Original Article

The Effect of Phenanthrene on Some Hematological Indices Yellowfin Seabream (*Acanthopagrus latus*)

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ABSTRACT

Background: We examined the effects of phenanthrene (Phe) on hematological parameters of yellowfin seabream (*Acanthopagrus latus*).

Methods: The research was carried out in Jan 2016 at Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran. Fish were injected with different concentrations (0, 2, 20 and 40 mg/kg) of Phe and blood, samples were taken from fish 1, 4, 7 and 14 d after injection.

Results: Results of Phe-treated fish showed a decrease in red blood cell and white blood cell counts, hematocrit amount after 4 d and in hemoglobin concentration after 7 d (P<0.05). Mean corpuscular hemoglobin concentration was enhanced in fish exposed to Phe up to day 4 (P<0.05). Phe-exposed fish showed an increase in the *percentage of* neutrophils with a decrease in the *percentage of* lymphocytes (P<0.05) and did not represent any effects on *percentage of* monocytes and eosinophils, mean corpuscular volume and mean corpuscular hemoglobin concentration.

Conclusion: Changes in hematological parameters after exposure to Phe result in a decrease of non-specific immunity.

Keywords: Blood Cells, Fish, Polycyclic Aromatic Hydrocarbons, Toxicity.

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INTRODUCTION

Anthropogenic activities such as oil extraction and transportation cause an increased discharge of oil compounds into aquatic environments [1]. The presence of polycyclic aromatic hydrocarbons (PAHs) in aquatic environments is a *crucial environmental problem* because of their accumulation in marine animals that can cause a disturbance of the homeostasis in animals [2]. The concentration of PAHs is recorded more than 1700 µg/L in seawater polluted with oil spills [3]. Phenanthrene (Phe) is one of the most abundant compounds of PAHs in the aquatic environment because of both petrogenic and pyrogenic sources. It is among 16 PAHs that are on the United States Environmental Protection Agency (USEPA) priority pollutant list [1, 4]. The concentration of recorded from 15.438 to 632.682 ng/g in the sediment of the Persian Gulf, is situated at the south of Iran [1]. This compound could cause structural damage to the hematopoiesis tissues [4]. Phe and its metabolites cause oxidative stress by inducing a high level of reactive oxygen species, which leads to the destruction of the blood cells [4].

Alterations of hematological parameters can provide important information on conditions of hematopoietic organs and be a valuable tool for forecast and primary diagnosis in disease or stress [5]. The hematological indices are also important parameters for determining the general status of the physiological fish to assess the oxygen-carrying capacity of the blood in fish exposed to *pollutants* [6]. Little information has been published on the role of hematological parameters as biomarkers of physiological changes during exposure of marine fish to Phe. Hematological indices like red blood cell (RBC), white blood cell (WBC), differential leukocyte count (DLC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) has regularly been used to illustrate the health status of fish under stress conditions [7].

Yellowfin sea bream (*Acanthopagrus latus*) are widely used for immunotoxicology studies due to

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their worldwide distribution and high commercial importance, especially in the Persian Gulf [8, 9]. Many studies have been performed on the hematological indicators of different fish species exposure to several types of pollutants [6,7]. However, no study on toxicological effects of Phe on the hematological indicators of yellowfin seabream is available.

This study aimed to investigate the effects of short-term exposure to Phe on some hematological indicators of yellowfin seabream.

MATERIAL AND METHODS

Animals and Treatments

This research was carried out at Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran in Jan 2016. Immature yellowfin seabream *(*81.11±12.1 gr mean body weight and 15.78 ±0.2 cm mean body length) were used as an experimental animal [10].

Fish were caught from the wild populations in the Musa Creek, located in the northwest of the Persian Gulf, Iran. Fish were acclimatized in fiberglass tanks (300 L capacity) containing filtered, aerated and ultraviolet-treated sea water (water temperature 25±1 ºC; salinity 46.1±0.01 g/L; dissolved oxygen 7.12±0.10 mg/L; pH 7.2±0.06; photoperiod 12/12 h light/dark) for 10 d before the exposure test. Fish were fed twice daily (2% of the body weight) until 24 h before sampling. Water in the tanks renewed daily.

Following acclimation, the experimental fish were divided randomly into five groups (twelve fish per group). They were anesthetized with the 2-phenoxyethanol solution (0.2%) and injected intraperitoneally with 0 (vehicle control), 2, 20 and 40 mg/kg- body weight of Phe (Code: P11409, 98% pure, Sigma-Aldrich Chemical Co) dissolved in coconut oil (10 µL/g body weight)**.** Choosing of Phe concentration was based on the previous studies on the PAHs toxicological effects on fish [11-14]. In addition, the dose of Phe purposed to display an actuated biological response in an acute exposure and a rapid peak in effects. Phe-treated groups were compared with control group (without injection). The experimental period was 14 d.

Sampling

Three fish were sampled from each group after 1, 4, 7 and 14 d of injection. They were immediately anesthetized with the 2-phenoxyethanol solution (0.2%), and blood samples were taken from the caudal vein by heparinized syringes. Finally, Fish were killed by a blow to the head, followed immediately by the severing of the spinal cord.

Hematological Analyses

Heparinized blood samples were diluted with Natt–Herrick's staining solution (1:30) and then WBC and RBC were counted using hemocytometer [15]. DLCs were conducted using blood smears stained with Giemsa stain [16]. The percentage of Ht was determined by centrifuging heparinized blood in capillary tubes at 10000 rpm for 5 min using a microhematocrit centrifuge (Hettich, Germany) [16]. Hb level (g/dL) was determined colorimetrically based on the measuring the cyanomethemoglobin formation [16]. RBC indices including, MCV (fl/cell), MCH (pg/cell) and MCHC (g/dL) were calculated [16].

$$\mathrm{MCH(pg/cell)} = \frac{\mathrm{Hb} \times 10}{\mathrm{RBC}} \qquad \mathrm{MCV(fl/cell)} = \frac{\mathrm{Ht} \times 10}{\mathrm{RBC}}$$

$$\mathrm{MCHC(g/dL)} = \frac{\mathrm{Hb} \times 100}{\mathrm{Ht}}$$

Statistical Analysis

All results were recorded as the mean ± standard deviation (SD). Data normality was controlled by Shapiro-Wilk test. The treated groups were compared with the control groups and data were calculated using one-way analysis of variance (ANOVA) followed by Turkey's post hoc test by SPSS 16.0 software (Chicago, IL, USA) at $P<0.05$ level.

Ethical Considerations

This research was performed according to the convention of animal rights and approved by the Ethics Committee of Khorramshahr University of Marine Science and Technology. We tried to use fish without causing them unnecessary suffering if it could be avoided.

RESULTS

No mortality was found in control and experimental groups during the experiment. In addition, there was no significant difference in all factors between control groups throughout the experiment.

Changes in hematological parameters in control and experimental groups are shown in Figure. 1 and 2. The number of RBC and WBC, Hb content and Ht amount decreased significantly in Phe-exposed fish compared to control in all sampling d ($P<0.05$). However, there was no significant difference in the number of RBC and WBC between control and Phe-treated fish at the end of the experiment.

Moreover, there was no significant difference in Hb content between control and fish treated with 2 mg kg^{-1} of Phe at days 7 and 14 and fish treated with 20 mg kg^{-1} of Phe at day 14 compared to control. The number of RBC and WBC and percentage of Ht decreased dose dependently up to day 4 and Hb content decreased in fish exposed to Phe up to day 7. Then, these parameters increased up to the end of the experiment (day 14).

There was no significant difference in the mean values (±SD) of MCV and MCH between control and Phe-exposed fish in all sampling days. The mean value (±SD) of MCHC increased significantly in fish exposed to 2 and 40 mg/kg of Phe up to day 4 (P<0.05) compared to control fish and then, decreased up to the end of the experiment. There was no significant difference in the mean value of MCHC between fish treated with 20 mg/kg of Phe throughout the experiment. In addition, no significant difference was detected in the mean value of MCHC between control and Phe-exposed fish at days 7 and 14.

Differential count of WBC showed obvious changes in Phe-exposed fish compared to control. The number of neutrophils increased significantly after exposure to Phe, while the number of lymphocytes decreased significantly after exposure to Phe in comparison with control (Figure 2) (P<0.05). However, no significant difference was found in the number of neutrophils and lymphocytes between control and fish exposed to 2 mg/kg of Phe in all sampling days and fish exposed to 20 mg/ kg of Phe at days 1 and 14. In addition, there was no significant difference in the number of eosinophils and monocytes in Phe-exposed fish in all sampling days compared to control.

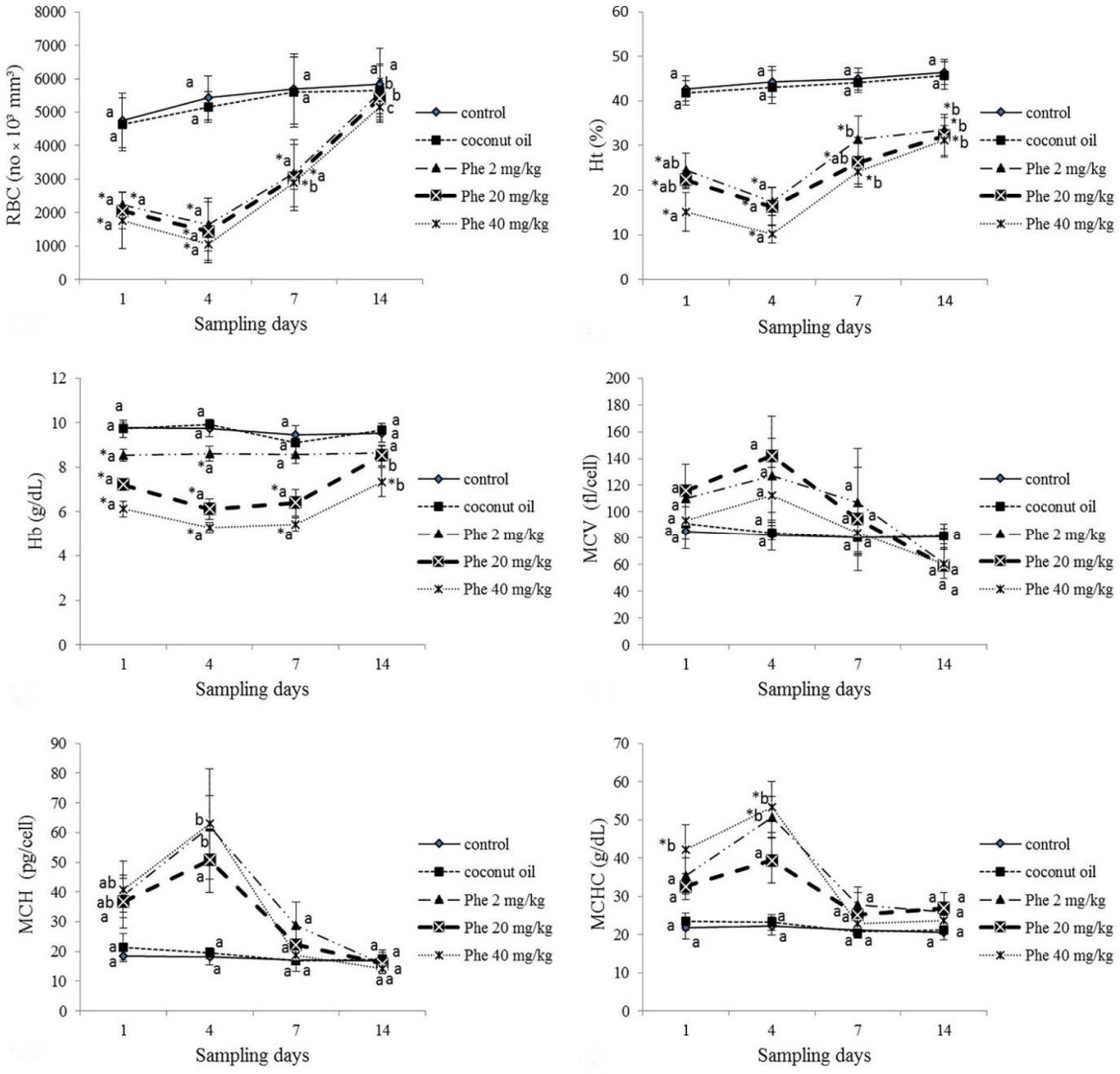

Figure 1. The alteration of hematological parameters in *A. latous* exposed to different concentrations of Phe (Mean±SD). Different letters show a significant difference between fish in different sampling days for the same groups. The star (*) indicates the significant difference between control and treated fish (P<0.05).

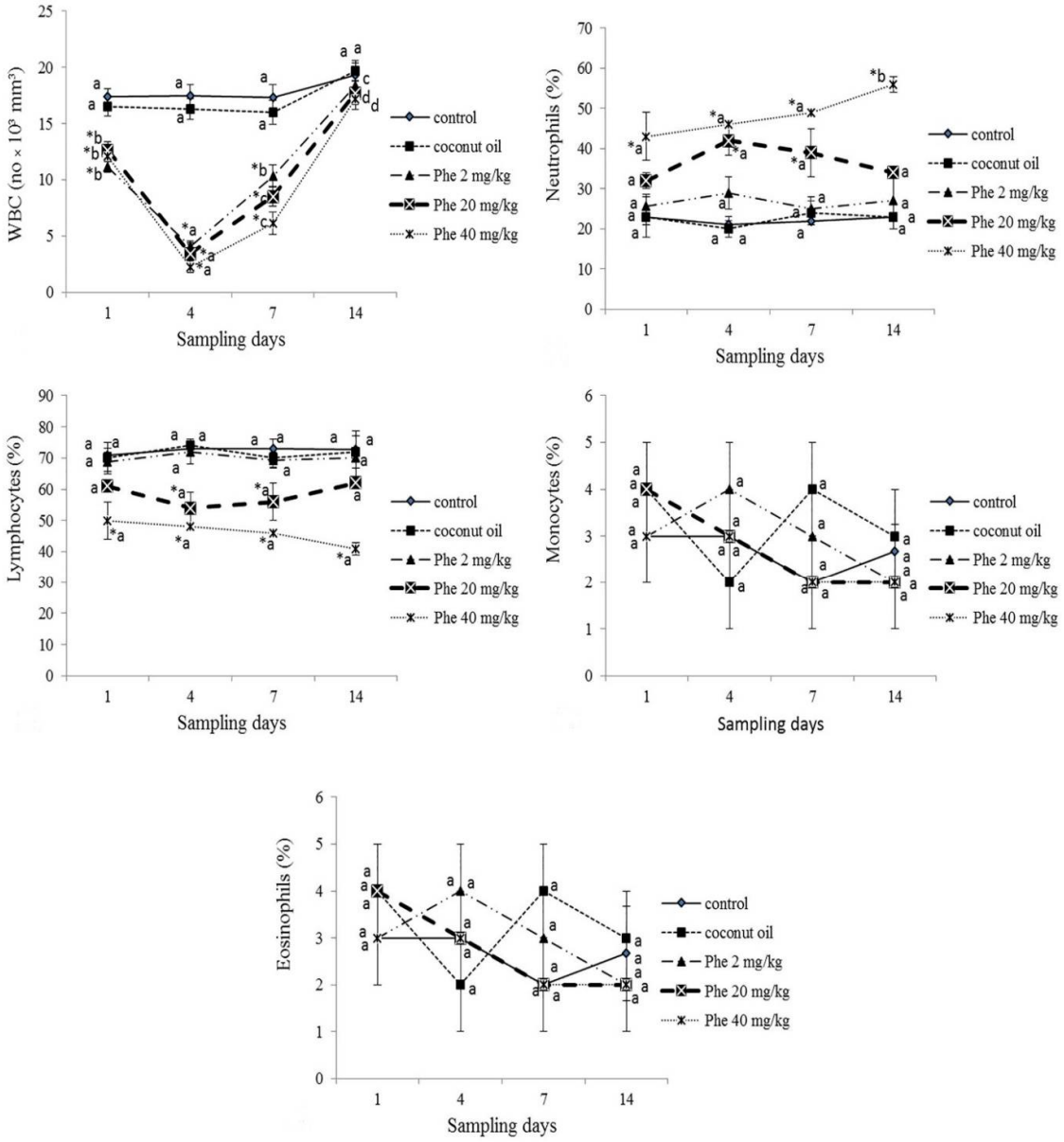

Figure 2. Changes in the number of white blood cells in *A. latous* exposed to different concentrations of Phe (Mean±SD). Different letters show a significant difference between fish in different sampling days for the same groups. The star (*) indicates the significant difference between control and treated fish (P<0.05).

DISCUSSION

The present study showed that the exposure to Phe decreased the amount of hematologic (including WBCs, RBCs, Ht, and Hb) in yellowfin seabream. These changes were similar to those observed in fish treated with nickel [17], nonylphenol [18] and crude oil [7]. The decrease in blood cells in Phe-exposed fish in the present study may be due to the migration of these cells into the damaged area, inability hematopoietic tissues to production and proliferate blood cells, internal hemorrhage and death of blood cells [19, 20]. The decrease in blood cells may affect other immune functions such as phagocytic activity and respiratory burst of blood cells [19], leading to immune system suppression and the increase of the fish susceptibility to diseases [21].

Decreased levels of Hb and Ht in animals exposed to xenobiotic might be due to erythrocyte destruction, irreparable damage of the gill morphology and function, dysfunction of enzymes related to methaemoglobin reduction in the blood, failure in erythrocyte synthesis in hematopoietic tissues and changes in erythrocyte plasma membrane because of acetylcholine hydrolysis in the body fluids by cholinesterase [22, 23]. Hb consists of protein and iron and the liver is the main

source of iron and the protein synthesis in organisms [23]. Then, pathological changes in the liver by xenobiotic may lead to decrease in Hb synthesis [23, 24]. On the other hand, pollutant exposure may cause serious structural and functional alterations such as the decrease in erythropoietin synthesis in the kidney that leads to the reduction of Hb production in hematopoietic organs [25]. These changes adversely affect oxygen supply to various organs and then result in slow metabolic rate and low energy production [23].

In the present study, levels of hematological factors increased 7 d after exposure, possibly due to Phe metabolism in tissues, especially in the liver. A half-life of 4 d for Phe was reported in zebrafish (*Brachydanio rerio*) [26].

According to our results, the amount of MHCH increased dose-dependently up to day 4 of experiment and then, decreased at the end of the experiment. Conversely, there was no significant effect on MCV and MCH values of fish. These results were in accordance with the previous study on silver carp (*Hypophthalmichthys molitrix*) exposed to diazinon [27], and diazinon-treated *Oncorhynchus mykiss* [28]. Changes hematological indices including MCV, MCH, and MCHC have been usually related to alterations in the shape, size and hemoglobin content of erythrocytes [29]. The increase in MCHC value represents large size RBCs with less hemoglobin content [30].

Our results showed the dose-dependent increase and decrease in neutrophil and lymphocyte counts in Phe-exposed yellowfin seabream, respectively. An increase in neutrophils and a decrease in lymphocytes of great sturgeon were exposed to diesel oil [31]. The neutrophilia, an increase in the number of neutrophils, might be due to increase in phagocytosis of damaged cells. Lymphopenia, a decrease in the number of lymphocytes, presumably resulted from the disintegration of the cell membrane [6]. The differentiation of immune cells in hemopoietic organs was suppressed in common carp with high plasma level of cortisol; however, myeloid cell production increased in these fish [32]. It was possible because of the decrease in the cytokine synthesis responsible for regulating the differentiation processes of lymphomyeloid cells [32].

CONCLUSION

Phe in the assayed concentrations caused changes in the hematological parameters of yellowfin seabream that resulted in a decrease in the defense mechanism of fish. Assessment of these indices could provide a useful indicator of Phe toxicity on marine fish.

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