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Effects of Clove Oil as An Anesthetic on Some Hematological Parameters of *carassius auratus*

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Abstract

Two levels of clove oil concentrations (0, 75, and 150 ppm) were prepared in 3 separate aquariums, each include 15 fish goldfish, *Carassius auratus* (average weight of 65 ± 5 g). Fish were exposed to different concentrations of clove oil and kept in aquariums at 18° C until they reach to stage 4 of anesthesia. Blood samples were taken from caudal vein at 0, 4, and 24 hours after anesthesia. Red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (PCV), white blood cell count (WBC) and the differential leukocyte count (leukogram) were determined by standard hematology method. Results showed that there were no significant differences between values of Hb, PCV and leukogram in each treatment in compare to the same control group (P> 0.05); however, WBC was significantly lower at 4 hours for 150 ppm clove oil treatment group and then returned to normal level 24 hours post anesthesia (P< 0.05). Moreover, RBC in this group was increased significantly after 24 h post anesthesia (P< 0.05). The induction time was less for 150 than 75 ppm clove oil treatment group (90 and 180 seconds respectively). Our results verified that using clove oil up to 75 ppm dose not have irreversible hematological side effect to the goldfish.

Keywords: Clove oil, Hematology, Goldfish, Anesthesia induction time

Introduction

A variety of anesthetics are using in aquaculture industry to aid fish handling, transportation and artificial propagation. They are used mainly in order to reduce stress level and to prevent fish damage during their handling. To select an anesthetic agent for a particular propose in fisheries science, several factor such as; the convenience for use, safety to the fish, human and environment, effectiveness, physiological perturbations and its cost may be consider by user [1]. The most commonly used fish anesthetic is tricaine methanesulfonate (MS-222) [2]. Other chemical agents such as, benzocaine (ethyl para-aminobenzoate) [3], 2-phenoxyethanol, metomidate [4], and carbon dioxide [1] were used to anaesthetize fish in aquaculture or biological researches. Each anesthetic has some advantage and disadvantage to use in aquaculture. For example MS-222, beside its relative expensive cost, is regarded as a carcinogenic and also a 21-day withdrawal period is required if the fish is intended for human consumption [1]. Clove oil is an essential oil distilled from stems, leaves and flower buds of the clove plant, Eugenia caryophyllata. The most active component of clove oil is eugenol (4-allyl-methoxyphenol) makes up 70 to 90% by weight of clove oil [4-5]. Clove oil efficiency has recently been examined as fish anesthetic for several fish species; *Anguilla reinhardtii* [6]; *Salmo salar* [7]; *Oncorhynchus mykiss* [1]; *Centropristis striata* [8]; *Sparus aurata* [9]; *Cyprinus carpio* [10]; *Solea senegalensis* [4]. Clove oil as a fish anesthetic have been assessed by a number of authors and most have reported it to be safe and an effective anesthetic [11]. Its main advantages lie in its low cost, limited regulatory or withdrawal time requirements, and its relative safety to both fish and humans [12].However the disadvantage of clove oil is its relatively low therapeutic index, i.e. the ratio between the therapeutic and the toxic concentrations [13]. The goldfish *Carassius auratus* is used commonly as an aquarium fish. The most important characteristic of an ideal anesthetic is no persistent effect on fish physiology. The purpose of this study was to assess this character of clove oil through measurement of multiple blood parameters of goldfish.

Material and Methods

The experiment was carried out at the Fish biology laboratory, University of Kurdistan in 2009. Goldfish *Carassius auratus* were purchased from a local ornamental fish dealer. They acclimatized for two weeks in 500-l circular tanks. During this period, fish were fed by hand twice a day. Forty five fish (mean body weight 65 ± 5 g) from this batch were selected 48 h before the experiment and randomly divided into three groups and stocked at a density of 15 fish per 121 liter aquariums. Water temperature was 18 ± 2° C and continuously aerated with a 10 cm air stone. The fish were fasted for 24 h prior the experiment. The clove oil was offered by the SHFA Company (Sanandaj, Iran) and used in two concentrations and control, 0 , 0.075 and 0.15 milliliter per liter (0, 75 and 150 ppm). For each treatment, 15 fish were placed in a well-aerated anesthetic bath containing the above mentioned concentration of clove oil. Fish were emergence to anesthetic bath until they reach to stage 4 of anesthesia; Loss of reflex activity, no reaction to strong external stimuli [14]. Fish then were returned to recovery aquariums. From each treatment group, blood samples were taken at 0, 4 and 24 hours after fish anesthetizing from 5 individual. In order to reduce effects result from fish maintaining during the experiment, the value from each treatment were compared with the same time value from the control group. Blood was drawn from the caudal vein using a needle and syringe. EDTA was used as anticoagulant. The indices used to evaluate the hematological profile were included; white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), hematocrite (PCV) and the differential leukocyte count (leukogram). The procedures were

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based on methods described for fish hematology [15]. The values of Hb, PCV, RBC and WBC were expressed in g/l, l/l, T/l (Tera = 10^{12}) and G/l (Giga = 10^{9}) respectively. Statistical analysis was performed using the MSTAT-C software program (Michigan State University). Two ways analysis of variance followed by Duncan's multiple range tests was used to investigate possible differences between treatments. Significant differences were accepted if the P value was less than 0.05.

Results

The results showed that applied clove oil concentrations were effective enough to make anesthetic in goldfish. The induction time to reach the stage 4 of anesthesia was 180 and 90 seconds for 75 and 150 ppm clove oil concentrations respectively. Effects of clove oil on the hematological indices of goldfish are showed in Figure1a and Figure1b (Table 1). Values of hematological indices in two experimental groups of 75 and 150 ppm were compared with the corresponding values in the control group (0 ppm clove oil). The RBC was significantly increased 24 h post anesthesia within 150 ppm clove oil treatment group in compare with control group ($p < 0.05$). A significant decrease of WBC in the 150 ppm treatment group was also observed 4 h after anesthesia in compare with control group ($p < 0.05$). This value was returned to the normal level after 24 h of anesthesia. The other indices (Hb, PCV and leukogram) in treatment groups were not significantly differed with the control group ($p > 0.05$).

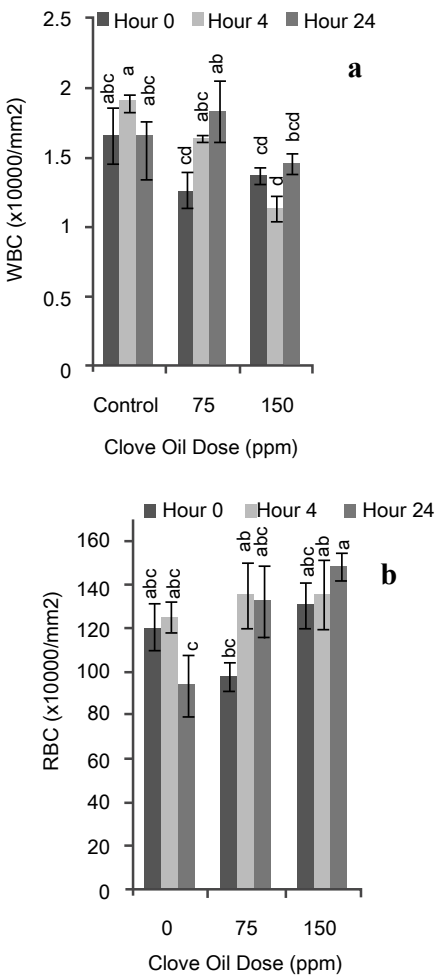

Figure 1: Effect of different clove oil concentrations on WBC (a) and RBC (b) values (mean ± SEM) of gold fish after 0, 4 and 24 hours exposes. Different letters indicate a significant difference for each variable ($P < 0.05$).

Clove oil Dose (ppm)	0 (control)			75			150		
Time of sampling (h)	0	4	24	0	4	24	0	4	24
Hb (g/l)	76.51 ± 9.58 a	102.5 ± 8.85 a	94.23 ± 5.00 a	75.83 ± 6.23 a	121.0 ± 17.70 a	115.1 ±3.89 a	88.30 ± 7.21 a	111.5 ± 11.67 a	88.13 ± 8.94 a
PCV (l/l)	0.3540 ± 0.02 a	0.3240 ± 0.02 a	0.3480 ± 0.02 a	0.3140 ± 0.01 a	0.3850 ± 0.01 a	0.3820 ± 0.02 a	0.3800 ± 0.02 a	0.3740 ± 0.02 a	0.3780 ± 0.04 a
Monocyte (%)	2.40 ± 0.40 ab	2.60 ± 0.40 a	2.00 ± 0.44 ab	1.80 ± 0.48 ab	2.40 ± 0.40 ab	2.60 ± 0.24 a	2.00 ± 0.25 ab	2.40 ± 0.24 ab	1.50 ± 0.24 b
Neutrophile (%)	12.20 ± 1.32 a	13.80 ± 2.53 a	12.60 ± 1.60 a	15.80 ± 1.11 a	15.20 ± 2.15 a	12.40 ± 1.46 a	14.2 ± 0.94 a	16.60 ± 1.69 a	13.80 ± 0.80 a
Lymphocyte (%)	85.00 ± 1.43 a	84.00 ± 2.83 a	85.00 ± 1.60 a	82.00 ± 0.92 a	82.00 ± 2.44 a	85.00 ± 1.46 a	84.00 ± 1.13 a	81.00 ± 1.58 a	85.00 ± 0.73 a

* Values are means ± SEM (n=5). Values followed by different letters indicate a significant difference for each variable ($P < 0.05$).

Table 1: Effect of different clove oil concentration as an anesthetic agent on hematological indices of gold fish*.

Discussion

Induction time is the number of minutes required to reach a given stage of anesthesia. Procedures that require long exposure may result in a prolonged recovery time sever oxygen debt, anoxia, or subsequent death of the subject [14]. Our results showed that increasing the anesthetic dose significantly decreased induction time. This was agreed with that reported about *Salmo salar* smolts [7], *Cyprinus carpio* [13], *Silurus glanis* [17]. In our study, time required to induction of anesthesia with 75 ppm clove oil was less than values reported for salmon [7], carp [13] and catfish [17], although they did not use 150 ppm clove oil concentration. This difference could be due to the different biological and environmental factors that might affect the efficacy of an anesthetic agent [7, 18]. Biological factors include; species kind, the stage of life cycle and age. Size and weight, lipid content, body condition and disease status all affect metabolic rate and therefore the pharmacokinetics of the anesthetic agents. Environmental factors such as temperature and pH, could affect the animal's metabolic rate as well as uptake across the gills [18]. The other important affecting factor seems to be the level of active ingredient of clove oil. Eugenol makes up 70 to 90% by weight of clove oil [4, 13] and so, different results may be due to differences in the proportion of eugenol content in clove oil. Hematological indices are closely related to response of the animal to the environment [19]. Previous studies showed that clove oil anesthesia at 30 mg/l concentration and 10 min exposing had no effect on the hematological profile of *Cyprinus carpio* [13] and *Oncorhynchus mykiss* [5] after 24 h. However, in Roach *Rutilus rutilus*, the 7-min exposure to clove powder caused a significant increase in hematocrit, hemoglobin and total erythrocyte count after anesthesia and then returned to normal level after 24 hours [20]. Moreover, the leukocyte counts of *Silurus glanis* exposed to 30 mg/l of clove oil were significantly decreased 24 hours after anesthesia [17]. Clove oil has been listed as a "Generally Regarded as Safe" substance by the United States Food and Drug Administration when administered at levels at which not exceed 1500 ppm in all food categories [11]. Less known about clove oil cytotoxicity and very few attempts have been made to determine active cytotoxic components of clove oil. In vitro toxicological studies have shown that clove oil exhibit high toxicity against human fibroblast and endothelial cells at a concentration of 0.03% [21]. Our results did not show any change in hematological indices of goldfish while clove oil was used at concentration of 75 ppm, however clove oil at concentration of 150 ppm could increased RBC value after 24 h. According to these results, clove oil at concentration of 75 ppm could be an efficient and

relatively safe anesthetic agent, but further studies are required to detect any possible toxicity effect on fish.

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