Phenol Toxicity Affecting Hematological Changes in Cat Fish (*Clarius lazera*)

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Abstract: Phenol and phenolic compounds are xenobiotics stressful environmental factors to which fish and animals are subjected to, and have become environmental problem due to anthropogenic impact on the environment The present study aimed to investigate the effect of phenol pollution on fish with special reference to the hematological, immunological, serum biochemical parameters, where fifty healthy *Clarius lazera* fish were divided into 3 groups. Fish of gp1 served as a control. Fish of gp. 2 & 3 were used for the determination of acute lethal concentration dose and the pathological effect of Phenol on the exposed fish. Blood samples were collected to obtain serum for biochemical studies and heparinized blood for hematological investigations. RBCs, Hb, HCt, and MCHC showed significant elevations, the serum GPT and GOT were increased significantly. L.D.H, glucose and cortisol were elevated, while serum cholesterol concentration was reduced significantly in high tem30^{o}C.
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1. Introduction

Fish plays an important role, not only in human food diets but also in animal and poultry rations. It is a palatabie and easily digested food which is rich in vitamins, calcium, phosphon.rs and iodine. In Egypt, fish is considered as a cheap food article if compared with other foods of animal origin. The flesh of healthy fish is considered as a marker for the natural aquatic environment (1-7).

Phenol and phenolic compounds are xenobiotics stressful environmental factors to which animals are subjected to serve animia due to phenol and have become environmental problem due to anthropogenic impact on the environment (8). They also are good research models of wide spread xenobiotics (9). Also, they are commonly present in industrial wastewaters and in non-specific pesticides, herbicides, bactericides and fungicides (10). Mukherjee *et al.* (11) reported that they are commonly found in the marine habitat and in fish tissues. Phenol induces toxic effects for fish health.

They induce genotoxic effect (12), carcinogenic effect (713), and immunotoxic effect (14).

Controversy, Stich (15) 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 (8 and 16). When the phenol is present in the aquatic environment, fish food consumption, mean weight and fertility are significantly reduced (17). For these reasons, phenol intoxication must be taken in consideration in the fish farming systems and also in natural aquatic habitat.

Fish metabolism was adversely affected by phenol (10 and 18). The phenol and its derivatives can alter protein metabolism by altering transamination rate of amino acids by enhancing the activity of aspartate aminotransferase (ASAT, EC 2.6.1.1) and alanine aminotransferase (ALAT, EC 2.6.1.2). Also, the carbohydrate metabolism was affected by phenol by altering the activity of lactate dehydrogenase (LDH, EC 1.1.1.27) thus, affecting the interconversion of lactate into puruvate (8). Gupta *et al* (10) recorded changed ASAT and ALAT activities in different fish tissues induced by phenolic compounds. The enzymes activities (ASAT or GOT and ALAT or GPT) catalyze the interconversion of amino acids and α-keto acids by transfer of amino groups. The ASAT catalyzes the transfer of this group from aspartate to α-ketoglutarate to form glutamate and oxaloacetate, while ALAT catalyzes the transfer of the amino group from alanine to α-ketoglutarate to form glutamate and puruvate (19). The measurement of transaminase activities in serum is frequently used as a diagnostic tool in human and animals (20 and 21). Damage to the liver, kidney and gills is evident from elevated transaminase activities (20).

The present study aimed to investigate the effect of phenol toxicity on fish with special reference to the haematological, immunological, serum biochemical parameters.

2. Material and Methods:

1- Fish:

Fifty healthy fish of both sexes and 150 ± 50 gm body weight, were obtained alive and transported immediately to the laboratory. They were kept in 5 glass aquaria (100 X 30 X 50 cm) that provided daily with a tap water and continuously with filtered air. The water temperature was adjusted at 15°C along the period of experiment using thermostatic heater. The fish were fed a balanced ration daily using the formula suggested by Ahmed and Matty (22). Fish were kept under observation for 2 weeks.

Fish were divided into 3 groups (gps). Fish of gp1 (10) served as a control with no treatment. Fish of gp. 2 & 3 (20, each) were used for the determination of acute lethal concentration dose (LD_{50} /72 hr, gp2) and to investigate the pathological effect of phenol on the exposed fish (gp3).

2-Experiments:

A. Determination of acute lethal concentration dose:

To determine lethal concentration dose, fish of gp. 2 were subdivided into 5 equal subgroups. Subgroup 1 served as a control. Other 4 subgroups exposed to 35, 75, 150 and 300 mg/L of phenol; respectively. Each dose was dissolved in the distal water of each aquarium. The number of dead fish was recorded within 72 hrs post-exposure and the acute lethal concentration dose was calculated according to the formula of Brown (23).

B- Long term exposure:

Fish of gp3 were exposed to 1/100 of LD_{50} /72 hr (1.5 mg/L) of phenol for 2 weeks according to *Taylor et al* (24). The excreta were removed regularly and the water was replaced within 4 days interval. Fish were kept under observation along the 14 days of exposure.

3- Sampling:

Blood sample were collected from the caudal vein after 3, 7, 14 days of exposure, part of blood was left to clot and then centrifuged at 3000 r.p.m. to obtain serum for biochemical studies, the other part was heparinized for hematological investigations using the methods of Drabkin, 1949 (25).

4-Haematological examinations:-

The erythrocytic indices (RBCs, Hb, HCt & MCHC) were estimated according to Schalm et al (26).

5-Serum biochemical analysis:

Kits Biomericux France were used for the determination of serum glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), lactate dehydragenase (LDH), alkaline phosphatase (AP), serum glucose, serum cholestrol, and total protein. Serum cortisol hormone was analyzed by means of a gemmacoat 125-cortisol radio-imumnassay kit (Diagnostic corporation, USA). Serum Ig M was also measured according to Fuda, et al (27).

6-Statistical analysis:

The obtained data were statistically analysed according to Snedecor and Cochran (28) by T test.

3. Results

A-Determination of acute lethal concentration dose:

Experiment 1 revealed that, the acute lethal concentration dose was 150 mg/L during the 1st 72 hrs post-exposure.

B- Long term exposure:

The effect of phenol exposure on RBC's count, Hb level, HCt and MCHC values of exposed fish were recorded in Table (1). Polycythemia was observed on the 14 day ($p<0.01$). Blood Hb, HCt, and MCHC showed a significant elevation by 14 days of experiment.

Table (2) revealed the changes of some biochemical constituents in the blood of fish due to phenol exposure. The obtained data revealed that serum GPT activity increased significantly by 14 days of exposure. A significant elevation in serum GOT activity was also observed on the 14th day ($p<0.01$). L.D.H serum activity was elevated along the whole period of experiment especially on the day 14th. Hyperglycemia was constant findings from the beginning of the experiment until the end of the experiment. Serum cholesterol concentration was increased, on the 3rd day and the 7thday and was reduced significantly, on the day 14thday. Cortisol hormone was elevated along the whole period of experiment especially on day 14th.

4. Discussion:

The aquatic environment of the River Nile subjected to many stressful factors, phenol and phenolic derivatives are one of the serious pollutants which cause serve anemia in fish. This observed hepatomegaly may partially reflect the enhancement of the liver size due to destructive changes. Barse *et al.* (21) reported elevated HSI values of *Cyprinus carpio* subjected to 4-*tert*-butylphenol.

Regarding the impact of phenol on the

hematological profile of fish polycythemia accompanied by elevated hemoglobin level, HCt value and MCHC were observed. Similar findings were reported by Mckim et al *(*29), Hilmy et al (30) and Taylor et al *(*24) recorded polycythemia in *rasy barb*. But in contrary to our finding Hb level and MCHC were reduced in *Clarias lazera* exposed to copper (31). The increased RBCs count may be due to stimulation of erythropoietin by elevated demands for O_2 or Co_2 transport as a result of increased metabolic activity or distruction of gill membranes causing faulty gaseous exchange. The increase Hb content could be explained as a process where the body tries to replace the oxidized denatured Hb (32). The increase of HCt value and MCHC may be attributed to swelling of RBCs due to increased Co_2 in blood, hypoxia or stressful procedures (33 and 34).

Exposure of fish to sublethal concentration (1.5 mg/L) of phenol for 14 days resulted in a marked increase in the activities of serum GPT, GOT, LDH and ALP. The present findings agree with our microscopic findings, which revealed a marked degeneration and necrosis of hepatocytes as the elevation in transaminases activities may be attributed to the liver injury (35).

Serum cholesterol level, in the present study, showed a significant reduction that could be due to greater level of utilization of cholesterol during corticosteroidogenesis, as it is the precursor for steroid hormones (36). In addition, they reported a rise in the blood protein resulted in a high density of lipoprotein in the serum and was suggested to be the cause of hypocholesterolemia in exposed fish.

Our results showed similar findings as that of Gill et al, (37) and *Snieszko* (38), who reported that, exposure of fish to phenol had no significant increase on blood glucose of *salmo gairnei*. The blood glucose level reflected the changes in carbohydrate metabolism under hypoxia and stress conditions. Rise of glucose level indicated the presence of stressful stimuli eliciting rapid secretion of both glucocorticoids and catecholamines from the adrenal tissue and accompined by cortisol elevation (39). Concerning serum protein level, a significant increase was noted 14 days postexposure to phenol. The elevated protein concentration may be due to the induction of protein synthesis in liver.

The serum Ig. M was determined to find out information about fish immune system which was previously investigated in different species by many authors as *Fuda et al,* (27) *O`Neill*(40), in this work, the purified Ig. M was revealed a single perception against specific polyvalent antiserum to fish Ig, similar results was obtained by Bagee et al. (41) who found that, *Coho salmon* Ig was detected by specific anti Ig 14. Our study revealed a significant decrease in Ig. M level in fish exposed to pollution if compared within control groups. *Anderson et al.* (42) found a relation between cortisol and IgM as when cortisol increased IgM decrease. The significant increase in cortisol level in fish exposed to phenol could be attributed to stress factors and the intoxication of fish (43).

We can conclude the fish exposed to phenol cause serve anemia and suppress immunity in exposed fish

Table 1: Effect of phenol on some haematological parameters in *Clarius lazera* along the period of experiment (Mean+S.E.)

Parameter	3days		7days		14days	
	Control	Exp.	Control	Exp.	Control	Exp.
RBCs $10^6/mm^3$	3.3 ±0.22	3.3± 0.51	3.2 ±0.30	4.4 ± 0.74	3.6± 0.68	4.7± 0.68*
HB g/dl	7.40 ±0.52	8.2 ±0.10	7.4±0.26	8.7±0.8	7.2±0.33	8.4±0.74**
H.Ct%	18.70±1.30	21.9±1.34	18.95±0.19	26.4±1.44	22.7±2.64	28.7±1.84**
MCHc%	32.70±1.80	33.8±1.30	33.52±0.84	36.52±1.36	32.3±1.52	42.6±1.27**

Exp: experimental * Significant at p<0.01. ** Non-significant

Table 2: Effect of phenol on the serum biochemical parameters *in Clarius lazera* along the period of experiment (Mean+S.E.)

Parameter	3days		7days		14days	
	Control	Exp.	Control	Exp.	Control	Exp.
SGPT (I.U/L)	28.2±0.3	53.3±033	34.3±064	45.5±1.29	35.0±1.11	48.9±2.68*
SGOt (I.U/L)	38.3±2.2	42.7±3.0	41.8±1.2	48.9±2.48	39.32±1.0	55.40±3.74**
L.D.H (I.U/L)	182±4.3	192±3.94	192±4.3	192±5.23	195±3.40	199±5.28*
A.L.P (U/L)	3.6±2.3	3.2±1.64	3.3±1.84	4.7±1.90	2.83±1.60	5.93±2.25**
Glucose (mg / dl)	28.24±2.3	33.68±1.2	29.82±1.2	42.20±2.8	30.64±1.8	62.8±2.78**

Total protein (g/dl)	246 ± 0.43	2.43 ± 0.14	2.50 ± 0.64	3.1 ± 0.29	2.5 ± 0.55	4.70 ± 0.94*
Cholesterol (ng/dl)	161.4±3.4	167.6 ± 2.9	167 ± 3.0	163 ± 3.23	162.9 ± 2.4	198.4 ± 4.3*
Ig. M (ng/ml)	1.8 ± 0.13	1.60 ± 0.24	1.80 ± 0.42	1.43 ± 0.92	1.75 ± 0.12	0.3 ± 0.065*
Cortisol (ng/ml)	0.87 ± 0.24	1.53 ± 0.06	0.98 ± 1.24	1.86 ± 1.21	0.84 ± 0.73	1.95 ± 1.63*

Exp: experimental * Significant at p<0.01. ** Non-significant

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