

## IMPACT OF MERCURY CHLORIDE EXPOSURE ON SOME OF IMMUNOLOGICAL AND BIOCHEMICAL ASSAYS OF COMMON CARP, *CYPRINUS CARPIO*

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### ABSTRACT

This work was designed to evaluate the influence of mercury chloride on some of biochemical and immunological biomarkers in common cap, *Cyprinus carpio*. Around of 120 fish were randomly allocated into four groups (30 fish per group) in triplicates as follows; first group act as control group provided with water only without adding  $HgCl_2$ ; G1, G2 and G3 were exposed to waterborne  $HgCl_2$  at levels of 0.01, 0.05 and 0.1  $mg\ l^{-1}$  respectively. After one month exposure to  $HgCl_2$ , there were significantly decreased ( $P<0.05$ ) in lymphocyte transformation index and in phagocytic and lysozyme activities. Besides, biochemically, Albumin and globulin content exhibited significantly declined ( $P<0.05$ ) particularly at higher dose of  $HgCl_2$ . In contrast, blood glucose value and urea showed significantly increased ( $P<0.05$ ) especially in G3. On the other hand, variable changes were observed in total count of leucocytes included lymphopenia and neutrophilia in G1 and G2 compared to control. In conclusion, this investigation indicated that mercury chloride has immune suppressive effects and is extremely toxic to common carp.

**Keywords:** Heavy metal- Hematology- Phagocytic index- Albumin-Total protein

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تأثير التعرض لكلوريد الزئبق على بعض الاختبارات المناعية والكيميائية في اسماك الكارب الشائع

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المستخلص

هدفت الدراسة لتقييم تأثير كلوريد الزئبق على بعض المؤشرات الكيميائية والمناعية في اسماك الكارب الشائع (*Cyprinus carpio*). لهذا الغرض تم استخدام 120 سمكة كارب تقريبا قسمت بشكل عشوائي الى اربعة معاملات (30 سمكة/معاملة) على النحو التالي: المعاملة الاولى اعتبرت مجموعة سيطرة مزودة بالماء فقط دون اضافة كلوريد الزئبق؛ تم تعريض المعاملة الاولى والثانية والثالثة (G1 و G2 و G3) إلى كلوريد الزئبق بمعدلات 0.01 و 0.05 و 0.1 ملغم/لتر على التوالي. بعد شهر واحد من التعرض للزئبق اشارت النتائج الى انخفاض معنوي ( $P<0.05$ ) في مؤشر تحول الخلايا الليمفاوية وفي أنشطة البلعمة والليزوزيم. علاوة على ذلك، اظهرت نتائج الاختبارات الكيميائية انخفاض معنوي في مستوى كل من الألبومين والكلوبيولين بشكل ملحوظ خاصة في المعاملة الثالثة (G3). في المقابل، ارتفعت قيمة كل من الكلوكون في الدم واليورينا خاصة في المعاملة الثالثة (G3). من ناحية أخرى، لوحظت تغيرات كبيرة في العدد الكلي لكريات الدم البيض بما في ذلك اللمفاويات والنتروفيل في G1 و G2 مقارنة بمعاملة السيطرة. تم الاستنتاج من هذه الدراسة إلى أن كلوريد الزئبق له تأثير مثبط للمناعة وهو شديد السمية لاسماك الكارب الشائع.

الكلمات المفتاحية: المعادن الثقيلة- علم الدم-مؤشر البلعمة-الالبومين-البروتين الكلي

## INTRODUCTION

Ecosystem pollution with several of “anthropogenic sources” and chemical contaminants is becoming globally the biggest risk and the important issue (15, 16). Different pollutants such as heavy metals released into the water environment via the industrial activities, mining, agricultural waste and runoff. Some of the dissolved metals such as mercury occur normally in aquatic ecosystem in small amount; but via the industrial operations could be transported, concentrated and converted into other formulae consequently reinstated into the environment (27). As a result, fishes in polluted areas are frequently exposed to large amount and higher levels of these chemical compounds which are different than those naturally occurring in the environments (17). Mercury and its products have been shares of extensive pollutant of aquatic environment causing destructive impact on aquatic animals. The most frequently forms of mercury in the ecosystem is “elemental mercury”, organic like ethyl and methyl mercury and inorganic forms (5). Several investigations indicated the toxic impact of mercuric in fishes involving immunosuppressive effect (6, 9, 13, 19). Also, biochemical and hematological changes were studied in fishes by several researchers (3, 11). In shed lights of the possible risk of mercuric salts, this study was aimed to evaluate some of immunological and biochemical changes in common carp following exposure to waterborne mercury chloride.

## MATERIALS AND METHODS

### Design of the experiment

A proximately of 120 common carp, *C. carpio* (weight  $134 \pm 5.20$  g; length  $17.50 \pm 2.24$  cm) were purchased from private cages farm in “Babylon province/Iraq”. Fish were acclimatized for two weeks in glass tanks with continuous aeration (DO 7.2 mg/L, pH 7.4) before starting the experiment. During acclimatization period fish were fed a marketable diet at of 3% body mass. Following adaptation, 120 fish were stocked randomly into four treatment groups (30 fish/group) in triplicates (10 fish per 70 L aquarium/replicate) as follows; first group act as control group provided with water only without adding  $HgCl_2$ ; G1, G2 and G3 were

exposed to waterborne  $HgCl_2$  at levels of 0.01, 0.05 and 0.1 mg  $l^{-1}$  respectively, for 30 days. The above levels of  $HgCl_2$  were chosen according to our previous study (3). “Water quality was registered every day during the experimental period as follows: “DO  $6.8 \pm 1.4$  mg l<sup>-1</sup>; Temp.  $22.4 \pm 1.6$ ; pH  $7.2 \pm 1.2$ ”. After experimental period, blood samples were collected up from two fish/replicate for detection of immunological, and biochemical indices.

### Immunological parameters

Immunological parameters included phagocytic activity of the head kidney head was determined at least in one yeast cells as mean percentage according to procedure pronounced by El-Boshy and Ramdan (6). Lymphocytes transformation index was determined following the method described by to Barta (4). Serum lysozyme was detected by “turbidometric assay” by the technique of Parry et al. (18). Detection of respiratory burst activity was carried out using “nitrobluetetrazolium (NBT) assay” (2).

### Hematological indices

Differential count of white blood cells for blood samples were determined according to method described by Mustafa (14) by scoring 200 cells for each slide which stained by using “May-Grünwald Stain”

### Biochemical profiles

Biochemical profiles (viz., albumin, total protein, globulin glucose and urea) were determined spectrophotometric using a commercial kits (Sigma, UK) according to enclosed booklet.

### Statistical analysis

Statistical analysis was achieved to determine the significant differences among the treated groups by applying one way analysis of variance (“ANOVA using “IBM SPSS Statistics version 23” with LSD. P values less than 5% were considered significant.

## RESULTS AND DISCUSSION

Results of this investigation indicated that the exposure to waterborne  $HgCl_2$  for 30 days caused greatest disturbances in biochemical and immunological parameters of common carp. The phagocytic activity, lymphocyte transformation index, and NBT were exhibited significantly decreased ( $P < 0.05$ ) in all  $HgCl_2$  treated group in comparison with control

group. Furthermore, level of lysozyme activity showed significantly decreased ( $P < 0.05$ ) only at higher level of mercury (G3) in compare with G1, G2 and control group (Table 1). Biochemical profile (TP, Al, Glu) revealed significantly decreased in mercury treated groups (G1, G2 and G3) relative to control group. On the other hand, glucose and urea content indicated markedly increased in all mercuric groups relative to control (Table 2). The highest glucose value was found at higher level of mercuric exposed fish (i.e., G3). Besides, total count of leucocytes exhibited lymphopenia and neutrophilia in G1 and G2 compared to control. While, G3 showed neutropenia and lymphopenia with increased the percentage of monocyte in comparison to G1 and G2 (Table 3). The non-specific immune tests are valuable biomarkers to assess the fish health status and are also good indicator to evaluate the infectious diseases and the pollution in fish aquaculture (21). In current study, the immune-toxicological impacts of  $HgCl_2$  on immune assays of common carp were demonstrated by decreased in phagocytic activity and lymphocytic transformation index in all mercuric groups. Concerning the impaired immunity in common carp, several researches, using various fish species proved immunosuppression after exposed to heavy metals (6, 7). The mercury compounds were established to cause detrital disturbances in physiologic and immune functions and exploitive effect in specific and non-specific immune structure of fishes (1,6,9). Our study revealed significantly decreased in phagocytic activity, lymphocyte transformation index and respiratory burst capacity. These findings are in line with Sarmiento et al. (22) whom registered the decreased in phagocytic activity and in respiratory burst ability in seabass, "*Dicentrarchus labrax*" fish following mercury chloride exposure. The researcher credited this reduction to failure in activation or decrease in the activity of "macrophages activating factor". The present investigation recorded significantly decreased in plasma lysozyme activity in G3 group compared to

control. This result is in consistent with El-Boshy and Ramdan, (6) who also found significant decreased in phagocytic activity and lysozyme activity in Nile Tilapia, "*Oreochromis niloticus*" exposed to different doses of mercury chloride. In contrary, MacDougal et al. (10) documented that in blue gourami mercuric exposure was increasing lysozyme activity. Hypoalbuminemia and hypoproteinemia in mercuric exposed groups might be owing to impairment synthesis of protein by the liver or excess renal excretion. Numerous researchers also demonstrated decreased of plasma protein in diverse fish species following heavy metals exposure (25, 28). On contrast, Mansour et al. (12) and Haggag et al. (8) have been recoded increasing in plasma protein level in freshwater fish caught from sites polluted with heavy metals. The increasing of urea in our study could be indorsed to gill dysfunction (26). Our results found that an increase in blood glucose values was directly proportional to the high level of  $HgCl_2$ . Setiyowati, 2019 regard the higher value of blood glucose in mercuric exposed fish as a secondary stress hormones (viz; catecholamines and cortisol) from internal cells and prompt of gluconeogenesis. El-Boshy, and Ramdan (6) have been recorded the hyperglycemia in Nile tilapia exposed to mercury. Regarding the neutrophilia and lymphopenia in mercury exposed fish. Similar results have been recorded in various fish species (11,24). Roales and Perlmutter (20) attributed the immunosuppressive effects of mercury and lymphopenia in fish to inhibiting the proliferation of the white pulp of the spleen.

### Conclusions

Based on these results,  $HgCl_2$  is highly toxic to common carp also has immune suppression it causes variable changes in biochemical and immunological biomarkers included reduction in "phagocytic activity and serum lysozyme activity" with increased in blood glucose values which is directly correlated to the increase in the level of  $HgCl_2$  particularly at higher level  $0.1 \text{ mg L}^{-1}$ .

**Table 1. Immunological parameters (mean values  $\pm$  SE) in *C. carpio* exposed to different levels of mercuric chloride for 30 days**

Treatment Groups	Phagocytic activity%	Lymphocytes transformation index	Serum Lysozyme activity $\mu\text{g ml}^{-1}$	Respiratory Burst capacity (NBT)
Control	1.87 $\pm$ 0.01a	1.17 $\pm$ 0.10a	7.67 $\pm$ 0.53a	1.12 $\pm$ 0.08a
G1	1.22 $\pm$ 0.02b	0.51 $\pm$ 0.02a	7.42 $\pm$ 0.22a	0.53 $\pm$ 0.02b
G2	1.13 $\pm$ 0.01b	0.93 $\pm$ 0.04b	7.58 $\pm$ 0.40a	0.55 $\pm$ 0.05b
G3	0.58 $\pm$ 0.00c	0.87 $\pm$ 0.01b	5.62 $\pm$ 0.30b	0.40 $\pm$ 0.01c

Data are means $\pm$ SE. Different alphabetic small letters represented significant different at P<0.05

**Table 2. Biochemical tests (albumin, globulin, total protein, glucose and urea) of *C. carpio* exposed to different levels of mercuric chloride for 30 days**

Treatment Groups	Albumin g/dl	Globulin g/dl	Total protein g/dl	Glucose mg/dl	Urea mg/dl
Control	1.87 $\pm$ 0.01a	2.17 $\pm$ 0.10a	4.04 $\pm$ 0.53a	42.20 $\pm$ 6.0a	13.00 $\pm$ 1.0b
G1	1.22 $\pm$ 0.02b	1.15 $\pm$ 0.02a	2.37 $\pm$ 0.22a	55.11 $\pm$ 3.2a	24.10 $\pm$ 3.0a
G2	1.43 $\pm$ 0.01b	0.87 $\pm$ 0.04b	2.30 $\pm$ 0.40a	96.65 $\pm$ 7.0b	23.50 $\pm$ 2.0a
G3	0.58 $\pm$ 0.00c	0.90 $\pm$ 0.01b	1.48 $\pm$ 0.30b	119.30 $\pm$ 5.0c	26.00 $\pm$ 3.0a

Data are means $\pm$ SE. Different alphabetic small letters represented significant different at P<0.05

**Table 3. Differential leucocyte count of *C. carpio* exposed to different levels of HgCl<sub>2</sub> for 30 days**

Treatment Groups	Lymphocyte%	Neutrophils %	Monocyte %	Eosinophils%
Control	82.43 $\pm$ 1.02a	5.15 $\pm$ 0.02b	11.41 $\pm$ 1.22a	1.00 $\pm$ 0.02a
G1	76.90 $\pm$ 0.9b	9.00 $\pm$ 0.10a	13.10 $\pm$ 1.53a	1.00 $\pm$ 0.08a
G2	76.27 $\pm$ 2.0b	9.93 $\pm$ 0.04a	12.25 $\pm$ 0.40a	1.55 $\pm$ 0.05a
G3	78.11 $\pm$ 1.4b	4.87 $\pm$ 0.01b	15.62 $\pm$ 0.30b	1.40 $\pm$ 0.01a

Data are means $\pm$ SE. Different alphabetic small letters represented significant different at P<0.05

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