Dose Phenol Toxicity Affected Endocrine Status in African Catfish (*Clarias gariepinus*)

Mona S. Zaki[1], Nabila El-Batrawy[2] and Nadia M. Taha[3]

[1]Department of Hydrobiology, National Research Center Dokki, Cairo, Egypt
[2]Department of Microbiology, Veterinary, Zagazig University, Zagazig, Egypt
[3]Department of Physiology, Faculty of Veterinary, Cairo University, Cairo, Egypt
dr_mona_zaki@yahoo.co.uk

Abstract: The influence of dietary phenol on immunity, and hormonal profile was studied in catfish. The results revealed that, treatment of Catfish (*Clarias gariepinus*) with 12mg/l phenol for 3 months decreased IgM, Insulin, Thyroxin, however there was elevation in cortisol hormone level. Phenol may induce an immunosuppressive effect on humoral immune response of African Catfish which was suggested by reduction of immunoglobulin.
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1. Introduction:

Phenol and phenolic compounds are examples of toxic chemicals acts as endocrine disruptors; which mimic or antagonize hormones and disrupt the endocrine system. It is also has great potential for compromising the immune system and increases susceptibility of fish to secondary infections (Writer *et al*., 2010).

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)

2. Material and Methods

One hundred and twenty African Catfish **(***Clarias gariepinus***)** were used in the present study. Their live body weight was averaged 37.5 grams. 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 12mg/L (Verma, *et al*., 1980).

Analytical grade phenol, C_6H_5OH (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.

Samples:

Serum samples were collected 4 times at one month interval and Sera were frozen at -20^{0}C for later analysis. Serum cortisol, IgM, T4, and insulin were determined using kits.

1 - IgM determination:

The serum IgM was measured according to Fuda *et al*. (1991).

1-a. Preparation of antisera:

Antisera for catfish were prepared by immunizing rabbits with catfish antigen as described by Hara (1976).

Catfish IgM antibody:

The procedure for labeling antibody fragment with enzyme was performed according to the method of Bagee *et al*. (1993).

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.

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 492nm.

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).

Serum thyroxin was estimated using radioimmunoassay (RIA) using coat (A) count provide by diagnostic product corporation Los Angelos U.S.A. (Defetoff, 1979).

Insulin was determined by RIA according to the method described by Sundly (1991).

Statistical analysis:

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

3. Results

Table (1) showed the influence of phenol on IgM. Highly significant decrease of IgM levels was detected in treated group with phenol.

Table (2) showed the serum hormonal changes in infected fish treated with phenol. The results revealed decrease level of insulin, and thyroxin while a highly significant elevation of cortisol level was observed

Table (1): Effect of Phenol toxicity (12mg/l) on IgM level of Catfish (*Clarias gariepinus*)

Groups/Duration (months)	1 Month	2 Months	3 Months
Control	1.84± 0.72	1.80± 0.45	1.23± 0.40**
Phenol (12mg/l)	1.20± 0.14	1.14± 0.19**	0.98**± 0.84

**P<0.01 M Month Number of catfish each group 60

Table (2): Effect of phenol toxicity(12mg/l) on some Hormonal profile in Catfish (*Clarias gariepinus*)

	Insulin µg/dl			Thyroxin			Cortisol µg/dl		
	1M	2M	3M	1M	2M	3M	1M	2M	3M
Control	10.1 ± 0.40	10.2 ± 1.2	10.00 ± 1.50	0.0780 ± 0.065	0. 750 ± 0.059	0.740 ± 0.054	0.764 ± 0.1286	0.752 ± 0.113	0.740 ±0.103
Phenol (12mg/l)	10.00* ± 0.16	15.00 ± 0.24	18.40 ± 4.8	0.0654 ± 0.072	0.0554* ± 0.081	0.0500* ±0.044	0.870 ± 0.65	0.945* ± 0.70	0.982** ± 0.84

*P<0.05 **P<0.01 M Month

4. Discussion

IgM level was determined to find out information about catfish 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 preciption 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).

Serum thyroxin (T4) concentrations in the serum of Cat fish species decreased in the intoxicated group. It has been shown that intoxication, and chronic stress in a marked long lasting depression of serum T_4 levels in catfish (Osborn *et al*., 1978 and Milne and Leatherland, 1980). The response of thyroid gland of tested catfish needs further investigation with particular attention to possible relationship between the HPI axis and pituitary thyroid axis. Mooreoud *et al*. (1977); Osborn *et al.* (1978) and Milne and Leatherland (1980) using histological approach concluded that cortisol reduced thyroidal activity in sock eye salmon. The significant decrease of insulin values may be attributed to phenol which may somehow reduce the metabolic activities in the phenol treated catfishes. The decrease in body weight was observed, while detectable agrees with Ostrowski (1984), Hilton *et al.* (1987) and Sundly *et al*. (1991) as they observed a detectable decrease in body weight of duck infected with phenol.

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)

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.

Corresponding author
Mona S. Zaki
Department of Hydrobiology, National Research Center Dokki, Cairo, Egypt
dr_mona_zaki@yahoo.co.uk

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