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The Efficacy of Clove Powder as an Anesthetic and its Effects on Hematological Parameters on Roach (*Rutilus rutilus*)

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Abstract: The anesthetic effects of clove powder were studied in juvenile Roach, *Rutilus rutilus*. The effects of exposures to various dosages of clove powder were measured.Times to induction and recovery from anesthesia were measured. The times to achieve anesthesia exhibited a negative exponential response to dose. The hematological parameters was assessed before, immediately after 7 min of anesthesia and 24 h, after the anesthesia at the concentrations of 175, 225, 275 and 350 mg L^{-1} clove powder. The 7-min exposure to clove powder caused the significant increase ($p<0.05$) in the hematocrit, hemoglobin and total erythrocyte count after anesthesia .These values returned back to normal within 24 h .Clove powder anesthesia had not effect on other hematological parameters. Results of the examinations suggest that the use of clove powder at the concentrations of 175, 225, 275 and 350 mg L^{-1} does not cause irreversible damage of the blood parameters in Roach.

Key words: Anesthesia, clove powder, hematological parameter, *Rutilus rutilus*

INTRODUCTION

A range of anesthetics are used by fish biologists to aid the capture, handling and transport of fishes. Anesthetics are used routinely during commercial fish culture (Marking and Meyer, 1985) and also by marine biologists during experiments in the field or laboratory. Anesthetics are physical or chemical agents which induce first a calming effect, then a successive loss of equilibrium, mobility, consciousness and finally, reflex action in an organism exposed to higher concentrations of the agent, or exposed for longer periods of time (Summerfelt and Smith, 1990). Anesthesia is achieved by placing the fish into an anesthetic solution that is absorbed through the gills and enters the arterial blood, from where it acts on the central nervous system (Ross and Ross, 1999). Some anesthetics reduce or block the activation of the Hypothalamic-Pituitary-Interrenal (HPI) axis associated with stressors and thus decrease or prevent the release of the stress hormone cortisol to the bloodstream of fish (Hoskonen and Pirhonen, 2006). After return of the anesthetized fish to the freshwater, the anesthetics or their metabolites are excreted via the gills (Ross and Ross, 1999). Hematological parameters are closely related to the response of the animal to the environment (Fernandes and Mason, 2003). Anesthesia may affect blood parameters and hemolised tissues (McKnight, 1966). Evaluation of the hemogram involves the determination of the total erythrocyte count (RBC), total White Blood Cell count (WBC), Hematocrit (HT), Hemoglobin concentration (Hb), erythrocyte indices (MCV , MCH , MCHC), white blood cell differential count and the evolution of stained peripheral blood films (Campbell , 2004). Clove powder is produced from the dry flowers and flower stalks of the clove tree *Eugenia aromatica* and is used for short-term immobilization of fish. It is soluble in water, available and very cheap. The goal of this study was to determine the efficacy of clove powder as an anesthetic and its effects on hematological parameters on Roach (*Rutilus rutilus*).

MATERIALS AND METHODS

Juveniles of Roach (11.47±2.53 g mean body weight and 12.43±1.97 cm mean total length) were used. One hundred eight fishes were divided into three groups and examined: Control group A (without the anesthesia), group B (after 7 min of anesthesia at the concentrations of 175, 225, 275 and 350 mg L^{-1}) and group C (24 h after 7 min of anesthesia at the same concentrations). The fishes were anesthetized and blood samples were collected by cutting of the caudal peduncle from group B. The

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observation of stages 3 and 5 anesthesia onset (Keene *et al.*, 1998) were made using different concentrations of clove powder under the same experimental condition. The times to stage 3 and stage 5 anesthesia onsets in groups B and C were also recorded. Group C were placed in freshwater tanks after 7 min of anesthesia for recovery and 24 h after anesthesia blood samples were taken. During this recovery period, fish behavior was observed and times to stages 2 and 5-anesthesia recovery (Keene *et al.*, 1998) were recorded. Blood sample in group A was taken without anesthesia. Samples preserved in disodium salt of Ethylene Diamine Tetra-acetic Acid (EDTA) bottles for analysis. The count of erythrocytes and leukocytes were enumerated in an improved Neubaeur haemocytometer, using Hayem and turck diluting fluids (Blaxhall and Daisley, 1973). The resultant value is the number of erythrocytes in TL^{-1} (T-tera $=10^{12}$) and number leukocytes in GL^{-1} (G-giga= 10^9). The amount of hemoglobin was determined according to cyanomethemoglobin procedure (Blaxhall and Daisley, 1973). Non-clotted blood (20 μL) was diluted with Drabkin solution (5 mL) and left standard 5 min. The absorbency of the mixture was read at 540 nm and the amount for hemoglobin was calculated from a parelly rumed hemoglobin standard. Hemoglobin content was expressed in g per dl. Hematocrit was determined by the standard microhematocrit method and expressed in percentage (Snieszko, 1960). The hematological in dices (MCV, MCH and MCHC) were calculated according to Seiverd (1964). The Mean Corpuscular Volume (MCV) was calculated as a quotient of Ht and RBC. The value of MCV was expressed in femtolitres (fl). The Mean Corpuscular Hemoglobin (MCH) expresses the average hemoglobin concentration in individual erythrocytes and is calculated as a quotient of Hb and RBC. The resultant value is given in Picograms (Pg). The Mean Corpuscular Hemoglobin Concentration (MCHC) was calculated as a quotient of Hb and Ht and expressed in gdL^{-1}. To estimate the differential leukocyte count, blood smears were prepared, air-dried, fixed in methanol and stained using may-Giemsa solution. Leukocytes in a blood smears were categorized in to lymphocytes, monocytes, neutrophils and eosinophils (Blaxhall and Daisley, 1973). Hematological data were analyzed with one-way analysis of variance by using SPSS11.5 for windows. Differences between means were determined using Duncan's multiple test ($p<0.05$).

RESULTS

The times to achieve stages 3 and 5 (Fig.1) onsets for clove powder exhibited a negative exponential response to dose. Stage 5 was induced faster at high concentration.

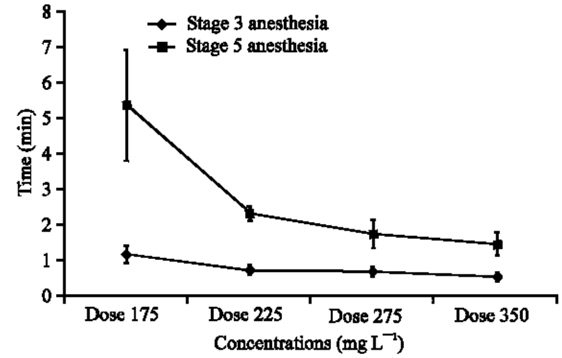

Fig. 1: Time to stages 3 and 5 anesthesia in Roach exposed to clove powder

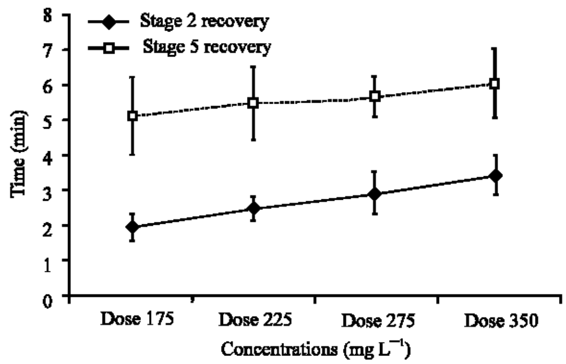

Fig. 2: Time to stages 2 and 5 recovery in Roach, 7-min of anesthesia exposed to clove powder

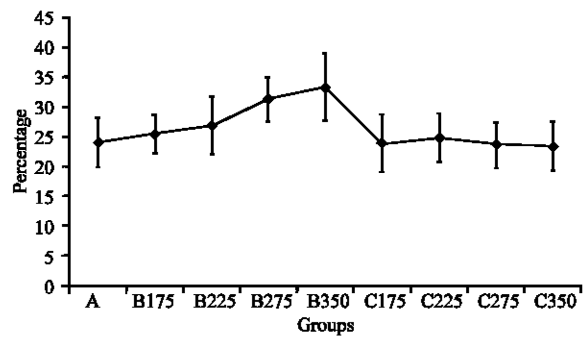

Fig. 3a: Effects of clove powder anesthesia on hematocrit. Statistical significance $p<0.05$ at groups B275 and B350

The response to high concentration was less variable than other concentrations, exhibiting a smaller Standard Deviation of the mean for the experimental dosages tested (Fig.1).

Fish exposed to high concentrations took longer to achieve recovery. Recovery stages 2 and 5 (Fig.2) fish exposed to the highest concentration require >7 min to

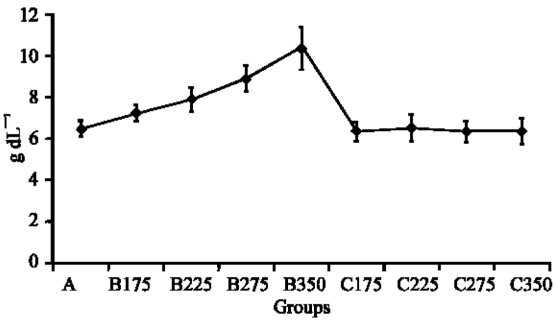

Fig. 3b: Effects of clove powder anesthesia on hemoglobin. Statistical significance $p<0.05$ at groups B175, B225, B275 and B350

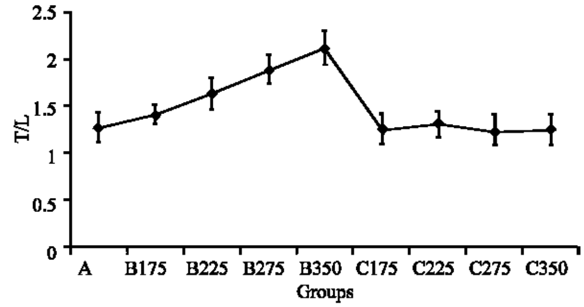

Fig. 3c: Effects of clove powder anesthesia on RBC. Statistical significance $p<0.05$ at groups B175, B225, B275 and B350

Table 1: Effects of exposures to various dosages of clove powder on hematological parameters on *Rutilus rutilus*

Group	A	B			
parameter	0	175	225	275	350
Wight (g)	11.53±2.61^{a}	11.6±2.56^{a}	11.23±2.6^{a}	11.42±2.71^{a}	11.49±2.49^{a}
Length(cm)	11.63±1.72^{a}	11.6±1.53^{a}	12.2±1.71^{a}	12.55±2.59^{a}	12.72±1.46^{a}
Number	12	12	12	12	12
MCV (fL)	191.75±51.78^{a}	179.61±22.48^{ab}	165.41±36.58^{ab}	166.92±29.8^{ab}	158.82±33.17^{b}
MCH (pg)	50.87±7.06^{a}	51.01±2.14^{a}	48.27±2.80^{ab}	47.16±1.67^{b}	48.79±2.10^{ab}
MCHC (gdL^{-1})	27.57±5.20^{b}	28.76±3.41^{ab}	30.36±6.06^{ab}	28.84±3.79^{ab}	32.09±7.48^{a}
WBC (gL^{-1})	4.94±0.54^{a}	4.93±1.27^{a}	5.17±1.54^{a}	5.13±0.82^{a}	5.25±1.05^{a}
Lymph. (%)	77.08±4.16^{a}	77.5±5.82^{a}	78.83±5.23^{a}	75.08±6.78^{a}	77.41±6.24^{a}
Neutro. (%)	17.08±3.42^{a}	16.66±6.27^{a}	14.08±4.52^{a}	18±5.22^{a}	15.75±7.72^{a}
Mono. (%)	3.75±2.52^{a}	4±3.10^{a}	5.08±3.60^{a}	5.50±3.50^{a}	5±3.35^{a}
Eos. (%)	2.08±1.16^{a}	1.83±1.19^{a}	2±1.34^{a}	1.41±1.08^{a}	1.83±1.02^{a}

Table 1:Continued

Group	C			
parameter	175	225	275	350
Wight (g)	11.15±2.76^{a}	11.89±2.65^{a}	11.4±2.68^{a}	11.5±2.5^{a}
Length(cm)	12.18±2.1^{a}	13.42±1.83^{a}	13±2.26^{a}	12.85±2.2^{a}
Number	12	12	12	12
MCV (fL)	189.73±25.42^{a}	189.46±25.55^{a}	191.19±27.35^{a}	188.46±24.44^{a}
MCH (pg)	50.90±4.29^{a}	49.72±2.81^{ab}	51.58±4.89^{a}	51.52±4.08^{a}
MCHC (gdL^{-1})	27.18±3.40^{b}	26.65±3.63^{b}	27.23±2.71^{b}	27.61±2.89^{b}
WBC (gL^{-1})	5.84±1.07^{a}	5.46±1.25^{a}	4.89±0.64^{a}	4.99±1.01^{a}
Lymph. (%)	75.91±8.47^{a}	75.16±6.04^{a}	78.91±7.97^{a}	78.16±6.56^{a}
Neutro. (%)	18.16±6.68^{a}	18.41±6.92^{a}	14.08±9.48^{a}	14.83±7.82^{a}
Mono. (%)	3.83±3.85^{a}	4.25±3.43^{a}	5.08±3.42^{a}	5.16±3.40^{a}
Eos. (%)	2.08±1.31^{a}	2.16±1.33^{a}	1.91±1.08^{a}	1.83±1.26^{a}

Values are mean ±S.D; Values followed by different superscript letters in each row are significantly different at $p<0.05$ (ANOVA). MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Hemoglobin, MCHC- Mean Corpuscular Hemoglobin Concentration, WBC- number of leukocytes, Lymph- Lymphocytes, Mono- Monocytes, Eos- Eosinophils and Neutro- sum of neutrophil granulocytes.

achieve stage 5 recovery. The times to achieve recovery stages 2 and 5, exhibited a positive response to dose.

Effects of clove powder on the hematological parameters are shown in Table 1 and Fig. 3. The exposure to clove powder 7-min after anesthesia at concentrations of 175, 225, 275 and 350 mg L^{-1} caused a significant ($p<0.05$) in the Ht, Hb and RBC and exhibited a positive response to dose (Fig. 3).

Their values returned back to normal within 24 h. The rest of the indices (WBC, MCV, MCH, MCHC, Lymphocytes, Monocytes, Neutrophile and Eosinophils) were at comparable levels in all groups (Table 1). Results of the examinations suggest that the use of clove powder at the concentrations of 175, 225, 275 and 350 mg L^{-1} does not cause irreversible damage of the blood parameters in Roach.

DISCUSSION

In this study, the significant increase ($p<0.05$) of hematocrit value , hemoglobin concentration and red blood cells after 7-min clove powder anesthesia was observed and their changes were concentration-dependant. On the other hand, Adamek *et al.* (1993) found increased count of RBC and hemoglobin concentration in *cyprinus carpio* following 2-phenoxyethanol (0.30 mL $^{-1}$) anesthesia. Velisek *et al.* (2007) also found increased of hematocrit value and actual count of *cyprinos carpio*. On the other hand, Velisek *et al.* (2005) reported that the 10-min exposure to clove oil at concentration of 30 mg L^{-1} had no effect on the hematological indices. The observed progression through the various stages of anesthesia was consistent with the descriptions by Keene *et al.* (1998) modified from the original descriptions by Jolly *et al.* (1972) and Hikasa *et al.* (1986) for respective onset and recovery states from chemical anesthesia. It has been demonstrated that onset times of individual stages of clove powder anesthesia as well as recovery times (Fig. 1 and 2) were concentration-dependant. The same effect of anesthetic concentration levels on anesthesia onset times has been described by Hirata *et al.* (1970) for the Crucian carp (*Carassius carassius)*, Hamackoua *et al.* (2004) for Tench (*Tinca tinca*), Grush *et al.* (2004) for Zebra fish (*Danio rerio*) and Velisek *et al.* (2005) for common carp (*Cyprinus carpio*).

CONCLUSION

The data presented in this study reveal that use of clove powder at low concentrations does not cause irreversiable damage of the hematological parameters in Rooach. The recommended dose to elicit stage 5 anesthesia in juvenile Roach is 225-275 mg L^{-1} clove powder. This will induce rapid anesthesia with a relatively short time for recovery. Its main advantages lie in its low cost and its relative safety to both fish and humans.

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