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Anesthesia of *Epinephelus marginatus* with essential oil of *Aloysia polystachya*: an approach on blood parameters

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ABSTRACT

This study investigated the anesthetic potential of the essential oil (EO) of *Aloysia polystachya* in juveniles of dusky grouper (*Epinephelus marginatus*). Fish were exposed to different concentrations of EO of *A. polystachya* to evaluate time of induction and recovery from anesthesia. In the second experiment, fish were divided into four groups: control, ethanol and 50 or 300 μL L^{-1} EO of *A. polystachya*, and each group was submitted to induction for 3.5 min and recovery for 5 or 10 min. The blood gases and glucose levels showed alterations as a function of the recovery times, but Na^+ and K^+ levels did not show any alteration. In conclusion, the EO from leaves of *A. polystachya* is an effective anesthetic for dusky grouper, because anesthesia was reached within the recommended time at EO concentrations of 300 and 400 μL L^{-1}. However, most evaluated blood parameters showed compensatory responses due to EO exposure.

Key words: anesthetic efficacy, blood gases, glucose, hemoglobin, plasma ion levels.

INTRODUCTION

Several procedures of fish culture such as handling, blood sampling, transporting and vaccination often generate a stress response in the animals (Kiessling et al. 2009, Zahl et al. 2012). Firstly, activation of the hypothalamic–pituitary–interrenal axis occurs, with subsequent release of catecholamines and cortisol. As a consequence, glucose and lactate increase and osmoregulatory disturbances occur (Zahl et al. 2012). Anesthetics obtained from

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plants, such as the essential oil (EO) of *Lippia alba* (Cunha et al. 2010, Azambuja et al. 2011, Becker et al. 2012, Heldwein et al. 2012, Salbego et al. 2014), *Ocimum gratissimum* (Silva et al. 2012), *Hesperozygis ringens* (Silva et al. 2013, Toni et al. 2014) and *Aloysia triphylla* (Gressler et al. 2014, Parodi et al. 2014, Zeppenfeld et al. 2014) showed efficacy and safety for use in aquaculture procedures.

The EO used in the present study was obtained from leaves of *A. polystachya* (Griseb.) Moldenke (Verbenaceae), an aromatic native plant widely distributed in subtropical regions of South America, mainly in Paraguay and North Argentina, and popularly known as "burrito", "poleo de Castilla" or "poleo riojano". This plant is referred to as a sedative (Del Vitto and Petenatti 1997) and is also used against gastrointestinal pain in folk medicine (Filipoy 1994). Studies with mice and rats indicated that the hydro-ethanolic extract from the aerial parts of *A. polystachya* has anxiolytic and antidepressant-like effects (Mora et al. 2005, Hellión-Ibarrola et al. 2006, 2008).

The dusky grouper, *Epinephelus marginatus* (Serranidae) has a wide distribution, occurring along the Mediterranean Sea and in the Indian Ocean to the southeast of the African continent (Fennessy 2006). On the west coast of the Atlantic Ocean, the dusky grouper occurs from Rio de Janeiro to the New Gulf region in Argentinean Patagonia (Figueiredo and Menezes 1980, Irigoyen et al. 2005). Since *E. marginatus* is a target species for aquaculture (Cunha et al. 2013, Cavalli 2014, Sanches et al. 2014), the present study investigated the anesthetic potential of the EO of *A. polystachya* in dusky grouper juveniles. Some blood parameters were also analyzed, aiming to evaluate possible side effects of this EO.

MATERIALS AND METHODS

PLANT MATERIAL AND ESSENTIAL OIL EXTRACTION

Aloysia polystachya (Griseb.) Moldenke (Verbenaceae) was cultivated in the medicinal plant garden of "Nature's Pharmacy", Municipality of Jardinópolis, SP, Brazil. The leaves were harvested in September 2012 at 10 am and dried in an oven with forced air circulation at a temperature of 45 °C for 48 h. The voucher specimen (UPMU No. 1213) was identified by Dr. Rossi from the Institute of Botany of São Paulo, and a voucher was deposited in the Herbarium of Medicinal Plants at the University of Ribeirão Preto, SP, Brazil. The EO was extracted from dried leaves by hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia (2007).

ESSENTIAL OIL ANALYSIS

The EO samples were analyzed by GC–MS with an Agilent 6890A gas chromatograph equipped with a 5973C mass selective detector using a non-polar HP5-MS fused silica capillary column (5% phenyl, 95% methylsiloxane, 30 m x 0.25 mm i.d. x 0.25 μm film thickness) and electron ionization mode at 70 eV. Helium was used as carrier gas at a flow rate of 1.0 mL min^{-1}; the injector and detector temperatures were set at 250 and 280 °C, respectively. Oven temperature was kept at 40 °C for 4 min and then gradually raised to 320 °C at 4 °C min^{-1}. Injections were performed in split inlet mode (ratio 1:100). Kovats retention indices were calculated using a homologous series of C7–C31 n-alkanes injected under the same conditions. The EO constituents were identified by comparison of the mass spectra and Kovats retention indices with literature data and with the National Institute of Standards and Technology (NIST) Mass Spectral

Library (NIST 2008, Adams 2009). FID analysis was performed in an equivalent column and using the same oven parameters as described for GC–MS. Both injection and detection temperatures were set at 300 °C and the split inlet mode ratio was 1:50. The percentage of EO compounds was calculated by under peak area integration.

ANIMALS AND WATER CONDITIONS

Dusky grouper (82.0 ± 2.3 g; 16.7 ± 0.1 cm) juveniles obtained from a fish culture in Rio Grande, southern, Brazil, were maintained for one week in 250 L continuously aerated tanks to acclimate to laboratory conditions. The animals were fed once a day with commercial feed and kept fasted for a period of 24 h prior to the experiments that were conducted in accordance with the Ethical Committee and the Animal Welfare Committee of UFSM (process number 074/2014). The water parameters were measured as follows: dissolved oxygen (6.20 ± 0.11 mg L^{-1}) and temperature (26.29 ± 0.12 °C) with a YSI oxygen meter (model DO 200A), pH (7.2 ± 0.1) with a pH meter (Hanna Instruments, Woonsocket, RI, USA; model HI 8424), total ammonia nitrogen (0.25 ± 0.06 mg N L^{-1}) measured by the salicylate method (UNESCO 1983), nitrite (0.08 ± 0.03 mg L^{-1}) determined as described by Bendschneider and Robinson (1952) and alkalinity (149.75 ± 0.67 mg $CaCO_3$ L^{-1}) by the method of Baumgarten et al. (1996). In addition, salinity was maintained throughout the experiment at 29 ppt.

EXPERIMENT 1: ANESTHESIA INDUCTION AND RECOVERY

The water conditions for this experiment were similar to those reported for acclimation. Juveniles were transferred with a net to a 10 L aquarium with the EO from the leaves of *A. polystachya* at 50, 75, 100, 200, 300 or 400 µL L^{-1}, firstly diluted in ethanol (1:10). Moreover, the possible anesthetic effect of ethanol was tested with the highest concentration used to dilute the EO. The EO concentrations were chosen based on the study of Parodi et al. (2014) with the EO of *A. triphylla*. To evaluate the time required for anesthesia induction, six (n = 6) juveniles were individually tested using aquaria at the respective concentration. Each animal was used only once and the anesthesia stages were determined according to Small (2003): Stage 1: sedation – decreased reactivity to external stimuli; Stage 2: partial loss of equilibrium and erratic swimming; Stage 3: total loss of equilibrium and cessation of locomotion. The maximum observation time was 30 min. After the induction of anesthesia, juveniles were transferred to anesthetic-free aquaria to measure the recovery time. Animals were considered to have recovered when they demonstrated normal swimming and reaction to external stimuli.

EXPERIMENT 2: BLOOD ANALYSIS OF ANESTHETIZED AND RECOVERED FISH

Animals were divided into the following groups (n = 6 per treatment and time of collection): control (without anesthetic), ethanol, 50 or 300 µL L^{-1} *A. polystachya* leaf EO. The collection times for each group were: exposure (3.5 min) and recovery times (5 or 10 min). Each fish was sampled only once. Recovery was performed in anesthetic-free aquaria. After exposure or recovery times, blood was collected from the caudal vein of each fish by heparinized 1 mL syringes and immediately analyzed using an i-STAT portable clinical analyzer with CG8+ cartridge (Abbott Laboratories, Chicago, IL, USA). The parameters measured were: sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), bicarbonate (HCO_3^-), pH, gases (PvO_2, $PvCO_2$), glucose, hemoglobin (Hb) and hematocrit (Hct). The clinical analyzer temperature was corrected to the water temperature according to the manufacturer's specifications. The efficacy of i-STAT measurements has been proved for several

fish species (Cooke et al. 2008, Kristensen et al. 2010, Paust et al. 2011).

STATISTICAL ANALYSIS

All data are expressed as mean ± SEM. The homogeneity of variances between treatments was calculated with Levene's test. As the data exhibited homogeneous variances, comparisons between different groups and times were made using two-way ANOVA and Tukey's test. Analyses were performed using Statistica ver. 7.0 software (StatSoft, Tulsa, OK, USA) with the minimum significance level set at $P < 0.05$.

RESULTS

CHEMICAL COMPOSITION

A total of 19 compounds were identified in the EO obtained from the dried leaves of *A. polystachya* (Table I). The main constituents found in this EO were carvone (58.76%) and α-limonene (33.68%).

INDUCTION AND RECOVERY TIMES FROM ANESTHESIA

As expected, by increasing EO concentration there was a proportional decrease in the time required for sedation and anesthesia induction, but not for recovery. Fish exposed up to 75 μL L^{-1} *A. polystachya* EO reached sedation (Stage 1), but no evidence indicated a possible deep anesthesia (Stage 3) during the evaluation time (maximum 30 min). Concentrations above 100 μL L^{-1} EO were able to induce sedation and anesthesia. Recovery time was significantly faster at 200 and 300 μL L^{-1} EO than at 100 μL L^{-1}, and the highest concentration tested (400 μL L^{-1} EO) presented the fastest recovery time (Table II). Mortality was not observed throughout the anesthesia induction procedure. Ethanol added to the water did not produce any anesthetic effect.

BLOOD PARAMETERS

Blood pH was not significantly affected by treatments. The PvO_2 and $PvCO_2$ values increased and decreased, respectively, in fish placed in the simulated recovery, as well as in those recovering from ethanol exposure for 5 min compared to those exposed to ethanol. Groupers exposed to both EO concentrations presented higher PvO_2 values than control fish. The PvO_2 values were also higher in fish exposed to 50 μL L^{-1} EO than in those exposed to ethanol. The PvO_2 values of fish recovered from 50 μL L^{-1} EO exposure were significantly lower than in exposed fish, but in those recovered from 300 μL L^{-1} EO exposure, these values were higher than in exposed fish and in the recovered control and ethanol groups. Groupers exposed to 300 μL L^{-1} showed significantly higher $PvCO_2$ values than the control group. In addition, fish recovered for 5 min presented significantly higher $PvCO_2$ values than the control and ethanol groups, but after 10 min recovery these values were significantly lower than in the ethanol group. The HCO_3^- concentration decreased in all groups at both recovery times when compared to exposure, but was not affected by treatments (Table III).

The levels of glucose, hemoglobin and hematocrit were not significantly different between exposure times in the control group (Fig. 1). Glucose levels also did not differ between times in the ethanol group. The groups treated with 50 or 300 μL L^{-1} EO showed higher glucose levels after 10 min recovery compared to other times. Fish exposed to 50 μL L^{-1} EO had increased glucose levels after 10 min recovery compared to the control and ethanol groups, but those exposed to 300 μL L^{-1} EO had decreased levels after 5 min recovery compared to the ethanol group (Fig. 1a).

Hemoglobin and hematocrit levels in the ethanol and 50 μL L^{-1} EO groups were lower after 5 min recovery compared to the other exposure times. Groupers exposed to 50 μL L^{-1} EO showed lower

TABLE I
Chemical composition of the essential oil from dried leaves of *Aloysia polystachya*.

Peak	RT	Compound	RI experimental	RI literature	Source	%
1	10.23	α-Pinene	931	937	N	0.75
2	11.94	β-Pinene	974	975	N	0.45
3	12.65	β-Myrcene	991	990	N	1.57
4	14.05	α-Limonene	1027	1028	N	33.68
5	14.52	β-E-Ocimene	1039	1038	N	0.40
6	14.91	β-Z-Ocimene	1049	1051	N	0.21
7	16.93	β-Linalool	1100	1098	N	0.93
8	17.69	E-p-Mentha-2,8-dienol	1121	1118	N	0.19
9	18.16	Limonene epoxide	1134	1136	N	0.27
10	20.28	α-Terpineol	1191	1190	N	0.40
11	20.43	1,6-Dihydrocarveol	1195	1195	N	0.29
12	20.50	Dihydrocarvone	1197	1199	N	0.29
13	21.70	E-Carveol	1231	1233	N	0.24
14	22.16	Carvone	1244	1242	N	58.76
15	23.22	Perillal	1275	1274	N/A	0.17
16	23.86	Thymol	1293	1292	N	0.16
17	27.99	β-Caryophyllene	1420	1419	N	0.80
18	29.05	α-Caryophyllene	1454	1455	N	0.15
19	30.74	β-Bisabolene	1509	1509	N	0.29
		Total identified:				100.00

RT = retention time (in min); RI experimental = calculated Kovats retention index; RI literature = reference Kovats retention index; N = NIST Mass Spectral Library (2008) and A = Adams (2009).

hemoglobin and hematocrit levels than the ethanol group and lower hematocrit than the control group. After 10 min recovery, fish that were exposed to 300 μL L^{-1} EO presented lower hematocrit and hemoglobin levels than exposed fish. Fish exposed to 300 μL L^{-1} EO showed higher hemoglobin and hematocrit levels after 5 min recovery compared to the fish that recovered from ethanol exposure for the same amount of time (Fig. 1b, c). Na^+ and K^+ levels did not differ between groups or exposure times (Fig. 2a, b). The lowest Ca^{2+} levels in the control group were observed after 10 min recovery. Additionally, lower Ca^{2+} levels were observed in animals anesthetized with 50 μL L^{-1} EO after 10 min recovery compared to the ethanol group at the same time (Fig. 2c).

DISCUSSION

The main constituents found in the EO obtained from leaves of *A. polystachya* were the monoterpenes carvone (58.76%) and α-limonene (33.68%). Other studies also demonstrated the presence of carvone (González et al. 2010) and limonene in

TABLE II
Time (in seconds) required for induction and recovery from anesthesia using the essential oil of *Aloysia polystachya* leaves (EO) in *Epinephelus marginatus.*

EO concentration ($\mu L\ L^{-1}$)	Sedation	Anesthesia	Recovery
50	429.3 ± 12.2	–	–
75	285.8 ± 15.3	–	–
100	210.0 ± 3.5	1555.7 ± 46.7	565.0 ± 9.3[a]
200	96.3 ± 3.4	313.4 ± 16.8	421.4 ± 17.3[b]
300	50.7 ± 0.7	163.7 ± 16.5	480.8 ± 8.7[b]
400	46.5 ± 1.6	90.2 ± 3.2	330.5 ± 13.0[c]
Equations	$y = 684.4\ e^{(-0.01x)}$ $r^2 = 0.9529$	$y = 6424.5\ e^{(-0.01x)}$ $r^2 = 0.9759$	–

Values are represented as mean ± SEM (n = 6). Different letters indicate significant differences between essential oil concentrations in the recovery stage ($P < 0.05$). Stages of anesthesia induction are according to Small (2003), where sedation corresponds to Stage 1 and anesthesia corresponds to Stage 3. The equations fitted above represent a relationship between the times of anesthesia and concentrations of EO, where y = time to reach the stage and x = concentration of EO of *A. polystachya* (in $\mu L\ L^{-1}$).

TABLE III
Blood parameters of *Epinephelus marginatus* evaluated after exposure (3.5 min) and recovery times from anesthesia induction by essential oil of *Aloysia polystachya.*

	pH	PvO_2 (mm Hg)	$PvCO_2$ (mm Hg)	HCO_3^- ($mmoL\ L^{-1}$)
Control				
Exposure	7.40 ± 0.06[a]	20.03 ± 0.95[a]	14.25 ± 0.80[a]	9.89 ± 0.54[a]
Recovery 5 min	7.37 ± 0.04[a]	37.66 ± 3.40[b]	8.79 ± 0.81[b]	5.98 ± 0.32[b]
Recovery 10 min	7.39 ± 0.07[a]	35.05 ± 3.63[b]	8.79 ± 0.90[b]	5.98 ± 0.42[b]
Ethanol				
Exposure	7.32 ± 0.08[a]	25.74 ± 4.34[a]	15.12 ± 1.93[a]	8.38 ± 0.60[a]
Recovery 5 min	7.42 ± 0.06[a]	40.81 ± 3.68[b]	8.29 ± 0.72[b]	5.36 ± 0.24[c]
Recovery 10 min	7.38 ± 0.03[a]	25.05 ± 4.28[a]	11.24 ± 0.78[a]	6.55 ± 0.07[b]
EO 50 $\mu L\ L^{-1}$				
Exposure	7.49 ± 0.03[a]	60.97 ± 3.39[a]*#	11.89 ± 0.83[a]	9.18 ± 0.66[a]
Recovery 5 min	7.46 ± 0.05[a]	42.79 ± 5.90[b]	8.74 ± 1.05[a]	5.25 ± 0.43[b]
Recovery 10 min	7.40 ± 0.05[a]	36.08 ± 3.70[b]	9.20 ± 0.68[a]	5.72 ± 0.32[b]
EO 300 $\mu L\ L^{-1}$				
Exposure	7.28 ± 0.06[a]	37.48 ± 3.16[a]*	19.53 ± 0.78[a]*	8.93 ± 0.81[a]
Recovery 5 min	7.19 ± 0.05[a]	61.20 ± 4.69[b]*#	11.85 ± 0.93[b]*#	4.65 ± 0.46[c]
Recovery 10 min	7.46 ± 0.07[a]	44.24 ± 0.95[c]*#	8.30 ± 0.3[6c]#	6.58 ± 0.48[b]

Values are represented as mean ± SEM (n = 6). Different letters indicate significant difference within the same treatment ($P < 0.05$). * indicates significant difference from the control group at the same time and # indicates significant difference from the ethanol group at the same time ($P < 0.05$).

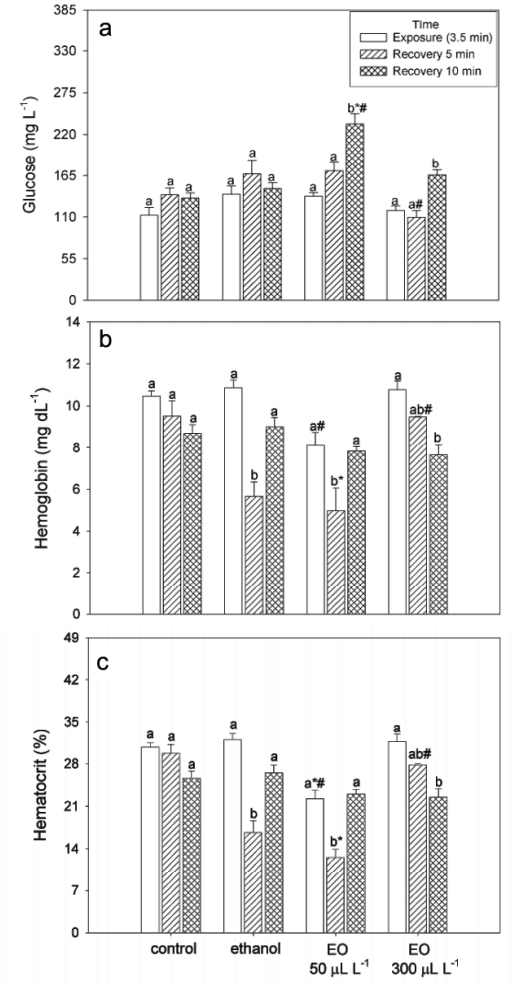

Figure 1 - Glucose (**a**), hemoglobin (**b**) and hematocrit (**c**) levels in *Epinephelus marginatus* after exposure to essential oil (EO) of *Aloysia polystachya* added to the water (n = 6). Values are represented as mean ± SEM. Different letters indicate significant differences between times in the same treatment ($P < 0.05$). * indicates significant difference from the control group at the same time and # indicates significant difference from the ethanol group at the same time ($P < 0.05$).

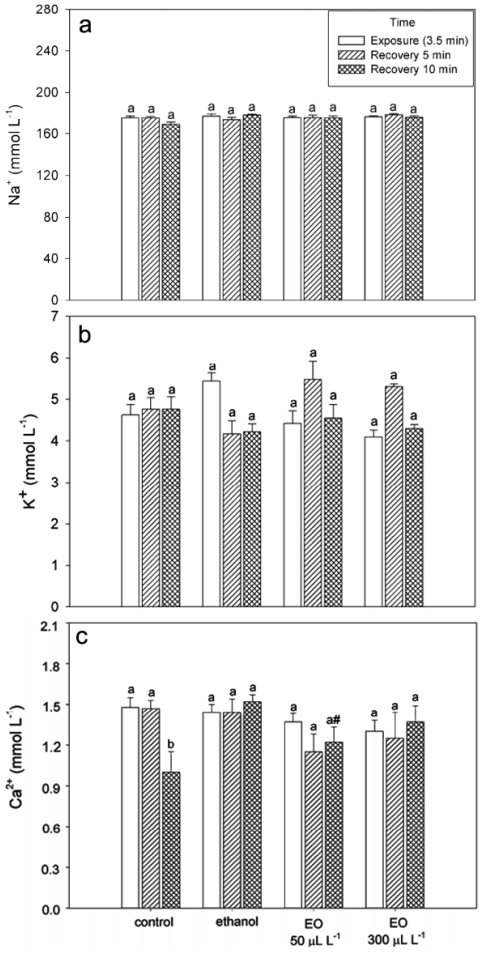

Figure 2 - Plasma Na^+ (**a**), K^+ (**b**) and Ca^{2+} (**c**) levels in *Epinephelus marginatus* after exposure to essential oil (EO) of *Aloysia polystachya* added to the water (n = 6 animals per group). Values are represented as mean ± SEM. Different letters indicate significant differences between times in the same treatment ($P < 0.05$). * indicates significant difference from the control group at the same time and # indicates significant difference from the ethanol group at the same time ($P < 0.05$).

this EO (Cabanillas et al. 2003). High contents of α-thujone and β-thujone (which were not found in the present study) were previously detected in this EO (Cabanillas et al. 2003, Duschatzky et al. 2004).

The present study demonstrated that *A. polystachya* EO has an anesthetic effect on grouper juveniles. This EO induced sedation at all concentrations tested and anesthetized animals within 3 and 1.5 min (300 and 400 μL L^{-1}, respectively). Recovery time for both concentrations was about 8 min and 5 min, respectively, and no mortality was observed as a result of anesthesia induction. These findings are in accordance with literature criteria (Marking and Meyer 1985, Gilderhus and Marking 1987, Keene et al. 1998, Park et al. 2009). Parodi et al. (2014) tested the anesthetic effect of the EO obtained from another species of *Aloysia*, namely *A. triphylla*, in concentrations ranging between 20 and 800 μL L^{-1} on two strains (albino and gray) of silver catfish (*Rhamdia quelen*), and 200 μL L^{-1} EO was the best concentration to induce anesthesia in the albino strain, while for the gray strain it was 400 μL L^{-1} EO. An emulsified mixture composed of *Mentha spicata* EO and methyl salicylate oil (containing 28.4% L-carvone) anesthetized common carp (*Cyprinus carpio*) within the recommended time at 395 μL L^{-1} (Roohi and Imanpoor 2014) and Atlantic salmon (*Salmo salar*) at 257 μL L^{-1} (Danner et al. 2011), but *M. spicata* EO alone induced anesthesia in less than 3 min only at 5000 μL L^{-1} (Roohi and Imanpoor 2015). Interestingly, increased EO concentration promoted recovery time decrease. Other EOs produced higher recovery times as the EO concentration increased (Cunha et al. 2010, Heldwein et al. 2012, Silva et al. 2012, 2013, Parodi et al. 2014). Since EOs are complex mixtures of compounds, a particular biological activity such as induction and recovery times from anesthesia depends on the specific chemical characteristics of each EO, including the qualitative composition and the proportions of each component in the oil (Raut and Karuppayil 2014).

The sedative and anesthetic activity of the EO of *A. polystachya* can be explained mainly by the combined action of its major components, the monoterpenoid-derived compounds carvone and limonene, which account for 92.44% of its total chemical composition. Carvone has a central nervous system (CNS) depressant effect detected in different pre-clinical studies in mice (Sousa et al. 2007), while limonene acts as an agonist for adenosine A2A receptors, and consequently can induce sedative effects (Park et al. 2011). Limonene also inhibited stimulant-induced behavioral changes in mice and rats, by regulating dopamine levels and 5-HT receptor function (Yun 2014). However, it is likely that a contribution to the activity detected in groupers is made by the minor components, such as limonene epoxide, for which an anxiolytic-like effect was detected in mice. The CNS effects of this compound were reversed by flumazenil, indicating a GABAergic mechanism of action (Almeida et al. 2012).

The blood pH values of the groupers, regardless of exposure time or group, were similar to or slightly lower than those reported for red pacu (*Piaractus brachypomus*) exposed to MS-222 and eugenol at 50, 100 and 200 mg L^{-1} (Sladky et al. 2001), yellow perch (*Perca flavescens*), walleye pike (*Sander vitreus*) and common carp anesthetized with buffered MS-222 (150 mg L^{-1}) (Hanley et al. 2010); silver catfish transported for 4 h in plastic bags with eugenol (1.5 or 3.0 μL L^{-1}) and *L. alba* EO (10 or 20 μL L^{-1}) added to the water (Becker et al. 2012). The pH values observed in the blood of groupers were not affected by *A. polystachya* EO exposure, in accordance with the lack of effect of eugenol and *L. alba* EO on this parameter in silver catfish (Becker et al. 2012).

The PvO_2, $PvCO_2$ and HCO_3^- values were within the range reported by other studies (Sladky et al. 2001, Souza et al. 2001, Hanley et al. 2010,

Becker et al. 2012). The handling (simulated recovery) of control groupers increased PvO_2 and decreased $PvCO_2$ and HCO_3^- values, probably due to hyperventilation. Exposure to 50 μL L^{-1} *A. polystachya* EO (and 300 μL L^{-1} compared to control fish) increased PvO_2, as was also observed by Hanley et al. (2010) in perch, walleye and common carp anesthetized with MS-222. $PvCO_2$ only increased in groupers exposed to 50 μL L^{-1} *A. polystachya* EO, as observed by Sladky et al. (2001) in red pacu exposed to MS-222 and eugenol. However, as in groupers exposed to 50 μL L^{-1} *A. polystachya* EO, Hanley et al. (2010) reported that $PvCO_2$ did not change in perch, walleye or common carp anesthetized with MS-222.

In the present study, the handling of simulated exposure did not induce a significant increase in blood glucose levels, but groupers anesthetized with 50 μL L^{-1} *A. polystachya* EO after 10 min recovery showed increased levels compared to those after exposure. Repetitive blood sampling increased blood glucose levels in rainbow trout (*Oncorhynchus mykiss*) and anesthesia with clove oil or MS-222 did not change this pattern (Wagner et al. 2003).

Hematocrit values found in this study (26–31%) in fish exposed to control conditions were similar to those reported by Becker et al. (2012) (26–33%) and Carneiro et al. (2009) (27–30%) in silver catfish. The lower hematocrit and hemoglobin values in recovered ethanol and 50 μL L^{-1} EO (5 min) and 300 μL L^{-1} EO (10 min) groups compared to exposed fish indicate possible hemodilution or decreased red cell number. Contrary to our results, anesthesia with clove oil or MS-222 reduced hematocrit decrease produced by repetitive sampling in rainbow trout (Wagner et al. 2003).

Ionoregulatory homeostasis is important to ensure proper cell function (Hwang et al. 2011). Moreover, stress due to usual aquaculture procedures is known to affect fish ionoregulation (Ashley et al. 2007). Addition of some sedatives to water can reduce ion loss in fish (Becker et al. 2012). Ionoregulation in groupers was not affected by anesthesia with *A. polystachya* EO, because the only change was lower Ca^{2+} in fish after 10 min recovery from 50 μL L^{-1} EO exposure compared to those recovered from ethanol exposure. Anesthesia with *H. ringens* and *L. alba* EOs (150, 300 or 450 μL L^{-1}) and recovery did not considerably affect plasma Na^+ and Cl^- levels either (Toni et al. 2014). Blood Na^+ also did not change, but K^+ increased in red pacu anesthetized with MS-222 and eugenol (Sladky et al. 2001).

CONCLUSIONS

The EO from the leaves of *A. polystachya* was an effective sedative and anesthetic for dusky grouper juveniles in concentrations similar to those found for other EOs in other species. Some of the tested blood parameters showed compensatory responses due to EO exposure, but most changes returned to control values after 10 min of recovery.

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