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Effects of 2-phenoxyethanol (2-PE) anesthesia on some haematological and biochemical indices of silver carp (*Hypophthalmichthys molitrix*)

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Abstract

In this study, the anesthetic effects of 2-phenoxyethanol (2-PE) on possible primary (cortisol level) and secondary (haematological indices and glucose level) stress responses were studied in silver carp (*Hypophthalmichthys molitrix*). Fish were first exposed to 0.1, 0.3, 0.5, 0.7 and 0.9 mL L^{-1} 2-PE, and the time of induction (deep anesthesia) and recovery were measured. At concentrations of 0.5, 0.7 and 0.9 mL L^{-1} 2-PE, all fish were anaesthetized within 3 min of exposure. For assessing possible stress effects caused by effective concentrations of 2-PE, the haematological indices, serum cortisol and glucose were determined in the deeply anaesthetized fish as stress indicators. 2-PE exposure resulted in a significant increase in red blood cell (RBC) amounts at 0.1 and 0.3 mL L^{-1}. A parallel increase in hemoglobin and Haematocrite amounts at 0.1 concentrations (p<0.05) was observed. The serum cortisol levels had the highest amount in 0.1 mL L^{-1} of 2-PE. Moreover 2-PE exposure resulted in a significant increase in glucose amounts first at the 0.1 and later in the 0.3 mL L^{-1} concentrations. This study shows that rapid induction of deep anesthesia with a relatively high concentration of 2-PE (0.5, 0.7 and 0.9 mL L^{-1}) was associated with the lowest effects on the haematological and serum biochemical indices in silver carp and so 0.7 mL L^{-1} could be suggested as a suitable dose for haematological studies in this species.

Keywords: Blood parameters, Anesthesia, Stress response, Silver carp

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Introduction

Anesthetics are physical or chemical operatives that make animals insensitive to pain by initially inducing a relaxing effect and afterwards inducing loss of balance, mobility, ability to perceive and reflex action (Summer and Smith, 1990). Anesthetics are greatly used in ordinary aquaculture performances to immobilize animals during transportation, spawning, vaccination and handling. Overdose of an anesthetic material may be utilized as a moral way to euthanize fish (Shaluei *et al*., 2012). The desirable characteristics of anesthetics used for finfish include: short induction and recovery time, non-toxicity to fish and humans, no long physiological effects, quick release from the body, steady under normal hatchery situations (light, heat) and non-foaming, readily attainable and cost efficient (Gholipour Kanani *et al.,* 2011).

The efficacy and security of any anesthetic factor change among species, life phases and environmental conditions. A perfect anesthetic should cause anesthesia quickly with minimum hyperactivity or stress. It should be easy to supply and should preserve the animal in the selected state. When the animal is eliminated of the anesthetic, recovery should be quick. The anesthetic should be efficient at low doses and the toxic dose should greatly exceed the effective dose so that there is a vast border of security (Coyle *et al*., 2004). The energetic material of 2-PE is ethylene glycol monophenyl ether, with the chemical formula $C_8H_{10}O_2$, molar weight 138.17 g L^{-1}, density 1.107 – 1.108; peroxide content less than 0.005% and the boiling point 245 °C. The anesthetic is a little soluble in water (26.7 g L^{-1}) but readily soluble in ethanol. 2-PE is a murky, greasy liquid. The solution is bactericidal and fungicidal, practical during surgery. It is comparatively inexpensive and remains active in the thinned state for at least 3days. 2-PE has a comparatively wide margin of security and has been announced to produce a range of effects from light sedation to surgical anesthesia at concentrations of 100-600 mg L^{-1}. 2-PE is not confirmed by FDA for use in fish in the U.S (Coyle *et al*., 2004). The anesthetic 2-PE is utilized not only for short-term immobilization of fish before reproduction, but also at any time fish are handled outside water, e.g. during veterinary interferences.

Estimation of blood cells, serum biochemistry and hormones are utilized as indicators of the physiological or sub lethal stress response in fish to endogenous or exogenous changes. In this regard, the rising of plasma cortisol and glucose levels is greatly used as a general indicator of stressful conditions (Hedayati *et al*., 2015).

Silver carp is one of the prime cultured fresh water fish in polyculture and of great economic importance. It is vital to understand responses of silver carp to anesthetics. Therefore, this study was carried out to appraise the efficacy of 2-PE as an anesthetic on silver carp and its effect on

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haematological and serum biochemical indices in this species.

Material and methods

Preservation of fish

105 silver carp (71.6±5.8 g, 20.3±3.1 cm) were supplied from the Bony Fish Propagation and Rearing Center of Sijeval (Bandar Torkaman, Gorgan, Iran). Fish were transferred to the aquaculture research center of Gorgan University of agricultural sciences and natural resources and acclimated to the laboratory conditions for 2 weeks. The average amounts for aerated and de-chlorinated tap water were used during the acclimation period, water pH was 7.79±0.50, dissolved oxygen 7.93±0.25 mg L^{-1}, temperature of 20.50±0.5°C and total hardness was 298±2.35 mg L^{-1} as $CaCO_3$. Water pH, temperature and dissolved oxygen were determined by Wagtech portable pH/temp meter and oxygen meter (Berkshire, UK). Water total hardness was determined using a portable photometer with commercial kits provided by the manufacturer (Wagtech Por-table Photometer 7100, Berkshire, UK). Throughout the acclimation and experiment periods, fish were held under natural photoperiod conditions (11:13 light–dark).

2-PE treatment

The 2-PE stock solution was prepared by dissolving pure 2-PE (Sigma Chemicals, St. Louis, MO, USA, 99.5%, d=1.107–1.108 g L^{-1}) in 95% ethanol (1: 10 ratio of 2-PE–ethanol). Concentrations of 2-PE tested were 0.1, 0.3, 0.5, 0.7 and 0.9 mL L^{-1}. Each mentioned concentration of 2-PE was added to a 100-L tank and the water well ventilated for 2 min prior to the experiment. Ten fish were separately exposed to each concentration and induction time (surgical or deep anesthesia phase; loss of responsiveness, lying on the bottom of tank and not reacting to handling) registered with a stop monitor (Shaluei *et al*., 2012). As soon as the deep anesthesia stage was reached, they were transferred to fresh and strongly-aerated tanks and the time for total recovery was recorded. Recovery time was registered as the time required for fish to regain balance and begin active swimming.

Haematological and biochemical analysis

For the haematological and serum biochemical tests, 10 fish were used for each efficient concentration (0.1, 0.3, 0.5, 0.7 and 0.9 mL L^{-1} 2-PE) and for the controls. Fish were separately exposed to each concentration. When fish arrived at the surgical or deep anesthesia phase, the midline posterior of the anal fin of the juveniles was cleaned with erasing paper to prevent possible pollution with water, and three milliliters of blood was drawn from the caudal vein. Blood collection was carried out by expert persons and lasted less than 20s immediately after netting the fish head with wet cloth to diminish stress (Chardeh Baladehi *et al.*, 2017).

Blood samples were rapidly collected from the caudal blood vessel by

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heparinized syringes and preserved without delay on ice (Abarghuei *et al.*, 2015). Estimation of the blood indices was carried out on fresh blood. Leukocyte and erythrocyte count was measured by diluting heparinized blood with Giemsa stain at 1:30 dilution and cells were counted using a Neubauer hemocytometer under the light microscope (Hedayati and Niazi, 2015).

Haematocrit amounts (Ht%) were determined after sampling by placing fresh blood in glass capillary tubes and centrifuging for 5 min at 10000 rpm in a micro Haematocrit centrifuge (Hettich, Germany). The packed cell volume was then calculated (Abarghuei *et al.*, 2015); Haematocrit readings were performed with the aid of a micro Haematocrit reader. Hemoglobin levels (Hb mg L^{-1}) were determined colorimetrically by measuring the formation of cyanomethemoglobin following standard methods (Hedayati and Niazi, 2015).

Blood samples were placed into tubes containing EDTA as an anticoagulant for the hematology tests. These parameters were determined: total white blood cell (Leukocytes) count, red blood cell (Erythrocytes) count, hemoglobin content and Haematocrite level. For the biochemical experiments, blood was put in tubes and allowed to clot at room temperature (25°C), for 30 min. Serum was removed from the clotted sample after centrifugation at 5,000 rpm for 5 min and frozen at -80°C until analysis. Glucose levels were calculated using a spectrophotometric method (WPAS2000-UV/VIS, Cambridge, UK) with reagents supplied in standard analyses kits (Pars Azmon, Iran). Cortisol was determined with a commercial kit (ELISA, DRG Diagnostics, Mountainside, NJ, USA) based on an enzyme immunoassay. Before using an ELISA kit for determining the serum cortisol level in silver carp, this diagnostic kit was validated for use by tests of linear response of the sample and cortisol overload (Weber *et al.*, 2011). Experimental kit, which was used for human samples, was functional to the measurement of cortisol content in fish. Prior studies approved that the kit could identify the cortisol molecule of the fish species (Hosseini *et al.*, 2015).

Statistical analyses

All results are expressed as mean±SD. Statistical analyses were carried out using the computerized package 18.0 for Windows. Normality of data was first estimated using a Kolmogorov–Smirnov's test, and homogeneity of variance was assessed with Levene's test. Based on these tests, all data were found to be normally distributed. To evaluate the effect of different concentrations of 2-PE on induction and recovery times and haematological and biochemical indices, all data were subjected to one-way ANOVA followed by Tukey's test at a 5 % significance level.

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Results

No mortality was monitored during the experiments. Effects of different concentrations of 2-PE on induction and recovery times are shown in Fig. 1. Induction times generally decreased significantly with increasing 2-PE concentrations (p<0.05). Contrary to this trend, recovery time from anesthesia increased as expected (Fig. 1).

Effects of different concentrations of 2-PE on the haematological indices of silver carp are given in Table 1.

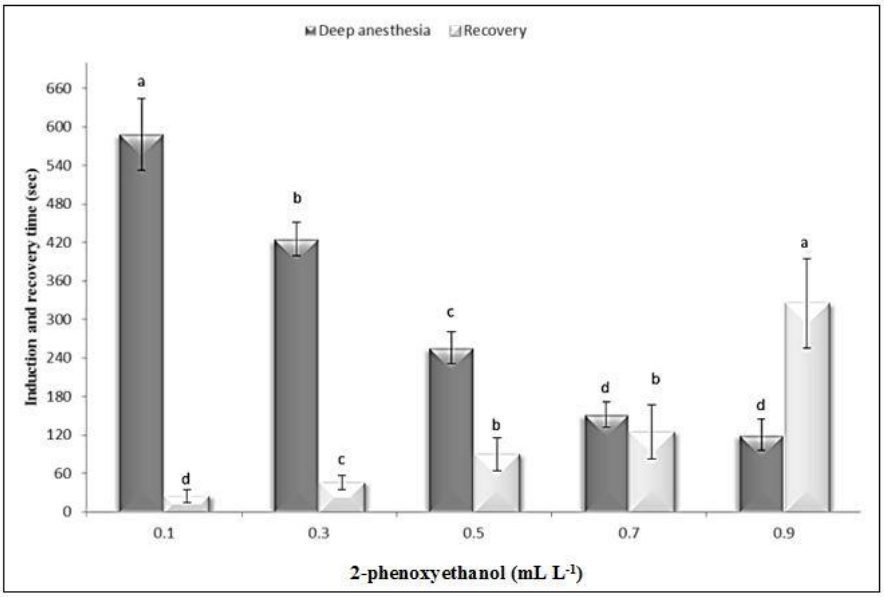


Figure1: Mean (±SD) induction time to anesthesia and recovery of silver carp (n =10) exposed to different doses of 2-PE.

2-PE exposure resulted in significant increase in RBC amounts at 0.1 and 0.3 mL L^{-1} and a parallel increase in hemoglobin and Haematocrite amounts were observed at 0.1 mL L^{-1} 2-PE (Table 1).

Results indicated that deep anaesthetization of fish using different concentrations of 2-PE led to an alteration in cortisol and glucose levels (Figs. 2 and 3).

The serum cortisol levels reached the highest level with 0.1 mL L^{-1} of 2-PE. Moreover, 2-PE exposure resulted in a significant increase in glucose amounts first at 0.1 and then at 0.3 mL L^{-1} concentrations.

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Table1: Haematological indices in silver carp exposed to different doses of 2-PE.

Parameters	Control	2-phenoxyethanol (mL L^{-1})				
		0.9	0.7	0.5	0.3	0.1
RBC ($\times 10^6$ cells mL^{-1})	1.91 ± 0.083^{c}	2.59 ± 0.11^{a}	1.90 ± 0.06 ^{c}	1.95 ± 0.070 ^{c}	2.00 ± 0.115 ^{c}	2.28 ± 0.11^{b}
WBC ($\times 10^3$ cells mL^{-1})	6.66 ± 0.24^{ab}	6.570 ± 0.27^{b}	6.77 ± 0.17^{ab}	7.18 ± 0.25^{ab}	7.04 ± 0.50^{ab}	7.35 ± 0.55^{a}
Hb (g dL^{-1})	6.90 ± 0.08^{b}	7.00 ± 0.45^{b}	6.95 ± 0.07^{b}	6.99 ± 0.02^{b}	7.26 ± 0.24^{ab}	7.66 ± 0.10^{a}
Hct%	20.87 ± 1.30^{bc}	20.30 ± 1.78^{c}	21.34 ± 1.56^{bc}	21.29 ± 1.07^{bc}	23.40 ± 0.50^{b}	27.63 ± 1.45^{a}
Hct%	20.87 ± 1.30^{bc}	20.30 ± 1.78^{c}	21.34 ± 1.56^{bc}	21.29 ± 1.07^{bc}	23.40 ± 0.50 ^{b}	27.63 ± 1.45^{a}

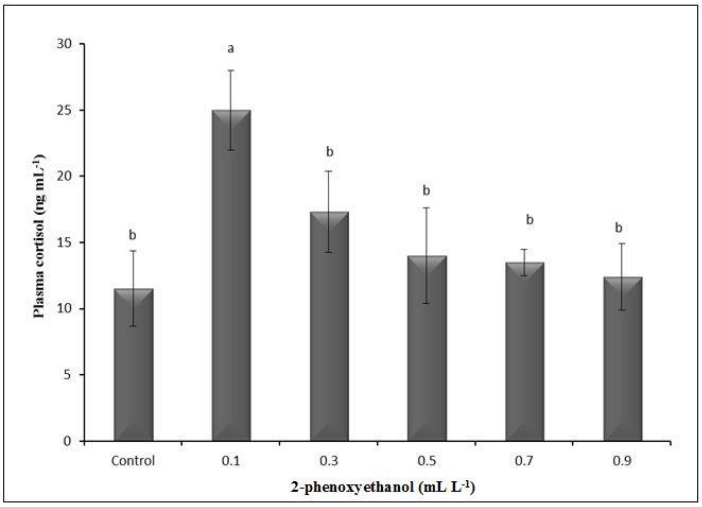


Figure 2: Serum cortisol levels in silver carp exposed to different doses of 2-PE.

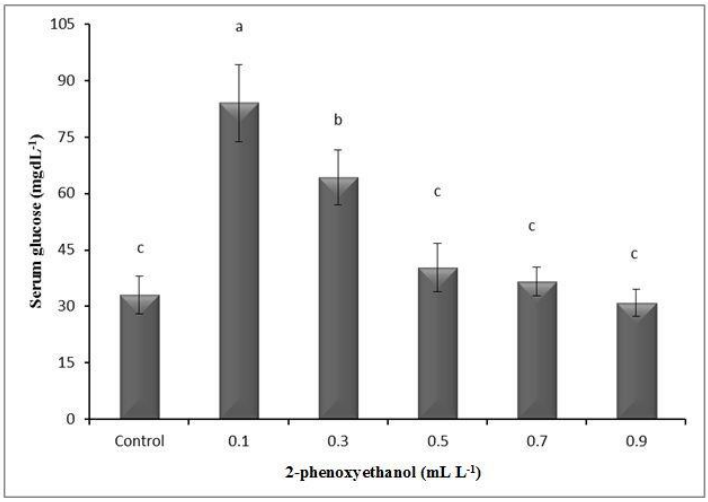


Figure 3: Serum glucose levels in silver carp exposed to different doses of 2-PE.

Discussion

Anesthetics are essential for many actions in aquaculture, because species may be differ greatly in their response to the same anesthetic. Screening of dosage of different anesthetics is often essential. The concentration of the anesthetic in the central nervous system determines the stage of anesthesia (Shaluei *et al.*, 2012). The fish takes up the anesthetic through a density gradient at the tissue/water interface,

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and some time is needed for the concentration of the anesthetic in the blood to arrive at the threshold level at which the fish becomes anaesthetized. Present results indicate 2-PE is an efficient anesthetic that can induce quick induction and fast recovery time in juvenile silver carp. Gilderhus and Marking (1987) contrasted the effectiveness of 16 anesthetic chemicals in rainbow trout, *Oncorhynchus mykiss*, and stated that 2-PE had all the criteria for efficacy. Weber *et al*. (2011) compared the effects of 2-PE, metomidate, clove oil and MS-222 in Senegalese sole, *Solea senegalensis*, and discovered that 2-PE was the most effective of the four anesthetic agents. McCarter (1992) announced no negative effects of anesthesia with 0.20 mL L^{-1} 2-PE on spermatozoa and their motility in grass carp (*Ctenopharyngodon idella*).

An appropriate anesthetic should cause anesthesia within 3 min or less, and permit recovery within 5 min or less. The maximum allowed time for the induction of deep anesthesia in fish is 10 min (Ross and Ross, 2008). There was no data in open literature on biochemical blood profile in silver carp anaesthetized with 2-PE. In a perusal of rainbow trout anaesthetized with 0.30 mL L^{-1} 2-PE, no significant negative influences of anesthesia on parenchymatous tissues were discovered (Veliekl and Svobodovan, 2004). The lowest concentration of the 2-PE utilized here that was capable of inducing deep anesthesia in silver carp was 0.1 mL L^{-1}. Anesthetics reactivate stress by causing a depression in the nervous system; dosage and time can influence the physiology and blood profile of the fish particularly stress indices (Veliekl and Svobodovan, 2004; Bolasina, 2006; Akbary *et al.*, 2016). Different stressors operate the hypothalamus– pituitary–inter renal (HPI) axis, resulting in a cortisol release that causes secondary stress answers. Stress hormones make active number of metabolic routes that result in alternation in blood chemistry and hematology indices. In the current study, RBC, hemoglobin concentration and haematocrite were raised significantly when fish were exposed to the 2-PE, first, at the concentration of 0.1 and then at 0.3. Adámek *et al*. (1993) discovered raised count of RBC and concentration of Hb in common carp (*Cyprinus carpio*) following 2-phenoxyethanol (0.30 mL L^{-1}) anesthesia.

This raise may be because of the release of unripe red cells from the spleen, to increase the oxygen supply to the main organs in answer to the higher metabolic requirements. In addition, this phenomenon could be a prompt response to the acute stress mediated by catecholamines (Tort *et al*., 2002). It is well known that there is a reduction in the count of leukocytes in ill fish which are exposed to any type of infection and expansion related to protection in leukocyte cells (Coyle *et al*., 2004). While there were no changes in rainbow trout right after the anesthesia, an increase is discovered 24h later (Velisek *et al*., 2007).

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In our study, the concentrations of glucose increased significantly in fish exposed to 2-PE, first at 0.1 and then at 0.3 mL L^{-1}. Under stressful conditions, increases in glucose levels occurred because of catecholamines and glucocorticoids release from adrenal tissues of fish (Shaluei *et al.,* 2012). Ortuno *et al.* (2002) announced a raise in glucose and cortisol amounts in *Sparus aurata* anaesthetized with 2-phenoxyethanol.

The raised plasma levels of glucose are compatible with those reported in Senegalese sole (*Soleasene galensis*) after exposure to 2-PE (500 mg L^{-1}) (Velisek *et al*., 2007). Ortuno *et al*. (2002) explained an increase of glucose in gilthead bream anaesthetized with 2-PE. Different findings have been reported in other fish anaesthetized using 2-PE (Velisek *et al.,* 2007). In the present study, a significant increase in cortisol level was observed in fish exposed to 2-PE at 0.1 mL L^{-1}. Molinero and Gonzalez (1995) showed that both the dose and exposure to 2-PE caused a cortisol reply in gilthead sea bream.

Weber *et al.* (2011) found a significant increase in plasma concentrations of cortisol in Senegalese sole after 20min exposure to 2-PE (500 mg L^{-1}). King *et al*. (2005) found cortisol response by juvenile and adult black sea bass because of netting and capture, but no significant changes in cortisol levels beyond the 10 to 30 min exposure to 20-PE (300 mg L^{-1}). Different responses were monitored in other fish after exposure to 2-PE and other anesthetics (e.g., King *et al*., 2005; Bolasina, 2006; Weber *et al*., 2011).

Generally, this study shows that rapid induction of deep anesthesia with a relatively high concentration of 2-PE (0.5, 0.7 and 0.9 mL L^{-1}) was related to the lowest effects on haematological and serum biochemical indices in silver carp and therefore can be suggested as suitable doses for haematological studies in this species. Fast anesthesia was observed with a higher dose (0.9 mL L^{-1}) but more recovery time was also recorded at the same dose, However in terms of blood and serum indices, by increasing the concentration of 2-PE, physiological parameters returned to levels at normal conditions (best in 0.9 mL L^{-1}), eventually because of long recovery time at higher concentration. Based on these results, 0.7 mL L^{-1} of 2-PE can be suggested as the best dose of anesthesia in silver carp.

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