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Water-borne kerosene as a stressor in the freshwater air-breathing fish, *Anabas testudineus* Bloch: Effects on interrenal and thyroidal activities

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Abstract

The effects of water-soluble fraction of kerosene on thyroidal and interrenal activities were investigated in the climbing perch, *Anabas testudineus.* The fish exposed to the selected concentrations of kerosene (3.33 and 6.66 ml/L) for 48 h showed elevated plasma glucose ($P<0.05$)and plasma urea ($P<0.01$). The plasma T_4 ($P<0.01$) increased and the plasma T_3 ($P<0.05$) decreased in the kerosene-exposed fish and these changes were reversed in the fish kept for 96 h recovery after 48 h kerosene exposure. The plasma cortisol concentration increased ($P<0.05$) after kerosene treatment and its level increased further during recovery. Our results demonstrate that water-borne kerosene activates thyroid and interrenal axes. Evidence is also presented that cortisol is involved in the post-stress recovery phase of the kerosene-exposed climbing perch, thus support the hypothesis that cortisol is involved in the regulation of both stress induction and stress tolerance in fish.

Keywords - *Anabas testudineus , Cortisol, Kerosene ,*T_3 *and*T_4

INTRODUCTION

The aquatic ecosystem is continuously being contaminated with untreated toxic chemicals from domestic, industrial and agricultural activities. When fish is challenged by stressors, a number of physiologic responses of reactive nature are engaged in an attempt to counteract the threat and to recover from the disturbed physiologic homeostasis. The stress response thus involves primary endocrine responses (secretion of ACTH, cortisol, and catecholamines) and secondary responses including an increase in plasma glucose, and tertiary or whole organism responses (Wendelaar Bonga, 1997; Barton, 2002).

As the primary link between the organism and the environment bringing out physiologic responses, the neuroendocrine system is critical in osmoregulatory adaptations (McCormick, 2001). Cortisol plays an important role in the ionoregulatory physiology of freshwater fish and modulate the ion transporting enzymes related to hypoosmoregulation, namely the H^+-ATPase (Lin and Randall, 1993) and the Na^+, K^+-ATPase (McCormick, 1995) with associated effects on branchial ionic influx in freshwater fish (Laurent and Perry, 1990; Perry and Laurenmt, 1993). In addition, cortisol promotes protein degradation and glycogen deposition in the liver, and suppresses the immune system, sex steroid secretion, and gonad maturation in stressed fishes (Stolte et al., 2008).

Like cortisol, thyroid hormones (THs) are involved in several physiologic processes of fish including metabolism and osmoregulation (Peter et al., 2000; Gavlik et al., 2002; Oommen et al., 2007). Many environmental chemicals have been known for its potential disrupting capacity on TH function particularly in developing embryos and juveniles of fishes (Yamano, 2005). However, the thyroidal control of stress response, especially on metabolic aspects of fish, has received little attention (Wendelaar Bonga, 1997; Peter et al., 2007).

In recent years much attention has been paid to the deleterious effects of petroleum spillage and the environment. These are ubiquitous environmental pollutants which can be environmentally dangerous (Hodson et al., 1997). Gurung et al., 2021 reported that crude oil exposure during organogenesis induced greater teratogenic effects on halibut, disturbances cardiovascular flow of embryonic Gulf killifish. Although the more toxic compounds in kerosene are volatile, fish can quickly absorb part of the WSF with adverse consequences to biological organization (Collier et al.,1996). Exposure to crude petroleum has shown to induce toxic symptoms in experimental animals (Akaishi et al., 2004; Khatun et al., 2021). Kerosene as one of the intermediate distillate products is well known source of harmful compounds (Peter et al., 2007).

The exposure of *Oreochromis niloticus* fingerlings to water soluble fraction of diesel fuel showed mortality even at low concentrations (Dede and Kaglo, 2001). Endocrine destruction has been found in many species of fishes including the perch *Percafluviatalia* and the roach *Rutilusrutilus* (Noaksson et al., 2003). The long-term exposure of rats to petroleum samples could increase anaemia through the reduction in hemoglobin content and pack cell volume levels (Dede et al., 2002).Exposure of *Hoplosternumlittorale*to Urucu crude oil affects gas exchange and ion regulation (Brauner et al., 1999).

Toxic compounds in crude oil lead to acute and chronic toxicity of aquatic animals (Sorhus et al., 2021). An elevated concentration of plasma cortisol has been demonstrated in some species (Thomas and Rice, 1987), indicating a corticosteroid stress response (Brown, 1993; Pickering, 1993). Changes in the cortisol levels after WSF of kerosene exposures have been shown to be dependent on dose and time in several teleost fish species (Alkindi et al., 1996;

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Brauner et al., 1999). Fish gills play an important role in ion regulation, gas exchange, acid-base balance and nitrogenous waste excretion and forms an interface of fish with its environment.

Materials and method

Adult climbing perch, *Anabas testudineus* (35 ± 5 g body mass) collected from a local supplier were maintained in the laboratory in 100 L glass tanks. Fish were acclimated to tap water at 28 ± 1^0Cunder natural photoperiod (12L/12D) for two weeks prior to the experiment. Fish were fed with commercial feed at the rate of 1.5% of body mass per day.

Experimental Protocol

Twenty-four laboratory acclimated fish were grouped into four of six each and kept in 60 L glass tanks. The untreated fish served as the control. The fish groups were exposed to kerosene 3.33 ml/L and 6.66 ml/L respectively for 48 h. A group of fish was first exposed to 6.66 ml/L kerosene for 48 h and then kept for 96 h recovery in clean freshwater.

Sampling and analysis - All the fish groups were sampled on the same day. Soon after the treatment, all the fish were anaesthetized briefly in 0.1% 2-phenoxyethanol (Sigma, St. Louis) solution and the blood was drawn from the caudal vessels, using a heparinised syringe. The heparinised blood was centrifuged at 10,000 g for 5 min at 4^0C and the plasma was separated and stored at -20°C until analysed.

Plasma glucose and urea

The concentration of plasma glucose was determined colorimetrically using GOD/ POD test kits (Span Diagnostics Ltd., NewDelhi). The level of plasma urea was estimated colorimetrically using standard method of DAM kit (Span Diagnostics Ltd., New Delhi).

Plasma cortisol, T_3 and T_4

Cortisol concentrations in the plasma samples were measured by competitive immunoenzymatic assay (DiaMetra, Foligno, Italy) and the values were expressed as ng ml^{-1}. The sensitivity and reliability of this method was examined and the values were comparable to RIA mothod reported earlier (Peter and Peter, 2007; Peter, 2007). In brief, plasma was deprotinized with ethanol phosphate buffer (1:9). Plate wells coated with mouse-anti-rabbit IgG were treated with standards and diluted samples (20 µl) and incubated with 200 µl cortisol-HRP conjugate at 37°C for 1 hour. After washing, 100 µl TMB-H_2O_2 was added and incubated at 20°C for 15 min in the dark. Absorbance was recorded on a plate reader (Span Autoreader 4011, New Delhi) at 450 nm after adding 0.15 moles sulphuric acid. The intra-assay coefficient of variation was 3%, and the inter-assay coefficient of variation was 9.32%.

Plasma T_3 and T_4 concentrations were measured by microwell enzyme immunoassay (EIA: magnetic solid phase) with kits (Syntron Bioresearch Inc, Carlsbad, California, Catalog # 3810-96 for T_3 and Catalog # 2210-96 for T_4). The sensitivity of this method was checked by comparison of results from RIA based on competitive binding of ^{125}I-labelled T_3 or T_4 (Peter et al., 2000) with the EIA results (Peter et al., 2007). Briefly, the anti-T_4 (goat anti-mouse IgG) coated wells were treated with 50 µl standards, control and samples. After adding 100 µl T_4-HRP conjugate the wells were incubated at 37°C for 1 hour. After washing, 50 µl of 0.05 M acetate buffer and TMB were added and incubated at 20°C for 15 min. Absorbance was read at 450nm after stopping the reaction with 1N HCl. The intra-assay coefficient of variation was 7.2 and inter-assay coefficient of variation was 9.0. Similarly, plasma T_3 was quantified as described for T_4 but used anti-T_3-antibody (goat anti-mouse IgG) and the T_3-conjugate HRP. The intra-assay coefficient of variation was 4.4 and inter-assay coefficient of variation was 8.5.

Statistics

Data were collected from six animals in each group and the statistical analysis was done using graphpad software. Data were analyzed using one-way analysis of variance (ANOVA) followed by SNK comparison test to find out whether there is any significant difference existed between the treatments. Significant differences between groups were accepted if P <0.05 and values were depicted as mean ± standard error of six fish.

RESULTS

Plasma glucose and urea

The plasma glucose concentration increased significantly (P<0.05) after the high dose (6.66 ml/L) of kerosene treatment but showed recovery (P<0.01) in the fish kept for 96 h in the clean water. The plasma urea content increased significantly after the low (P<0.05) and the high (P<0.01) dose of kerosene treatment (Table. 1).

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Plasma T_3 and T_4 andCortisol

The plasma T_3 showed a significant decrease after the high (*P*<0.05) dose of kerosene exposure whereas, plasma T_4 increased significantly (*P*<0.01) after the high dose of kerosene treatment (Fig. 2).The plasma cortisol increased significantly (*P*<0.05) after kerosene exposure and its level returned to basal level in the fish kept for recovery(Fig. 1).

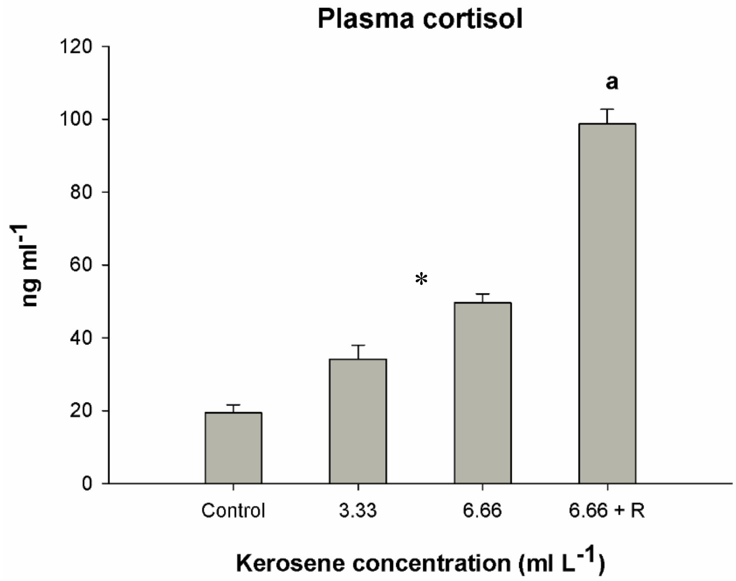

Fig.1 Plasma cortisol (ng ml^{-1}) in freshwater climbing perch after kerosene exposure for 48 h with or without 96 h recovery (R). Each column represents mean ± SEM of six fish. Statistical differences between fish groups were assessed after SNK test. **P*<0.05 compared with control a: *P*<0.05 compared with 6.66 ml L^{-1} kerosene-treated fish.

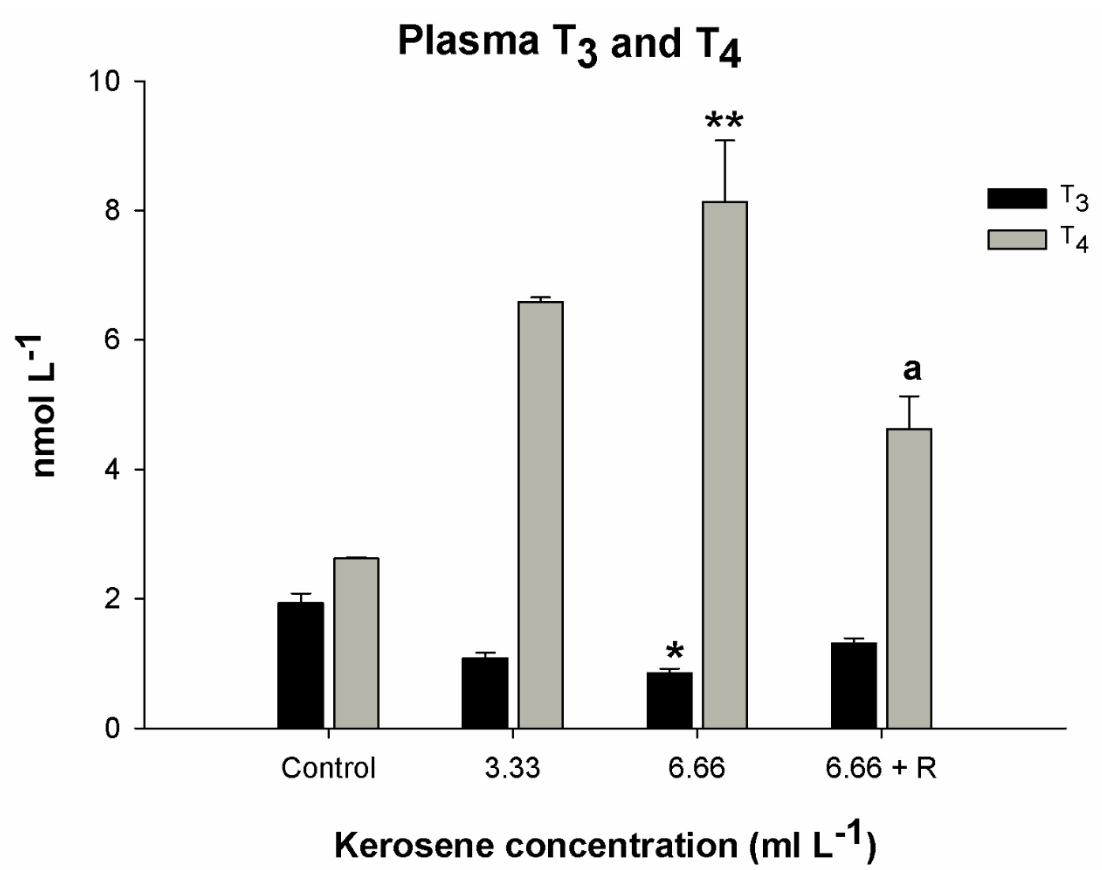

Fig. 2 Plasma T_3 and T_4 levels (nmol L^{-1}) in freshwater climbing perch after kerosene exposure for 48 h with or without 96 h recovery (R). Each column represents mean ± SEM of six fish. Statistical differences between fish groups were

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assessed after SNK test. * P<0.05, ** P<0.01 compared with control, a: P<0.05 compared with 6.66 ml L^{-1} kerosene-treated fish.

Table.1 *Plasma glucose (mg dL^{-1}) and urea (mg dL^{-1}) in freshwater climbing perch after kerosene exposure for 48 h with or without 96 h recovery (R).*

	Control	3.33 ml L^{-1}	6.66 ml L^{-1}	6.66 ml L^{-1} + R
Glucose	73.17 ± 0.84	83.04 ± 7.35	112.68 ± 4.91*	68.07 ± 4.13^{b}
Urea	6.91 ± 0.39	11.04 ± 0.29*	14.46 ± 2.51**	11.77 ± 0.76

Values are mean ± SEM of six fish
* P<0.05, **P<0.01 compared with control
b: P<0.01 compared with 6.66 ml L^{-1} kerosene-treated fish.

DISCUSSION

Our study clearly demonstrates that water-borne kerosene disturbs the hydromineral homeostasis and activates the interrenal and thyroidal axis in the climbing perch. The toxicity of petroleum is mostly related to its water soluble fraction (WSF) that contains, among other organic and inorganic compounds, the short chain polycyclic aromatic hydrocarbons (PAHs). PAHs are ubiquitous environmentalpollutants and can be environmentally dangerous even at low concentrations (Hodson et al., 1997). Several behavioural, physiologic and biochemical responses are expected from the fish exposed to sub lethal levels of WSF (Stott, 1980; Val, 1999). Freitas et al., 2020 have reported the effect of diesel and different lubricant oil on oxidative stress, histopathological alteration of tissues and growth.

The elevated plasma glucose, an indicator of sympathetic activation during stress (Randall and Perry, 1992), associated with an increased plasma cortisol; clearly indicate that kerosene induces a classic stress response in climbing perch. This implies an increased energy mobilization by kerosene in our fish. Glycogenolysis and subsequent hyperglycemia are the well documented responses in fish to various pollutants, revealing a toxic stress condition in fish (Peter et al., 2004, 2007). The hyperglycemic effect of kerosene is consistent with the hyperglycemic effect of the water soluble fraction of crude oil reported for European flounder (Alkindi et al., 1996). The hyperglycaemic effects of toxic and non-toxic stressors have been demonstrated in many fishes (Peter et al., 2004; Teles et al., 2005). Similarly, elevated plasma urea after kerosene exposure indicates an increased nitrogen metabolism as evidences have been presented that amino acids and aminotransferases are involved in the production of urea (Ray and Medda, 1976), and the liver is an important ureogenic tissue (Walsh and Mommsen, 2001). Changes in the plasma urea levels by other toxicants have also been reported in this fish (Leji et al., 2007).

The elevated cortisol level after kerosene exposure indicates a cortisol-driven stress response as has been reported earlier (Alkindi et al., 1996; Brauner et al., 1999; Kennedy and Farrell, 2005). Kerosene treatment produced a stress related increment of plasma cortisol in our study. Recent studies have demonstrated that cortisol has a direct effect on carbohydrate metabolism, stimulating glycogenolysis and gluconeogenesis, but that it also interacts with catecholamines which may exert dominant effects in the immediate stages of stress (Vijayan and Moon, 1994). The increased glycogenolysis and gluconeogenesis in liver associated with an increased plasma glucose and cortisol have been demonstrated in rainbow trout (*Oncorhynchus mykiss*) treated with beta-naphthoflavoneand benzo(a)pyrene (Tintos et al., 2008). The stress response to chemical threats is dependent on an intact hypothalamic-pituitary-adrenal axis, which also may be a target to these chemicals. Ranch mink (*Mustela vison*) showed development of adrenal hypertrophy after chronic oral exposure to low concentrations of bunker C fuel oil (Mohr et al., 2008).

Kerosene exposure showed a decline in the plasma T_3 but elevated the plasma T_4 in our fish. This implies that kerosene exposure demands an activated thyroid. Although cortico steroid hormones have been reported to depress thyroid function (Redding et al., 1986; Brown et al., 1991), a positive involvement of thyroid in energy metabolism during kerosene exposure has been shown earlier in climbing perch (Peter et al., 2007), where they reported that the fish thyroid responds to the action of petroleum products and influences the metabolic homeostasis of this air-breathing fish. Similarly, a number of studies have demonstrated that chemical stressors influence the interrenal and thyroid functions in fish. For example, exposure of catfishes *Heteropneustesfossilis* and *Clariasbatrachus,* to malathion and endosulfan caused changes in the circulating THs (Yadav and Singh, 1986; Sinha et al., 1991). A decrease in T_3 level has been reported in rainbow trout exposed to acidic water (Brown et al., 1990) and to starvation (Oommen and Matty, 1991). In European flounder, exposure to WSF of crude oil declined plasma T_3 and T_4 concentrations (Alkindi et al., 1996). The elevated plasma cortisol and the reduced plasma T_3 in response to kerosene exposure highlight the involvement of interrenal and thyroid in the stress tolerance mechanisms in fish as has been reported earlier (Peter and Peter, 2007).

Overall, our results demonstrate that water-borne kerosene activates thyroid and interrenal axis and produces disturbances in the hydromineral homeostasis in our fish as a part of the integrated stress response. The activated interrenal axis in the stressed and the post–stress recovered fish provide evidence that cortisol is involved in the regulation of both stress induction and stress tolerance in fish.

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