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Phenol Toxicity Affected *Tilipia nilotica* Fish.

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ABSTRACT

The influence of dietary phenol on immunity, and hormonal profile was studied in fish. The results revealed that, treatment of *Tilipia Nilotica* with 20 mg/l phenol for 21 days there was an elevation in cortisol hormone level. Our findings suggest that phenol may induce an immunosuppressive effect on humoral immune response of *Tilipia Nilotica* which was suggested by reduction of immunoglobulin.
Keywords: Phenol; Endocrine; *Tilipia Nilotica*

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INTRODUCTION

Among the different types of pollutants one of the most relevant to aquatic ecotoxicology. Exposure to pollutant and derivatives can induce a Varity of toxic symptoms in experimental animals. Phenols can act as a mediator in free radical generation in fish. Studies the goldfish Carassiusaurathus has shown an increase in antioxidant defenses in animals after exposure to different concentrations of the water-soluble fraction for various experimental times. Other studies have also indicated that the exposure of fish to water-soluble fraction causes different effects in cortisol plasma concentration suggesting that these contaminants might interfere in fish stress response. Phenols are discharged into water from the effluents of a variety of industries such as coal refineries, phenol manufacturing, pharmaceuticals, industries of resin, paint, dyeing, textile, leather, petrochemical, and pulp mill. Natural processes such as the decomposition of plant matter also contribute to phenol accumulations in the aquatic environment (BuBuikema *et al*., 1979 and Ali *et al*., 2011). Phenols are of growing concern due to their high persistent and toxicity in the aquatic environment in addition to the difficulty in detecting them given their lack of taste and odor (Tilak *et al*., 2007).Unfortunately, there is a lack of information regarding phenol pollution and its effect in the Egyptian aquatic environment. The record level of phenol in Egyptian waste water was 0.05 ppm (Nazih *et al.,* 2008). *C. gariepinus* was extensively used as fish model by many scientists to monitor microbial, pathological or environmental studies (Ibrahem *et al.,* 2011). Unfortunately, there is a lack of information about the toxicity and pathological consequences in *C. gariepinus* exposed to phenol (Ibrahem, 2011). Controversy, Stich (1991) reported that phenol may act as free radical scavengers and prevent genetic damage caused by other agents. They have a high bioaccumulation rate along the food chain due to its lipophilicity. Thus phenol pollution presents a threat against natural environment and also to human health (Nassr-Allah 2007). When the phenol is present in the aquatic environment, fish food consumption, mean weight and fertility are significantly reduced (Saha et al., 1991). For these reasons, phenol intoxication must be taken in consideration in the fish farming systems and also in natural aquatic habitat.

MATERIAL AND METHODS

Setting and methods

One hundred and twenty *Tilipia Nilotica* were used in the present study. Their live body weight was averaged 50gm. The fish were healthy and clinically free from external and internal parasites. They were maintained in tanks containing well aerated water at atmospheric temperature for two weeks before the experiments began. Fish were randomly distributed into two groups;, each of 60 fish. Group one not given any treatment and considered a control group, the second group treated with sublethal dose of phenol at a dose level of 20 mg/L (Verma, *et al*., 1980). Analytical grade phenol, C6H5OH (purity 99%; E. Merck, made in Germany) was used as test chemical. Test fish were not fed from 2 d prior to the end of the experiments. The test medium was replaced in both control and experimental tanks. The experimental fish were fed on ration composed of 16.3% crude protein, 2.5% crude fat and 14% crude fiber, the digestible energy was 26% cal/kg. The diet contained feed additives which included minerals, vitamins and amino acids. Body weight measured every month for four month. Serum samples were collected 3 weeks (21 days) interval and Sera were frozen at -200C for later analysis. Serum cortisol, IgM, T4, and insulin were determined using kits. The serum IgM was measured according to Fuda *et al*. (1991). Antisera for *Tilipia Nilotica* were prepared by immunizing rabbits with catfish antigen as described by Hara (1976).

EIISA assay procedure

Assays were carried out in 96-well polystyrene ELISA microtiter plates (Titertex, Horsham, PA). The micortiter plates were coated with rabbit antitilapia IgM which was fractionated by DE-52 according to the method described by Bagee *et al*. (1993).

Incubation of samples and standards

After washing as described above 100 µL of sample and standard were placed into the appropriate wells in the microtiter plates and incubated at room temperature. Incubation with peroxidase labeled antibody. After washings as described above, each well received 150µ1 of peroxidase labeled antibody 1: 1600 in PBS-BSA, followed by incubation for 12 hrs at room temperature.

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Enzymatic color reaction

The plates were washed as described above and O-phenylenediamine (3mg/ml O.1M citric acid-phosphate buffer (pH 5.0) containing 0.02% H_20_2) were added to each well for enzymatic color reaction. The reaction was stopped after 30min at room temperature by adding 100ul of 4N HCL. The results were recorded at absorbance of 492 nm. Double antibody sandwich Elisa according to the method of Matsubara *et al*. (1985) was used for determination of IgM. Cortisol was estimated using radio immunoassay technique according to the method of Wedemyer (1970) and Pickering and Potinger (1983).

Statistical analysis

The difference between the groups were calculated according to Snedecor and Cochran (1967) by t-test.

RESULTS

Table 1 shows the influence of phenol on IgM. Highly significant decrease of IgM levels was detected in treated group with phenol. Table 2 shows the serum hormonal changes in infected fish treated with phenol. The results revealed significant elevation of cortisol level was observed.

Table 1: Effect of Phenol toxicity (20mg/l) on IgM level of *Tilipia Nilotica*

Groups/Duration (Week)	1 week	2 week	3 week
Control	0.42 ± 0.24	2.2 ± 0.76	3.6 ± 0.54
Phenol (20mg/l)	1.95 ± 0.13	3.6 ± 0.10	6.4 ± 0.42

Table 2: Effect of phenol toxicity (20mg/l) on Cortisol µg/dl of *Tilipia Nilotica*

Groups	Cortisol µg/dl		
	1 week	2 week	3 week
Control	0.562 ± 0.23	0.543 ± 0.42	0.592 ±0.56
Phenol (12mg/l)	1.23 ± 0.70	1.96 ± 0.62	2.21 ± 0.94

DISCUSSION

IgM level was determined to find out information about *Tilipia Nilotica* immune system, which was previously investigated in different species by many authors as Matsubara *et al.* (1985) and Fuda *et al*. (1991). In this work the purified IgM revealed a single perception in this work reacted against specific polyvalent antiserum to catfish IgM a similar result was obtained by Bagee *et al*. (1993). They found that chum salmon (IgM) was detected by specific anti (IgM) antibodies. While the lower limit was 5 mg/ml reported, by Fuda (1991). There is a significant decrease in IgM level in fish treated with phenol in comparison with control group. Anderson *et al*. (1982) found a relation between cortisol and IgM as when cortisol increased IgM decreased. The significant increase of cortisol level in intoxicated group with phenol could be attributed to stress factors and the intoxication has examined response of fish to stress factors e.g. crowding, continuous handling infection. Wedemyer (1970); Strange (1978); Barton *et al*. (1980) and John *et al*. (1994), reported that the elevation of cortisol in phenol treated fish may be attributed to intoxication, and continuous handling of fish. These observations emphasizes the extreme care needed during design and analysis of experiments, involving the (HPI) axis of test fish due to extremely sensitive HPI axis. Similar results were reported by Pickering and Pottinger (1983). The perfuse skin mucous secretion was prominent in phenol intoxicated catfish. This can be explained by the fact that skin in among the first to be in close contact with the dissolved pollutants. Hence, reactions in the skin cells are spontaneous as a protection mechanism through increasing level of mucous secretion over the body surface, forming a barrier between the body and the toxic medium, minimizing its irritation effect, thus, scavenge or even eliminates toxicants through the epidermal mucous (Chebbi and David, 2010). Nervous manifestation; skin expressed perfuse mucous, black patches with skin erosion and ulceration in the later stages. All observation were correlated to the time and dose exposure ((Ibrahem, 2011). White or cream-colored nodules that may exceed several centimeters in diameter. That may

or may not bulges slightly above the liver capsule and are difficult to detect from external examination when they are embedded within the liver. Early stage neoplasms of hepatocellular origin may be similar to the bile duct tumors in gross appearances *i.e*. as white or crea-colored foci, or they may appear as pale foci just liver capsule. More advanced tumors may appear as white gray, cream-colored or reddish-tan colored masses bulging from, or as nodules within the liver tissues. In conclusions phenol reduces of the hormonal immune response as detected by decrease of IgM level and cortisol elevation. Suppress IgM, Thyroxin *(T4)* hormone and insulin levels.

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