood glucose levels. At the end of the recovery period, the blood glucose levels of silver catfish exposed to ethanol and both EOs (except 300 µL L^{-1} EO-L) remained higher than the initial values and higher than those of the control group (Figs. 3a-b).

In the control group, plasma cortisol levels decreased after 2 min and remained low until the end of the recovery period. In fish exposed to ethanol, cortisol levels decreased up to 5 min after exposure and returned to the initial values after 5 min of recovery. Overall, exposure to both EOs (except 100 µL L^{-1} EO-L at most times) reduced plasma cortisol levels compared to the control and/or ethanol groups (Figs. 3c-d).

Plasma creatinine values of control fish increased significantly after 5 min and remained high at the end of the recovery period. Fish exposed to ethanol and both EOs showed significantly higher creatinine levels 1 min after exposure compared to control fish and these levels returned to control values at the end of recovery only in those exposed to EO-L (Figs. 4a-b). Plasma urea levels in the control group remained constant at all evaluation times. Fish exposed to ethanol showed a significant increase in plasma urea levels when compared to control fish after 5 min of exposure and returned to control values at the end of the recovery period. Plasma urea was significantly higher with most anesthesia treatments and recovery times in fish exposed to both EOs when compared to the control and ethanol groups (Figs. 4c-d).

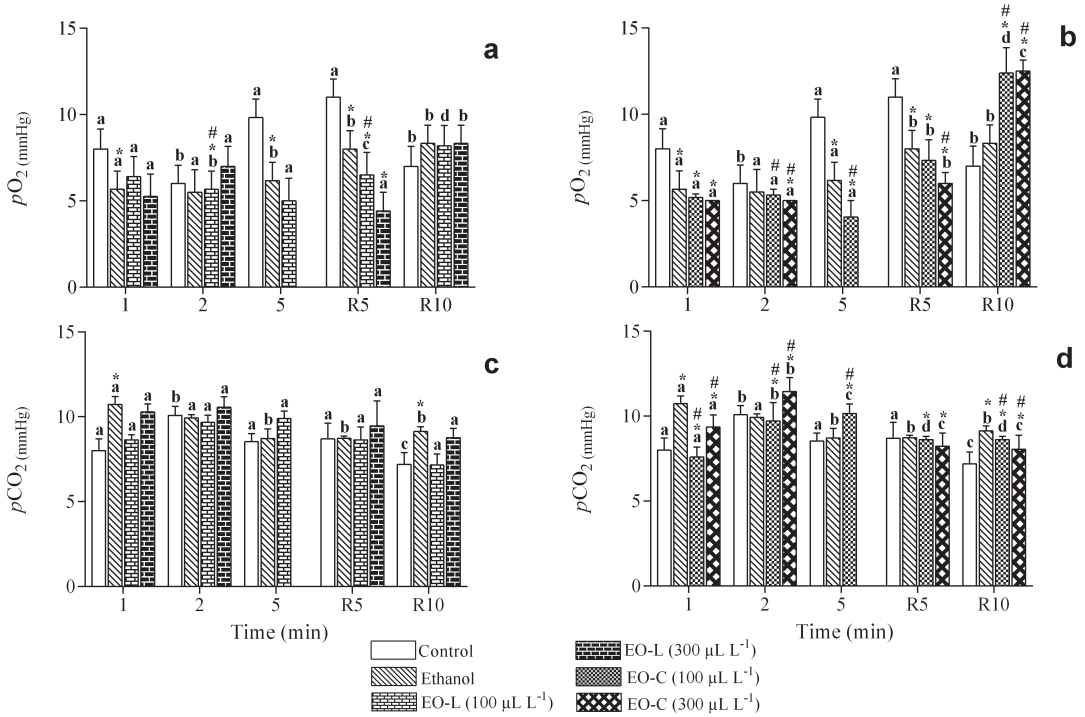

Fig. 2. Partial pressures of **a-b** oxygen (PO_2) and **c-d** carbon dioxide (PCO_2) in silver catfish (n = 6) (*Rhamdia quelen*) submitted to different concentrations of essential oils from the linalool (EO-L) and citral (EO-C) chemotypes of *Lippia alba*. Values are mean ± SEM. Different letters indicate significant difference between times within the same EO concentration. * indicate significant difference from control, # indicate significant difference from ethanol. Two-way ANOVA and Tukey's test or Scheirer-Ray-Hare extension of the Kruskal-Wallis test and Nemenyi test.

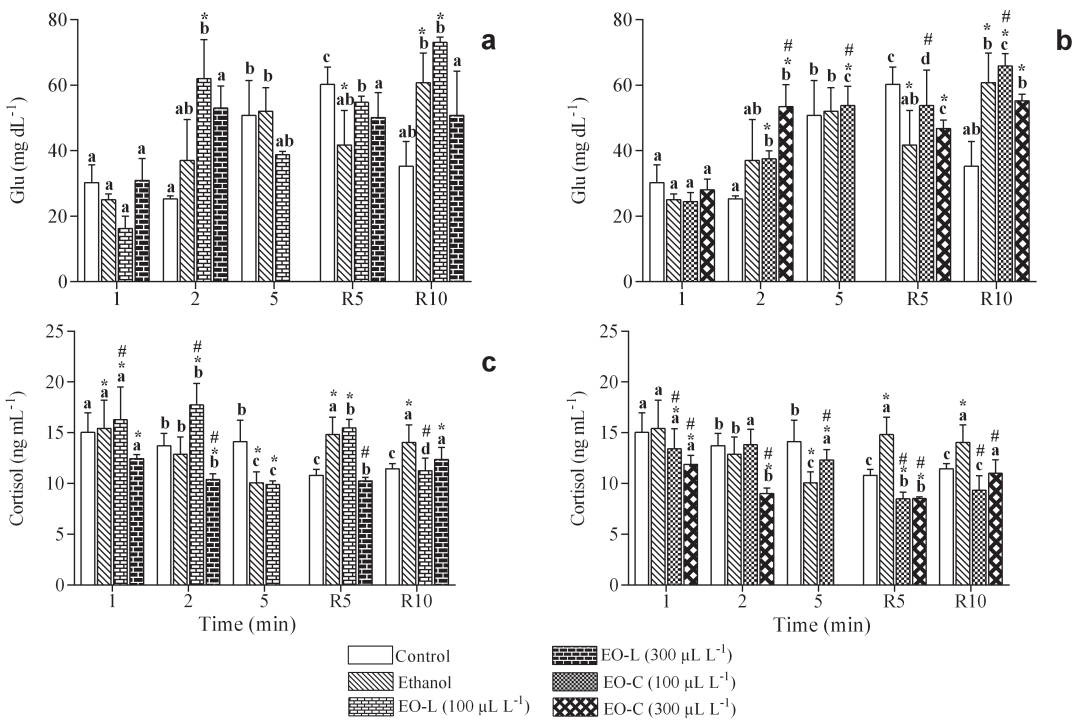

Fig. 3. a-b. Blood glucose (Glu) and **c-d.** plasma cortisol in silver catfish (*Rhamdia quelen*) (n = 6) subjected to different concentrations of essential oils from the linalool (EO-L) and citral (EO-C) chemotypes of *Lippia alba*. Values are mean ± SEM. Different letters indicate significant differences between times within the same treatment. * indicates significant difference from control, # indicates significant difference from ethanol. Two-way ANOVA and Tukey's test or Scheirer-Ray-Hare extension of the Kruskal-Wallis test and Nemenyi test were used to determine statistical significance.

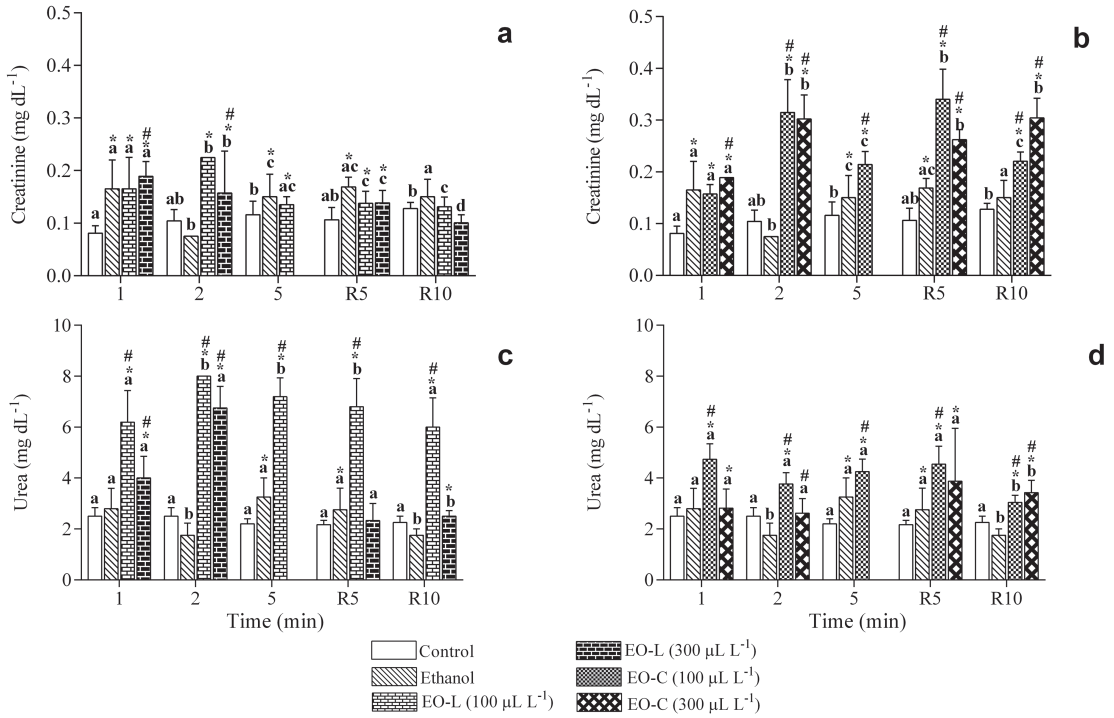

Fig. 4. a-b. Plasma urea and **c-d** creatinine in silver catfish (*Rhamdia quelen*) (n = 6) subjected to different concentrations of essential oils from the linalool (EO-L) and citral (EO-C) chemotypes of *Lippia alba*. Values are mean ± SEM. Different letters indicate significant differences between times within the same treatment. * indicates significant difference from control, # indicates significant difference from ethanol. Two-way ANOVA and Tukey's test or Scheirer-Ray-Hare extension of the Kruskal-Wallis test and Nemenyi test were used to determine statistical significance.

Discussion

Since EOs represent a chemical interface between plant and the surrounding environment, their syntheses are often affected by environmental conditions, thus expressing the occurrence of chemotypes or chemical races in the producing plant species (Gobbo-Neto, Lopes, 2007). Although there are many examples of the occurrence of geographic variations of EOs chemical composition in several plants (Figueiredo *et al*., 2008), the distribution of chemotypes is often not locally limited. In some species, different chemotypes can grow side by side (Schmidt *et al*., 2004).

The present study demonstrates that the EO from *L. alba* cultivated by our group in southern Brazil has linalool as its main compound (50.56%), and so it belongs to chemotype linalool. On the other hand, *L. alba* collected in northern Brazil can be classified in the chemotype citral, once this is the major compound of its EO (54.26%). Some authors indicated that geographical distribution and exposure to different soil and weather conditions, season of collection can affect the chemical composition of *L. alba* EO (Pascual *et al*., 2001; Hennebelle *et al.*, 2008; Maffei *et al*., 2011; Teles *et al.*, 2012). However, specimens from the chemotypes citral, linalool and carvone, collected in different regions of Brazil, cultivated in similar conditions, maintained the same chemical composition, indicating that differences are due to genotypic variations (Tavares *et al.*, 2005).

The EO-C anesthetized silver catfish within approximately 2 min at 300 µL L^{-1}, inducing anesthesia faster than EO-L. Anesthesia recovery was slower with EO-C, but it can be considered and adequate anesthetic for silver catfish, because an ideal anesthetic must induce anesthesia up to 3 min and enable the recovery in about 10 min (Park *et al*., 2008). The anesthetic effect of EO-L in silver catfish involves the modulation of the benzodiazepine (BDZ) site of the GABAergic system (Heldwein *et al.*, 2012). The EO-C blocks the excitability of rat sciatic nerves (Sousa *et al.*, 2015), but the anesthetic effect of the EO from *Aloysia triphylla* (which has citral as its major compound) in silver catfish is not related to a modulation of the BDZ site of the GABAA receptor (Santos *et al*., in press).

All parameters examined in this study are within the range previously observed for silver catfish (Barcellos *et al*., 2001; 2004). Blood Na^+, K^+ and Ca^{2+} of silver catfish were not affected by anesthesia with either EO tested. Similar results were found in the blood of *Amazon* catfish (*Leiarius marmoratus*) anesthetized with 10-200 µL L^{-1} eugenol (Honorato *et al*., 2014) and in the plasma of silver catfish anesthetized with 150 and 300 mg L^{-1} MS-222 (Gressler *et al*., 2014). However, silver catfish anesthetized with 150-450 µL L^{-1} of *Hesperozygis ringens* and *Lippia alba* (EO-L) showed altered plasma Na^+ and K^+ between 30-240 min of recovery (Toni *et al*., 2014) and anesthesia of tambaqui with 20 mg L^{-1} jambu extract induced blood ionoregulatory

changes 2 h after recovery from anesthesia and Na^+ levels did not return to control values, even after 72 h (Barbas *et al*., 2016). Apparently ionoregulatory effects of anesthesia in blood or plasma are significant only after a few hours of recovery, when they can be detected.

During fish anesthesia, opercular movement (respiration) generally decreases compared to conscious fish, explaining the lower blood pO_2 in silver catfish anesthetized with both EOs and the higher pCO_2 in those anesthetized with EO-C. Through anesthetic recovery from both EOs there was an increase in blood pO_2 levels and a reduction in pCO_2 levels in those exposed to EO-C, which is expected for the recovery period with normal return of opercular movements. These same oscillation patters in pO_2 and pCO_2 from fish anesthesia, were found for red "pacu" (*Piaractus brachypomus*) anesthetized with MS-222 (150 mg L^{-1}) (Hanley *et al.,* 2010) and "tambaqui" (*Colossoma macropomum*) anesthetized with waxy extract of "jambu" flowers (*Spilanthes acmella*) at 20 mg L^{-1} (Barbas *et al.,* 2016).

An increase in plasma levels of glucocorticoids such as cortisol is one of the main responses to stress (Barton, 2002). Plasma cortisol increases significantly in juvenile *R. quelen* 5 - 30 min after handling (Koakoski *et al.,* 2012), but surprisingly, handling was not sufficient to raise the plasma cortisol in the control group of silver catfish in our study. Toni *et al*. (2015) observed no increase in plasma cortisol levels of silver catfish exposed for 6 h to 30 and 50 µL L^{-1} EO from *Hesperozygis ringens* and they proposed that the primary stress reaction only took place in the first minutes after contact with the EO, as observed in fish exposed to EO-L in the present study.

A study by Gesto *et al.* (2014) using stressed rainbow trout (*Oncorhynchus mykiss*) showed that when catecholamines were released in the blood no changes in cortisol levels were observed as glucose levels increased. As plasma cortisol levels did not increase significantly, we suppose that the increase of blood glucose in silver catfish observed in the present study might be due to catecholamine release. According to Morgan, Iwama (1997), an increase in blood glucose occurs in response to a stressor, in order to provide most of the energy demand to cope with this stress. Our results corroborate those obtained by Inoue *et al*. (2011) and Honorato *et al*. (2014), who observed that anesthesia with eugenol increased plasma glucose compared to the sham control in "tambaqui" (20 and 60 mg L^{-1}) and Amazon catfish (10-200 µL L^{-1}). Anesthesia with 20 mg L^{-1} "jambu" extract also increased blood glucose levels in "tambaqui" (Barbas *et al*., 2016). Several studies testing a variety of anesthetics on multiple fish species also demonstrated increased glycemia after anesthesia induction (Ortuno *et al*., 2002; Deriggi *et al*., 2006; Barbosa *et al*., 2007; Park *et al*., 2008).

An increase in plasma urea levels in silver catfish during anesthesia and recovery was observed for both EOs when compared to the control group. The same result was obtained for plasma creatinine levels, but these levels were much higher in silver catfish anesthetized with EO-C than in control fish, and these levels returned to control values after 10 min recovery in those exposed to EO-L. Anesthesia of goldfish (*Carassius auratus*) with 50 µL L^{-1} nanoencapsulated clove oil also increased serum urea levels (Gholipourkanani *et al*., 2015). Increases in urea and creatinine levels together are probably due to lesions caused in the kidney of fish (Das, Mukherjee, 2003). Studies conducted by Borges *et al*. (2007) showed an increase in urea and creatinine levels in the serum of silver catfish exposed to cypermethrin, suggesting that these analyses can be useful for early detection of intoxication in fish. However, as nitrogen compounds are excreted as ammonia mainly through the gills (Nawata *et al.*, 2007), and time of exposure to the EOs was brief, the elevation of urea levels observed in both EO groups in our study may be due to changes in ammonia and creatinine gill excretion and not to renal lesions.

In summary, different chemotypes of *L. alba* were detected according to their place of cultivation. We suggest that the EO-L and EO-C can be safely used as anesthetics in silver catfish, because the alterations in most parameters returned to control values within 10 min. However, the EO obtained from different chemotypes of the *L. alba* presented different physiological responses in plasma creatinine and the use of EO-L is preferable because the high creatinine levels provoked by EO-C exposure. Additional studies with longer exposure and/or recovery times are necessary to improve our understanding of the effects of the EO of this chemotype on renal function.

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