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Original Article

Monitoring of Biochemical Effects of Phenol in the Carp (*Cyprinus carpio*) Fry

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ABSTRACT

This study was conducted to investigate the possible side effects of phenol on biochemical parameters of carp (*Cyprinus carpio*) fry with an average weight of 0.474±0.04 g. Fishes were treated with 0 (control), 5, 10 and 20 ppm of phenol during 24, 48, 72, and 96 hours. We have tested the effects of phenol on the biochemical profile, i.e., the total protein, lipid and glycogen levels, in the whole body of the carp samples. They showed change as total protein ($p<0.05$), glycogen ($p>0.05$) and lipids ($p>0.05$) content in the whole body. In view of results, the present study reports metabolic dysfunction in response to phenol toxicity in carp.

Keywords: *Cyprinus carpio*, Phenol, Glycogen, Total protein, Lipid

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INTRODUCTION

One of the most important problems of recent years that is influencing all living organisms and retrograding natural resources is water pollution (Khan, et al., 2000; Inyinbor et al., 2018). A great number of chemical compositions that get into water ecosystems can cause dangerous impacts on freshwater and marine organisms (Stegeman et al., 1992; Garg et al., 2009). Random dismissal of industrial wastes into aquatic ecosystems without any pretreatment causes serious problems to the non-target organisms (Mishra & Poddar 2013). Draining of wastes into freshwater systems decreases the dissolved oxygen level which causes respiratory problems and consequent mortalities in fish (Black 1955; Abu Aita 2014).

Conversely, even less is known about the ecotoxicological impacts of organic pollutants on terrestrial, wildlife and aquatic organisms, and a comprehensive revisal on ecotoxicological impacts is missing. Aquatic organisms are especially important targets, as they are exposed to wastewater residuals over their all life. Standard intense ecotoxicity information has been reported for a number of natural pollutants, but, such data alone may not be appropriate for specifically addressing the question of environmental impacts, and afterwards in the risk and hazard assessment (Escher 2001; Traas & Van Leeuwen 2007; Ma et al., 2019).

Phenol and phenolic compounds are xenobiotics, which are derived from the aquatic environment as a result of anthropogenic factors and they are stressful environmental agents. Animals such as humans, fish, rabbits, mice, which were exposed to those compounds suffer from anemia and some other pathologies (Hori et al., 2006; Zaki et al., 2011). There were no published reports on the biochemical effects of phenol on carp fry. The aim of this study is to investigate the various biochemical effects of phenol in carp fry with an experimental study design.

MATERIALS AND METHODS

Fish and experimental design

Carp (*Cyprinus carpio*) samples (0.474±0.04 g mean weight) used in this study were obtained from the Keban Fish Breeding Unit of IX. Region Directorate, the State Hydraulic Works in Turkey. Two weeks prior to the experiments, the fish were acclimatized in glass aquaria aerated with

air stones. The fish were fed with a commercial diet daily to satiation. Exposure chambers were cleaned as needed. For the duration of the study, the dissolved oxygen (Intellical™ LDO101), pH (Thermo Scientific™ Orion Star™ A111 pH Benchtop Meter), temperature (Checktemp®1 Pocket Thermometer), hardness (Premium Water Hardness Test Kit), salinity (Extech EC170 Salinity Meter), nitrite and ammonia levels (100 UV Visible Spectrophotometer) were monitored and maintained within acceptable ranges. The water quality characteristics were determined according to the American Public Health Association guidelines (APHA, 2005).

The fish were divided equally into four groups (20 fish/per groups). The first group was maintained in tap water as a control group. The fish in groups 2, 3 and 4 were exposed to 5, 10 and 20 ppm of phenol respectively for 96 hours. The entire experiment was repeated two independent times; each replicate for each group contained ten fish, and a total of 160 carp individuals were used during the experimental stage of this study. No mortalities were observed during the experiment. At the 96th hour of the test, the fish were anaesthetized in 50 ppm benzocaine solution and the whole bodies were isolated, washed with physiological saline (0.9 % NaCl) and stored at -40 °C until the biochemical assays, which were performed within one month after extraction.

Biochemical assays

Total protein activity

Protein extraction was carried out according to Plummer (1971). Briefly, carp fry (one individual internal organs removed: 0.464±0.02 g) were homogenized in the homogenizer by adding 10 ml of 10% trichloroacetic acid solution. These fish samples (10.464 ml) were centrifuged at 3500 rpm for 15 minutes at room temperature. The supernatant in the tubes was discarded and 2ml of 96% ethyl alcohol was added. The tubes were then mixed slowly and centrifuged at 3500 rpm for 15 minutes. After centrifugation, the supernatant was discarded and the pellet was re-dissolved by adding distilled water (10 ml). As described by Lowry et al. (1951), the amount of protein was determined using the Folin-phenol reagent. Bovine serum albumin was used as the standard protein. Spectrophotometer was read with absorbance at OD_{695nm}.

Lipid level

Total lipid extraction was carried out according to Folch et al. (1957). Carp fry samples (one individual internal organs removed: 0.464±0.02 g) were homogenized in homogenizer by adding 10 ml of chloroform methanol (2:1, v/v). These fish samples were centrifuged at 1200 *g* for 5 minutes at room temperature to separate the lipid containing organic layer from the aqueous layer. The organic layer of each sample was left in pre-weighed and prepared boiling tubes. These tubes were then evaporated at 37 °C under nitrogen and allowed to dry. In desiccator was cooled dried tube containing lipid and reweighed. The weight of the crude lipid was calculated by subtracting the initial weight of the empty tube from the weight of the tube containing the dried lipid.

Glycogen content

Glycogen extraction was carried out according to the method reported by Joseph et al. (1961). Glycogen extraction was carried out. Carp fry samples (one individual internal organs removed: 0.464±0.02 g) were homogenized in the homogenizer by adding 10 ml of 10% trichloroacetic acid solution. The homogenizer bottle was placed in an ice water bath and the temperature of the contents was kept below 15 °C during the homogenization period. These homogenates were filtered through filter paper and the total volume of liquid passing through the filter was measured. In order to precipitate the glycogen, aliquots were left in the prepared centrifuge tubes and 5 volumes of ethyl alcohol were added. After the solution was kept in the oven set at 35-40 °C overnight. These samples to precipitate glycogen were centrifuged (10.464 ml) at 3500 rpm for 15 minutes at room temperature. After centrifugation, the supernatant in the tubes was discarded. Pellets were resuspended by adding 2 mL of distilled water. As described by Nicholas et al. (1956), the proportion of glycogen was determined using the anthrone method. Spectrophotometer was measured with absorbance at OD_{620nm} where the glucose solution was used as standard.

RESULTS AND DISCUSSION

The results of this experimental study, which was carried out to determine the biochemical effects of phenol on carp fry, showed that the biochemical profiles of carp fry change according to the exposure amount of phenol.

The effects of phenol on the total protein level of carp were shown in Table 1. A significant (p<0.05) decrease of the protein level in fish exposed to all the doses of phenol was observed to 0 and 1 ppm groups had higher protein activity than those the other experimental groups (p<0.05). In these exposed groups the total protein was found as 4.33±0.11, 3.83±0.14 and 3,14±0.13 mg/ml for all experimental groups at the 96th hour respectively.

Administration of phenol at the dose of 5 and 10 ppm showed a significant decrease in the total lipid after 96th hour. In total lipid activity, the minimum levels observed were 0.37±0.04, 0.26±0.04, 0.19±0.05 and 0.37±0.03 mg/g for the experimental and control groups, respectively (Table 2).

There was no significant difference (p>0.05) in the activity of glycogen in the whole body of 1ppm phenol treated fishes after the 96th hour of exposure as compared with the control group (Table 3). But, administration of phenol at the dose of 5 and 10 ppm showed a significant decrease in the glycogen content after the 96th hour.

Table 1. Effect of phenol on the total protein content in whole body of carp.

	Group	24 h	48 h	72 h	96 h
Protein (mg/ml)	Control	4.58±0.15	4.57±0.11	4.59±0.13	4.60±0.13
	1 ppm	4.39±0.13	4.38±0.14^{a}	4.29±0.10^{a}	4.33±0.11^{ac}
	5 ppm	4.32±0.14	4,10±0.14^{a,b}	3.71±0.14^{a,b}	3.83±0.14^{a,b}
	10 ppm	4.08±0.03	3,72±0.14^{a,b,c}	3.12±0.10^{a,b,c}	3,14±0.13^{a,b,c}

Table 2. Effect of phenol on the total lipid content in the whole body of carp

	Group	24 h	48 h	72 h	96 h
Lipid (mg/g)	Control	0.38±0.05	0.39±0.04	0.37±0.03	0.39±0.01
	1 ppm	0.39±0.03	0.38±0.03	0.38±0.04	0.37±0.04
	5 ppm	0.36±0.04^{a,b}	0.30±0.02^{a,b}	0.26±0.04^{a,b}	0.27±0.05^{a,b}
	10 ppm	0.31±0.02^{a,b,c}	0.23±0.02^{a,b,c}	0.19±0.05^{a,b,c}	0.24±0.02^{a,b,c}

Table 3. Effect of phenol on the glycogen content in the whole body of carp

	Group	24 h	48 h	72 h	96 h
Glycogen (µg/g)	Control	2.72 ±0.5	2.69±0.4	2.69±0.3	2.73±0.6
	1 ppm	2.70 ±0.4	2.71±0.5	2.70±0.5	2.69±0.5
	5 ppm	2.67 ±0.4	2.68±0.5	2.63±0.4	2.59±0.5
	10 ppm	2.08 ±0.3^{a,b,c}	1.96±0.6^{a,b,c}	1.99±0.3^{a,b,c}	1.82±0.6^{a,b,c}

As stated above, experimental exposure to phenol with various doses for 96 h caused some biochemical alterations in carp fry. The phenol could alter protein metabolism by changing the transamination rate of amino acids by increasing the activity of alanine aminotransferase (ALAT, EC 2.6.1.2) and aspartate aminotransferase (ASAT, EC 2.6.1.1) (Hori et al., 2006).

Administration of phenol at the dose of 5 and 10 ppm showed a significant decrease in the total lipid after the 96th hour. The main energy source in fish is lipids. Fish swimming induces high energy demands (Fernández-Vega et al., 2015). Catabolism of such amount of lipid in *Cyprinus carpio* reflects a higher demand for ATP as a consequence of the detoxification process and elevated stress (Sannadurgappa et al. 2007). The reduced glycogen content in carp exposed to phenol may cause a lower energy accumulation in fish, and these fish will probably swim slower.

Hori et al. (2006) and Abdel-Hameid (2007) reported that slow swimming is a general response observed in fishes exposed to phenol. Glycogen decrease was to attend energetic consumption caused by phenol. Due to the absence of increased glycogen in the white muscle of the fish, glycogen synthesis was not done to determine glucose reduction.

CONCLUSION

An attempt was made to assess the phenol toxicity on carp fry by performing acute bioassays. Results of tolerance limits and response of test fish towards phenol confirm that phenol has an impact during acute toxicity. The swimming behavior of fish is slowed down due to changes occurred in biochemical parameters like the swimming behavior of fish was due to changes occurred in biochemical parameters like proteins, lipids and glycogen. Our study claims that phenol can affect the metabolism of test fish by hindering biochemical activity. This experimental study showed the toxic potential of phenol on carp, but the induction pattern of antioxidant enzymes under long-term and high dose exposure conditions still needs to be investigated.

Conflict of interests: The authors have no conflicts of interest to declare.

Ethics committee approval: All animal studies were approved by the Animal Ethics Committee of Kahramanmaraş Sütçü Imam University, Faculty of Agriculture (KSÜZİRHADYEK) and Research Institute (Protocol number: 2016/1-1).

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