Iranian Veterinary Surgery Association

IRANIAN JOURNAL OF VETERINARY SURGERY

Journal homepage: www.ivsajournals.com

ORIGINAL ARTICLE

Evaluation of the Anesthetic and Tranquilizing Effects of Clove Powder (*Syzygium aromaticum*) and Lavender Oil (*Lavandula officinalis*) in Convict Cichlid Fish (*Cichlasoma nigrofasciata*)

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Received: 11 July 2018
Accepted: 26 October 2019
Online: 26 October 2019

Keywords:

Lavender;

Clove;

Convict cichlid fish;

Anesthesia;

Hematological changes.

Abstract

Objective-The present study describes the anesthetic effects of essential oil of Lavender (*Lavandula officinalis*) in Convict cichlid fish (*Cichlasoma nigrofasciata*) in comparison with clove powder (*Syzygium aromaticum*).

Design- experimental study

Animals- 25 Juvenile Convict cichlid fish with unknown sex and an average weight of 4.2 ± 0.5 g were used (n = 5 for each experimental group per experiment).

Methods- The fish were divided into five groups, initially with five fish per group. Each group was subjected to one of four final concentrations [0.3 and 0.5 g/l] of clove powder and [1.8 and 3 cc/l] essential oil of Lavender and control group was for determination of the basal values for blood parameters. The times for sedation, pre-anesthesia, and anesthesia were recorded based on the behavioral events seen after exposing the fish to each aquarium. After induction of anesthesia, the fish were transferred to anesthetic-free aquariums, and recovery time was recorded. Blood samples were taken by dissecting peduncle just 30 minutes after fish were placed in water contain anesthetics.

Results- essential oil of Lavender had efficacy to anesthetize the fish, and its sedation was similar to the clove powder at a commensurate concentration in Convict cichlid fish. However, the anesthesia form was different.

Conclusions and Clinical Relevance- Lavender oil as a sedative and anesthetic agent can be used in fish.

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DOI: 10.30500/ivsa.2019.193913.1191

1. Introduction

Anesthetic agents are used in various agriculture manipulations such as choosing fish, sorting, take samples, tagging, artificial reproduction, and surgery. In aquaculture, fish is exposed to stress during handling and transporting; therefore, anesthetics are used to reduce stress-induced damages.[1] Anesthesia is a biological condition with partial or complete loss of feeling or voluntary neuromotor control that is induced by chemical or nonchemical methods.[2] There are many different types of anesthetics; the most commonly used is tricaine methane sulfonate (MS-222, TMS). It is relatively expensive, and its carcinogenic effects are well known, and the avoidance period for this drug is at least 21 days.[3] Substances such as quinaldine sulfate, 2-phenoxyethanol, metomidat, and benzocaine hydrochloride are also used in some cases for the induction of sedation, and anesthesia.[4] Due to the increase of aquarium fish breeding, the veterinarian knowledge in diagnosing, examination, and treatment of aquarium fish must be increased. In recent years, the use of plant compounds has been developed to induce anesthesia in fish. It has been demonstrated that phenol compounds such as eugenol found in the flower and stem of clove can be used for anesthesia in aquatic animals.[5] The use of anesthetic drugs and non-pharmacological methods have been used frequently in aquatic anesthetics. Previous research has indicated that clove extract as a potential source for stress-free transport of fish do not affect gill tissue and is suggested as an ideal anesthetic agent in ornamental fish transport.[6] One study by Grush *et al*. examined the efficacy of clove oil as an anesthetic for the zebra fish (*Danio rerio*) in comparison with MS-222 showed that eugenol could be an effective anesthetic for using in this species and its benefits include a lower cost, lower required dosage, improved safety, and potentially low mortality rates.[7] Wimalasena *et al*. studied the Antimicrobial activity of lavender (*Lavendula angustifolia*) oil against fish pathogenic bacteria isolated from cultured olive flounder and based on their study lavender oil used to treat bacterial infections in aquaculture.[8] The convict cichlid fish, *Cichlasoma nigrofasciata*, was selected for this study. This species was chosen because convict cichlid fish is one of the most popular ornamental fish that is abundantly replicated.

In the present study, the effects of anesthetic and tranquilizing of clove (*Syzygium aromaticum*) and Lavender (*Lavandula officinalis*) on the convict cichlid fish (*Cichlasoma nigrofasciata*) were evaluated. Based on our knowledge, the Lavender plant was used as an anesthetic agent in fish for the first time.

2. Materials and Methods

The essential oil of lavender was obtained from the fresh leaves by the hydro-distillation process with a Clevenger type apparatus for 2 hours. The flowers and stems of the clove were prepared, and then the clove was completely powdered. Twenty five convict cichlid fishes (4.2 ± 0.5 g), were housed in a pre-prepared and oxygenated aquarium (100 L). They remained for one week to equalize the environmental conditions (25.35 ± 0.65° C, pH 7.45 ± 0.09). An aquarium was also considered for the recovery of the anesthetized fish. A semi-static system was used, and 50% of the water volume was changed daily. The Fish were fasted 24 hours before the experiments. The number of animals used in each experiment was the lowest possible in deference to the policy of reduction of experimental animals. The fish were divided into five equal groups (n = 5). To determine the minimum concentration needed to produce complete anesthesia in fish, clove with concentrations of 0.1, 0.2 and 0.3 g/l, and lavender with concentrations of 0.6, 1.2 and 1.8 cc/l were used. In this regard, after preliminary tests, concentrations of 0.3 g/l of cloves and 1.8 cc/l of lavender were determined as the minimum effective concentration in anesthetizing. Fewer concentrations only induced sedation in fish and had no anesthetic effect. All of the groups were individually placed in the containers containing the determined concentration of extract of these plants. In group 1, the fish were placed in a water containing clove powder at the concentration of 0.3 g/l. In group 2, the fish were placed in a water containing clove powder at the concentration of 0.5 g/l. In group 3, the fish were placed in a water containing lavender at the concentration of 1.8 cc/l. In group 4, the fish were placed in water containing lavender at the concentration of 3 cc/l. In group 5, control group fish were kept in tanks and used for blood sampling. The induction of anesthesia in the fish were separated by three steps, namely, sedation, pre-anesthesia, and anesthesia. distinguishing each stage from the other one was adjusted from the six anesthetic stages of fish suggested by McFarland.[9] Shortly, sedation was appointed by stopping voluntary swimming and the incomplete loss of equilibrium (McFarland stage 3), whereas pre-anesthesia was determined as a complete loss of equilibrium and reaction to faint external stimuli by mild touching (McFarland stage 4). The anesthesia stage in fish is a stage that fish losses totally reaction to powerful external stimuli, such as strong tactile and vibration of the tail region (McFarland stage 5). Four different observers separately recorded induction times for each stage to reduce experimental error. The concentrations of clove (0.3 g/L, 0.5 g/l) and lavender (1.8 cc, 3 cc) in the water were prepared, and each group of fish was exposed to one of these concentrations and the time was recorded and compared. After anesthesia inducing and recording the time of different stages of anesthesia, they were transferred to the restoration aquariums.

Blood parameters

Blood samples were taken from vena caudalis by cutting the tail at the end of the body in the control group and 30 minutes after fish were placed in water containing anesthetics in other groups. Hematocrit was measured by the microcentrifugation method of Larsen and Snieszko.[10] Hemoglobin concentration was determined by the method of Oser.[11] After blood sampling, blood was transmitted to Eppendorf tubes containing heparin to measure erythrocyte and leukocyte counts. Morphologic indices for erythrocytes were calculated by the method of Weinberg *et al*.[12] Mean corpuscular hemoglobin (MCH) was calculated by dividing the hemoglobin (grams per 108 ml of blood) by the erythrocyte counts (millions per cubic milliliter) and multiplying by 10. Mean corpuscular hemoglobin concentration (MCHC) was calculated by dividing the hemoglobin (grams per 100 ml of blood) by the hematocrit (percentage) and multiplying by 100. To determine the amount of glucose by the enzymatic method and using glucose kit (Pars Azmoon Co., Tehran, Iran). Plasma protein has been assessed by the method of Wedemeyer and Yasutake.[13]

Statistical Analyses

All results were analyzed using SPSS version 19. Data were collected regarding the duration of sedation, pre-anesthesia, anesthesia, and recovery analyzed by use of a one-way ANOVA followed by a Duncan test when appropriate. All the measurements were expressed as mean ± SD, and differences were considered significant at a value of $p<0.05$.

3. Results

Anesthesia was produced in all fish in four groups by clove and lavender in different concentration. The sedation time in lavender concentrations was shorter than clove concentration (Figure 1). Pre-anesthesia time was short in lavender oil (3 cc) and clove (0.5 gr) (Figure 2). Also, anesthesia time was short in these groups (Figure 3). Recovery time from anesthesia in lavender (3 cc) group was greater than other groups (Figure 4). In comparison it can be said that the anesthetic effects of lavender are roughly the same as cloves, according to the time of induction and recovery. One of the benefits of lavender in comparison with clove can be noted that it does not change the color of the water, while the clove changes the color of the water to yellow. In comparison to the different concentrations used for each plant, it should be noted that if the concentration of anesthetic is increased, the time of anesthesia induction is decreased, but the recovery time of the anesthesia is increased.

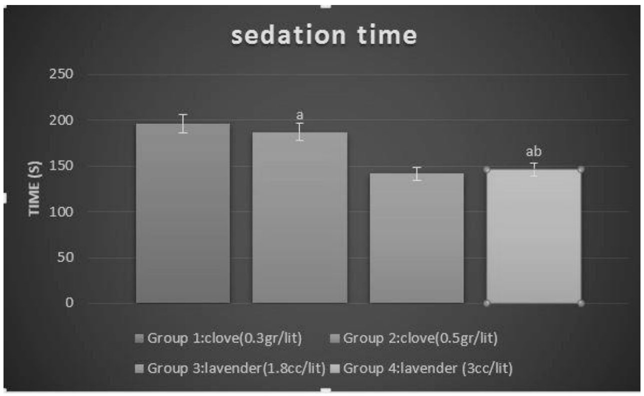

Figure 1. Influence of different concentrations of clove and lavender on sedation time in convict cichlid fish. a: $p<0.05$ vs clove (0.3 g/l), b: $p<0.05$ vs clove (0.5 g/l). Values are given as mean ± SD

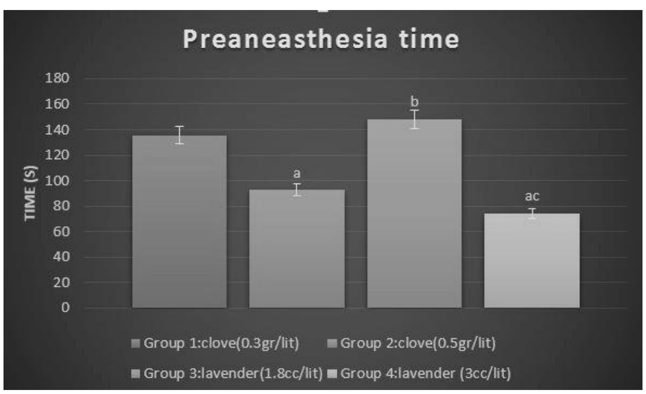

Figure 2. Influence of clove and lavender with different concentrations in each group on pre-anesthesia time in convict cichlid fish. a: $p<0.05$ vs clove (0.3 g/l), b: $p<0.05$ vs clove (0.5 g/l), c: $p<0.05$ vs lavender (1.8 cc/l). Values are given as mean ± SD.

According to Table 1, hematocrit increased significantly in all treatment and hemoglobin increased significantly in group 2 and 4 in comparison with the control group. Protein and glucose levels diminished significantly in all treatment in comparison to control group. Erythrocyte count, MCH, and MCHC rates of anesthetized and un-anesthetized fish did not vary significantly.

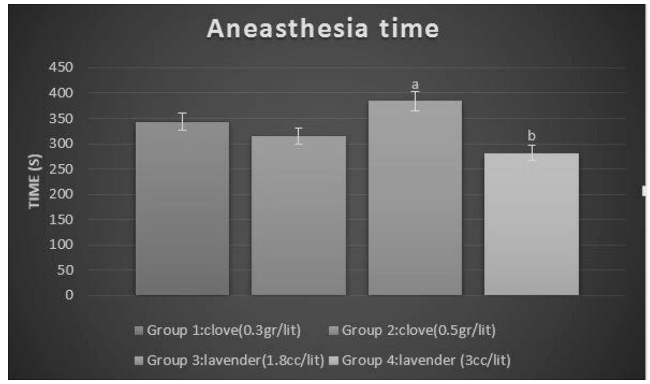

Figure 3. Influence of clove and lavender with different concentrations in each group on anesthesia time in convict cichlid fish. a: $p<0.05$ vs clove (0.5 g/l), b: $p<0.05$ vs lavender (1.8 cc/l). Values are given as mean ± SD.

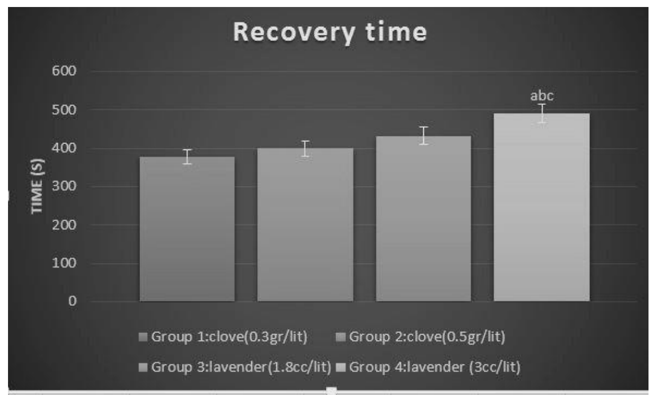

Figure 4. Influence of clove and lavender with different concentrations in each group on recovery time in convict cichlid fish a: $p<0.05$ vs clove (0.3 g/l), b: $p<0.05$ vs clove (0.5 g/l),c: $p<0.05$ vs lavender (1.8 cc/l). Values are given as mean ± SD.

4. Discussion

Chemical compounds can be injected intraperitoneally, intramuscularly, immensely, orally, or even through a diver for induction of anesthesia in aquaculture. Nevertheless, the method of immersing is the most commonly used anesthetic induction method in aquatic animals. Anesthesia abolishes pain in the fish and induces a calming effect followed by loss of equilibrium, mobility, and consciousness.[2] Clove oil is a natural product obtained by distillation of the flowers, stems and leaves of the clove tree *Syzygium aromaticum* (i.e. *Eugenia aromaticum* or *Eugenia caryophyllata*). It is a dark brown liquid with a rich aromatic odor and flavor. It has been used as a mild topical anesthetic agent since antiquity for a toothache, headaches and joint pains. The clove is relatively cheap,[14] and is more potent than other anesthetics which are used in the fish.[15,16] The active ingredients of the clove are eugenol and iso-eugenol, but raw clove oil also contains acetyl eugenol and a big amount of terpenoid compounds. Eugenol is quickly absorbed and metabolized after oral administration, and it is approximately completely excreted in the urine in 24-hours with no manifest ill effects in the fish.[17] Thus, eugenol has been used in laboratories as a safe mater.[18] Injecting clove oil is not effective because the elimination of the drug is too rapid to induce anesthesia.[19,20] Therefore, for the immense majority of procedures containing fish, clove oil is used by submerging in an anesthetic bath. Clove oil has recently been studied as a potential anesthetic agent for some ornamental fish and several farmed cold and warm water fish species.[21-24] In this study, the effects of clove powder on convict cichlid fish was similar to previous studies. Tomoe and colleagues showed that lavender extract was effective in reducing the airway allergic inflammation and mucosal hyperplasia in asthma, an animal model. In a study, Alnamer *et al.* investigated the sedation effects of aqueous and methanolic extracts of lavender on mice. They showed that both aqueous and methanolic extracts of lavender at low doses had a significant relaxing effect in compared to diazepam, they had drowsy effects at higher doses and indicated that the plant was effective in the treatment of insomnia.[25] Bradley et al. studied the anti-anxiety effects of lavender odor in mice with elevated plus maze method compared to diazepam, which showed that lavender decreased the anxiety in these mice and exploratory behavior in mice. The material indicated a greater reduction in anxiety in the female sex.[26] Lis-Balchin *et al.* studied the performance of lavender extract, and the results indicated that the mechanism of action of lavender is post-synaptic and is not similar to atropine, and the spasmolytic effect of the lavender extract is likely to be mediated by cAMP, not through cGMP.[27] Previously, the lavender extract was not used in anesthesia of fish. Surveys such as that conducted by Sudagara *et al.* have shown that clove powder significantly increased the hematocrit, hemoglobin, and total erythrocyte count after anesthesia.[28] In 1988, Iwama *et al*. published a paper in which they described the effects of five fish anesthetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout and resulted in a transient increase in hematocrit.[29] A study by Bolasina examined cortisol, and hematological response in Brazilian codling, *Urophycis brasiliensis* (Pisces, Phycidae) subjected to anesthetic treatment showed that the use of benzocaine significantly increased the erythrocyte number and hemoglobin concentration and glucose levels decreased significantly compared with the control group.[30] In a study conducted by Limsuwan *et al*. to

Table 1. Mean values (± SD) of hematological characteristics for convict cichlid fish exposed continuously to different levels of clove and lavender. Means followed by the same letter within a column are not significantly different ($p < 0.05$). Mean values that differ ($p < 0.05$) from the control are indicated by an asterisk.

Groups	**Hemoglobin (g/100 ml)**	**Hematocrit (%)**	**RBC (100 µl)**	**MCH (pg)**	**MCHC (g/dl)**	**Protein (g/100 ml)**	**Glucose (mg/ 100 ml)**
Control	7.66 ± 0.89	25.58 ± 2.35	1.04 ± 0.14	78.29 ± 1.74	32.78 ± 2.38	3.72 ± 0.33	68.08 ± 10. 67
Clove 0.3 g/l	8.42 ± 0.29 a	31.55 ± 2.13*a	1.12 ± 0.10 a	79.94 ± 3.48 a	29.45 ± 2.64 a	3.20 ± 0.10*a	29.69 ± 9.89*a
Clove 0.5 g/l	8.88 ± 0.24*b	36.10 ± 2.45*b	1.20 ± 0.22 a	79.08 ± 3.55 a	28.42 ± 4.86 a	3.24 ± 0.18*a	37.42 ± 4.15*a
Lavender 1.8 cc/l	8.36 ± 0.35 a	30.63 ± 2.81*a	1.15 ± 0.12 a	80.57 ± 2.49 a	30.11 ± 2.99 a	3.10 ± 0.11*a	33.57 ± 0.12*a
Lavender 3 cc/l	8.92 ± 0.13*b	34.04 ± 2.55*ab	1.10 ± 0.09 a	79.69 ± 2.57 a	30.91 ± 2.11 a	3.23 ± 0.10*a	45.38 ± 5.08*b

evaluate the stress response and blood characteristics of channel catfish (*Ictalurus punctatus*) after anesthesia with etomidate, it was shown that hemoconcentration, indicated by increased total erythrocyte count, hematocrit, and hemoglobin, resulted from anesthesia with 1-4 mg/E etomidate for 30-180 min.[31]

In this study, the lavender oil was used for induction of anesthesia in convict cichlid fish. The result of our study showed that lavender oil can induce anesthesia in convict cichlid fish similar to clove extract without any side effects. It is recommended to use it in fish anesthesia based on similarity to clove and appropriate induction and anesthetic time and good recovery.

Conflict of Interests

None.

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