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RESEARCH ARTICLE

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REPRODUCTIVE AND PATHO-PHYSIOLOGICAL RESPONSES OF BLUE TILAPIA *(OREOCHROMIS AUREUS)* EXPOSED TO CHROMIUM WITH OR WITHOUT CHELATING SUBSTANCES

ABSTRACT:

Fish may be exposed to Chromium (Cr) waste which is released into aquatic environments through electroplating tannery and textile industries. Therefore we examined the effect of hexavalent chromium on the reproductive and patho-physiological changes of blue tilapia (*Oreochromius aureus*) with trials to improve these hazard effects by addition other adsorbant substance. A total number of 270 *O. aureus* (40-45 g/fish) were divided into 9 equal groups (each subdivided into 3 equal replicates). The 1st group was used as control, meanwhile 2nd and 3rd groups received 1/8 and 1/4 LC_{50} of Cr (4.925 and 9.85 mg Cr /l), respectively. Other groups received the same doses of Cr, in addition to 0.3 g/ l of EDTA (group 4 & 5), super phosphate (group 7 & 8) and charcoal (group 8 & 9). Sampling was done from each at 7, 30 and 75 days after experiment start. Fish exposed to Cr showed significantly reduced growth; which was improved when EDTA, super phosphate and/ or charcoal were added with Cr. A significant reductions ($P<0.05$) in the blood parameters were found in fish exposed to Cr. These parameters were enhanced by addition of either chelating agents to the Cr. Significant increases in plasma glucose concentration, aspartate aminotransferase (AST) and alanine aminotrasferase (ALT) activities in plasma, liver and muscle were observed in fish exposed to Cr. The total lipids and total proteins in plasma, liver and muscle were decreased significantly of fish exposed to Cr alone. Addition of EDTA, super phosphate and charcoal to Cr polluted water enhanced the biochemical parameters of fish and the enzyme activities returned to the normal levels as in control fish group. The fertility of *O. aureus* was also reduced significantly after exposure to sublethal dose of Cr alone. On other hand, it was improved by the addition of chelating agents to the Cr polluted water. The observed histopathological changes varied with the dose of Cr and their period of application. It was improved with variant degrees based on the type of chelating agents used.

Key words: tilapia, chromium, *chelating agents*, haematology, biochemistry and histopathology

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INTRODUCTION

Natural water may be contaminated by untreated wastes of industrial, technological and agricultural origin containing various metallic compounds. Heavy metals due to their bio – accumulative and non-biodegradable properties constitute a core group of aquatic pollutants. Chromium particularly enters the aquatic media through effluents discharged from tanneries, textile, electroplating, metal finishing, mining, dyeing, printing, ceramic, photographic and pharmaceutical industries etc. Chromium exists mostly in two valence states in nature: trivalent (chromic chloride) and hexavalent (potassium dichromate). Hexavalent chromium (VI) is commonly used in industrial chrome plating, welding, painting, metal finishes, steel manufacturing, alloy, cast iron and wood treatment. Otherwise it and is a proven toxin, mutagen and carcinogen. The mechanism biochemistry of cytotoxicity of chromium (VI) is not completely understood. However, a large number of studies demonstrated that chromium (VI) induces oxidative stress, DNA damage, apoptotic cell death and altered gene expression. Conversely, trivalent chromium (III) is essential for proper insulin function and is required for normal protein, fat and carbohydrate metabolism, and is acknowledged as a dietary supplementin its trivalent form (Bagchi *et al.,* 2002). Baig *et al.* (2003) also postulated that the chromium hexavalent form is carcinogenic and toxic to aquatic life, whereas Cr (3+) is however comparatively less toxic.

The ability of bone charcoal to remove Cr from aqueous solutions by adsorption has been investigated. The adsorbent used was first studied as a function of time and amount . Tests were carried out with synthetic solutions whose Cr concentrations (500 mg L (-1)) were similar to those found in some effluents of moroccan tannery industries. Cr removal efficiencies higher than 90% were obtained at pH 3.5 using 3 gm of bone charcoal and a stirring time of about 30 min. Results of Cr removal by all sieved fractions of bone charcoal had shown the same interesting capabilities for Cr(III) retention. The cross interference with other elements was also investigated. Pre-treatment of bone charcoal by nitric acid led to an increase in its

specific surface area but induced a drastic reduction in its Cr elimination abilities (Dahbi, *et al*., 2002). Adsorption tests were also carried out using calcinated bone charcoal. The results obtained showed a similar percentage of Cr retention to those found with untreated bone charcoal. On the other hand, a double treatment of bone charcoal with HCl and NaOH provided an enhancement of Cr(III) retention. The role played by the mineral fraction of the solid phase of bone was thus evidenced (Dahbi, *et al*., 2002).

Recently , the removal of metals by synthetic anthropogenic chelating agents has received much attention. The literature to data reports a number of chelators that have been used for chelate-induced hyperaccumulation (Huang *et a*l., 1997). EDTA is the most commonly used chelator due to of its strong chelating ability for different heavy metals (Norvell, 1991) The use of EDTA (Ethylenediaminetetra acetic acid) and NTA (nitrilotriacetic acid) has especially been questioned because of their potential for increasing the solubilization and remobilization of heavy metals from aquatic sediments (Muller and Forstner, 1976). The present study was undertaken to investigate the effect of chromium on the path-physiological condition and reproductive performance of *O. aureus* and trails to reduce the toxic effect of chromium using chelating agents (EDTA, super phosphate and activated charcoal).

MATERIALS AND METHODS

The experimental work of this study was carried out an indoor wet Lab. in Central Laboratory for Aquaculture Research, Abbassa , Abo-Hammed, Sharkia , Egypt. Average weight of Blue tilapia " *Orechromius aureus* " of average weight 40-45 gm were acclimated in laboratory conditions for 2 weeks before the begging of the experimental work. Fish were distributed in twenty seventh glass aquaria of about 100-liter capacity each and stocked at a rate of 10-fish/ aquarium. The glass aquaria were supplied with dechlorinated tap water and continous aeration was adapted by using an air pump and air stones. Average water temperature was maintained at 27+ 2 C. The aquaria were divided into 9 groups with three replicates per group. The 1st group was left and kept as control, 2nd and 3rd groups received 1/8 and 1/4 LC_{50} of Cr (4.925 and 9.85 mg Cr /l) respectively according to Sesha and Balaparameswara (1996). Other groups received same doses of Cr, respectively in addition to 0.3 g/ l of EDTA (groups 4 & 5) , super phosphate (groups 7 & 8) and charcoal (groups 8 & 9. The treatments groups are illustrated in tables (1).

Fish of the experimental groups were fed on a pelleted fish diet containing 32 % CP. and the diet was fed at a rate of 3 % of live body weight twice daily for 90 days. Semi–dynamic method for removal of excreta was used every day by siphoning a portion of water from the aquarium and replacing it by an equal volume of water.

Sampling (blood and tissues) was done at 7, 30 and 75 days of the start of experiment. Blood samples were taken from the caudal vein of non anaesthetized fish by sterile syringe containing EDTA as an anticoagulant. Erythrocyte count according to Dacie and Lewis (1984), haemoglobin content according to Van kampen (1961) and haematocrit value according to Britton (1963) were detected.

Table (1): Experimental groups and their notation

Groups	Chromium		Chelating agents	
	LC_{50}	Dose (mg/ l)	Type	Dose (g/l)
1	-	-	-	-
2	1/8	4.925	-	-
3	1/4	9.85	-	-
4	1/8	4.925	EDTA	0.3
5	1/4	9.85	EDTA	0.3
6	1/8	4.925	uper phosphate	0.3
7	1/4	9.85	uper phosphate	0.3
8	1/8	4.925	Charcoal	0.3
9	1/4	9.85	Charcoal	0.3

Plasma was obtained by centrifugation of the blood at 3000 rpm for 15 min and the non haemolyzed plasma was stored in a deep freezer at-20°C till analysis. Plasma protein content was determined by Biuret method described by Wotton (1964). Glucose concentration was measured according to Trinder (1969) using Boehring Mannheium kits. Total lipids were determined colorimetrically using kits supplied by El Nasr Pharmaceutical Chemical Co. according to Joseph *et al.* (1972). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically using kits supplied by Diamond Diagnostics according to Reitman and Frankel (1957).

After 75 days of the experiment, decapitation of fish was done and the gonads were removed and weighted and the gonadsomatic index (GSI) was determined (Munkittrich and Dixon 1988). Egg numbers in the ovaries of the female were counted to determine the fecundity, (Munkittrich and Dixon 1988). Milt sample from the male were taken by striping before decapitation of males and sperm cell concentration counted (Cochran, 1987). Hormonal levels were estimated by using radioimmunoassay (RIA) for testosterone (Carlstrom *et al*., 1988) and estradiol-17 β (Abraham, 1976).

Tissue specimens (gills, liver, kidneys, spleen and muscle) from all experimented groups were collected at 7, 30 and 75 days of the experiment. They were immediately fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol , cleared in xylene, blocked in paraffin section at 5 um thickness and stained with hematoxyline and eosin stains (Carliton 1976).

The data were analyzed statistically using T- test (Harold and Larson, 1982) to compare the means of treated groups against that of the corresponding control.

RESULTS AND DISCUSSION

Haematological variables:

The results of erythrocyte count (RBCs), haemoglobin content (Hb) and haematocrit value (Hct) obtained from the fish exposed to sublethal doses of chromium (1/8 and 1/4 LC_{50}) (4.925 and 9.85 mg Cr /l) alone or with 0.3 g/l of different absorbing substance are given in table (2). The RBCs, Hb and Hct were reduced significantly in fish exposed to sublethal doses of Cr for 7, 30 and 75 days when compared to the control fish groups. The RBCs count was also significantly decreased when fish exposed to mixture of high dose of Cr with supper phosphate for 30 days. However, Hb content decreased significantly in fish exposed to high dose of Cr with supper phosphate for 30 and 75 days. These parameters may be used as sensitive diagnostic indicators of Cr poisoning. The bad effect of Cr on heamatological parameters agreed with the finding of Khangatrot *et al*. (1999) who reported that freshwater catfish exposed to sublethal levels of Cr for 28 days had shown a significant decrease in RBCs, WBCs, HB and Hct. The reduction of these parameters in Nile tilapia, *O. niloticus,* at sublethal levels of cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of haemosynthesis that affected by pollutants (Wintrobe, 1978). The decrease in RBCs count may be also attributed to haematopathological or acute haemolytic crisis that results in sever anemia in most vertebrates including fish species exposed to different environmental pollutants (khangarot and Tripathi, 1991) or to reduction of growth and other food utilization parameters which results in sever anemia (James and Sampath, 1999). These results are in agreement with those of Prabakaran *et a*l. (2006), they indicated that chronic exposure of *O. mossambicus* to 5 mg l(-1) of chromium (VI) decreased both nonspecific and specific parameters of the immune system, which resulted in a lower disease resistance to A. hydrophila. They also revealed a concentration-dependent modulation of the immune system by chromium (VI), as demonstrated by suppressive or stimulatory effects on lymphocytes, lysozyme, phagocytic killing mechanisms, and disease resistance in *O. mossambicus*. The histopathological examination in the present study, explained the significant decrease in the RBCs, Hb and Hct values where marked degeneration was seen in the liver, spleen and kidney of Cr exposed groups together with depletion of hematopietic tissue and the marked gill lesions.

Addition of adsorbents reduced the toxic effect of Cr, blood parameter were improved in fish exposed to Cr with different chelating agents. The RBCs count was significantly increased when fish exposed to mixture of Cr with activated charcoal or supper phosphate for 7 and 90 days, respectively. However, Hb content increased significantly in fish exposed to low dose of Cr with charcoal for 30 days.. Since the RBCs ranged from 0.85 to 1.02 at Cr alone versus 1.30 to 1.96 at Cr with adsorbents. The most of the blood parameters (RBCs, Hb and Hct) in fish exposed to mixture of Cr with EDTA or activated charcoal was same as the control group (Tables 2). These results are in agreement with those Hilmy *et al*. (1986) who noticed of that EDTA therapy caused considerable decreases in all blood parameters (RBCS, HB and Hct) in toads, *Bufo regularis* after administration of 6.2 mg Cd / Kg. The histopathological findings supported the previous improvements in the hematological parameters where the chelating agents improved the gill structure and body defense with minimal lesions.

Table (2): The erythrocyte count, haemoglobin content and haematocrit value of the *O. aureus* after exposure to chromium and addition chelating

Parame	Erythrocyte count (C/mm^3)			Haemoglobine Content (g/100ml			Haematocrit value (%)		
Groups / Days	7 days	30 days	75days	7 days	30 days	75days	7 days	30 days	75days
1	1.33 ±0.03	1.68 ±0.03	1.57 ±0.08	4.67 ± 0.36	8.64 ± 0.45	6.02 ±0.16	18.9 ± 1.80	23.66 ± 1.55	19.0 ±1.44
2	1.00** ±0. 08	1.02*** ±0.03	1.31* ±0.06	3.57* ± 0.21	4.56** ± 0.22	4.89*** ±0.22	12.8*** ±1.05	13..5*** ±1.19	14.66** ±0.55
3	0.92*** ±0.02	0.85*** ±0. .06	1.13** ±0.05	3.27** ± 0.21	3.94** ± 0.19	4.04*** ±0.13	12.0*** ±0.96	12.33*** ±0.91	14.00*** ±0.36
4	1.30 ±0.06	1.43 ±0.12	1.53 ±0.0.5	5.88* ± 0.25	7.65 ± 0.27	5.89 ±0.13	18.66 ±1.17	20.33 ±1.17	17..99 ±1.08
5	1.30 ±0..64	1.806 ±0.06	1.60 ±0.08	4.52 ± 0.35	7. 50 ± 0.23	6.12 ±0.08	14.00 ±1.26	20.66 ±0.55	16.66 ±0.55
6	1.32 ±0.04	1.62 ±0.07	1.97 *** ±0.09	4.77 ± 0.33	7. 07** ± 0.28	5.81 ±0.13	16.66 ±0.55	21.66 ±0.76	19.00 ±1.31
7	1.49* ±0.06	1.42** ±0.04	1.53 ±0.09	4.28 ± 0.47	6.24** ± 0.61	4.41** ±0.13	13..33* ±0.91	19.57* ±0.84	15.00* ±0.81
8	1.32 ±0.02	1.77 ±0.03	1.47 ±0.08	4.77 ± 0.3	13.78** ± 0.10	6.61 ±0.98	14.02 ± 1.42	21.51 ±0.67	16.25 ±0.39
9	1.56*** ±0.01	1.32 ±0.27	1.34 ±0.08	4.03 ± 0.41	7.46 ± 0.31	5.37 ±0.36	12.45** ± 1.03	24.00 ±1.29	15.00* ±0.36

Data are represented as means ± S.E
* Significant at P<0.05 **Significant at P<0.01
*** Significant at P<0.001

Biochemical changes:

Results of table (3) show that addition of different absorbance substance to Cr contaminated media reduced significantly the Cr level in the water and helped in elimination of the metal from the fish body and in turn improved the biochemical parameters as compared to fish exposed to two doses of Cr.

The plasma glucose concentration showed higher significant values (P<0.01) in fish exposed to Cr doses alone, at all periods as compared to the control group (57.99 ±1.23 , 57.05 ±1.42 and 80.22 ±3.74 mg % for 7, 30 and 75 days, respectively). These values were also increased significantly in fish expose to mixture of high dose of Cr and 0.3 mg super phosphate/L. An increase in the glucose concentration of plasma of fishes exposed metallic stress was observed by many investigators. Sastry and Tyagi (1982) found that the plasma glucose concentration was elevated in freshwater teleost fish, *Channa punctatus*, after exposure to hexavalent chromium for 30 days. These results were in agreement with those of Nath and Kumar (1988) in freshwater teleost, *Colisa faciatus,* after exposure to sublethal dose (48 mg/l) hexavalent chromium even

for 6h only. Also, the plasma glucose concentration increased significantly in European eel (*Anguilla anguilla* L) after exposure to Cr for 24 hours (Teles *et a*l., 2005). The increase in glucose concentration in fishes may be due to the prevalence of hypoxic or anoxic conditions, which normally enhance the glycogen utilization. The increased liver glycogenolysis and glycolysis may be due decreased of glycogen which breakdown to glucose (Ambrose *et al.,* 1994). On the other hand, this elevation of plasma glucose concentration was insignificant varied in fish exposed to Cr with adsorbent substances. However, the reduction of the glucose concentration in plasma of fish treated with EDTA or charcoal were due the removal of Cr by those chelating agents. These results are in agreement with those of Verma *et al.* (1981) who found a reduction of Cr from the organs (liver, kidney, gills and brain) in freshwater fish, Notopterus *notopterus*, treated with *EDTA*. The histopathological alteration in the gills of chromium exposed fish decreased the gas exchange and consequently resulted in hypoxia which predisposed the significant increase in glucose together with the marked degeneration of the pancreas. The addition of chelating agents improved the condition of gills and pancreas as reduce the glucose concentration in the plasma (Table 3).

Table (3): The changes of glucose concentration and lipid content in plasma of blue tilapia *(O. aureus*) after exposure to chromium and addition of chelating agents.

Parameter	Plasma glucose			Plasma lipid		
Days / Group	7 days	30 days	75days	7days	30 days	75 days
1	57.99 ±1.23	57.05 ±1.42	80.22 ±3.74	7.82 ±0.33	8.13 ±0.43	8.98 ± 0.45
2	76.85*** ±1.314	174.88*** ±8.90	103.84*** ±2.97	6.66* ±0.24	6.85 ±0.41	6.74** ±0.52
3	89.64*** ±0.84	180.96*** ±7.55	105.88*** ±6.62	5.85** ±0.10	4.51*** ±0.49	6.58*** ±0.21
4	50.45 ±4.10	53..31 ±2.54	82.83 ±1.81	8.94 ±0.66	10..53 ±0.98	8.67 ±0.37
5	56.42 ±2.3	84.85 ±4.24	84.73 ±4.96	7.28 ±0.41	8.1 ±0.91	8.36 ±0.22
6	53.39 ±5.01	56.29 ±2.58	88.87 ±3.02	7.09 ±0.31	9.59 ±0.46	7.55 ±0.37
7	54..91 ±0.71	42.88* ±1.00	85.90 ±2.90	6.99 ±0.41	7.88 ±0.44	7.636 ±0.35
8	58.62 ±2.52	53.99 ±1.45	72.45 ±3.23	7.23 ±0.34	10.81 ±1.04	10.35 ±808
9	63.32 ±1.3	104.25* ±2.14	94..91* ±4.60	5.34** ±0.82	7.21 ±0.21	6.43*** ±0.44

Data are represented as means ± S.E

*Significant at P<0.05 **Significant at P<0.01

*** Significant at P<0.001

The plasma total lipid concentration after 7 days showed a significant decrease (P<0.01) to 6.66 ±0.24 and 5. 85 ± 0.10 mg/l after exposure to 1/8 and 1/4 LC_{50} of Cr when compared to the control fish group (7.82 ±0.33). These values showed also significant decrease in fish exposed to sublethal dose of Cr with charcoal for 7, 30 and 75 days of exposure (Table 3). These results are in agreement with those of Vutukuru (2003) who record significant decrease of the total lipids in liver , gills, muscle and kidney of *Labeo rahita* after exposure to hexavalent chromium. The decrease in tissue lipid and protein might partly due to their utilization in cell repair and tissue organization with the formation of lipoprotein which is important cellular constituents of cell membranes and cell organelles present in cytoplasm (Harper, 1963). The significant decrease in the plasma lipid by addition of the chelating agents could be due to the improvement in the pathological changes in the liver which initiate the process of phosphorilization. Also the improved gill to carry more oxygen so the body contributes in more metabolic processes.

The plasma total proteins showed no significant variation nearly of all fish under investigation for 7 days. After 75 days, the plasma total protein decreased significantly to be 1.88 ±0.03, 1.60 ±0.04 and 1.91 ±0.13 in 2nd, 3rd & 9th fish groups respectively versus the control group (2.73 ±0.13). Similarly, muscle and hepatic total protein were significantly decreased in fish exposed to 1/8 and 1/4 LC_{50} of Cr alone , mixture of 1/4 LC_{50} of Cr with 0.3 mg EDTA, activated charcoal or super phosphate /l in all periods when compared to the control groups (Table 4). The total protein decrease in plasma, liver and muscle of fishes exposed to metal stress was observed by many investigators (Ambrose *et a*l., 1994, Vutukuru, 2003). The depletion in tissue protein of *Labeo rohita,* may be due to impaired or low rate of protein synthesis under metallic stress as reported by Syversen (1990), Prasanta and Milan (1996) and Vutukuru (2003) or due to their utilization in the formation of mucoprotein which is eliminated in the form of mucus. Further, direct or indirect utilization of protein and lipids for energy needs was also reported (Nagai and Ikeda, 1971).

The present study also showed that Cr is toxic to *O. aureus* and induced alteration at the biochemical processes, more pronounced histopathological changes in different fish groups and it was time and dose dependent. Also, the metal induced alteration

Table (4): The changes of total protein (g/100g) in plasma, liver and muscle of blue tilapia (*O. aureus)* after exposure to chromium and addition chelating agents.

Parameters	Plasma			Liver			Muscle		
Days / Group	7 days	30 days	75days	7days	30 days	75 days	7 days	30 days	75days
1	2.05 ± 0.14	2.48 ± 0.04	2.73 ± 0.13	21.89 ±0.76	23..38 ± 0.55	45.67 ± 1.48	14.33 ± 0.94	11..2 ± 0.181	22.66 ± 1.271
2	1.91 ±0 .06	2.25 ± 0.22	1.88*** ±0.03	14.86*** ± 0.52	15.74*** ± 0.53	29..37*** ± 1.14	9.18*** ± 0.302	9.06*** ±0.293	10.56*** ± 1.89
3	1.79 ± 0.13	1.45*** ± 0.06	1.60*** ± 0.04	16.76** ± 0.98	12.143** ± 0.63	28.585*** ± 0.70	7.5*** ± 0.143	8.95*** ±0.33	8.86*** ± 1.033
4	2.00 ±0 .01	2.79 ± 0.20	2.45 ± 0.11	18.76 ± 1.25	22..55 ± 0.47	41..23 ± 0.62	12.62 ± 0.115	13..28 ± 1.12	17..55 ± 1.29
5	4.36*** ± 008	2.423 ± 0.14	2.1*** ± 0.0 3	23.66 ± 1.17	16.71** ± 1.16	38.28** ± 0.34	10..24** ±0.145	15.14* ± 0.109	14.61** ± 1.02
6	4.34*** ± 0.17	2.59 ± 0.24	2. 69 ±0.05	26.41* ± 1.47	32..92*** ± 1.00	44.48 ± 1.43	12..33 ±1.23	15..9** ± 0.365	14.18** ± 1.22
7	2.05 ± 0.36	2.01 ± 0.15	2.54 ± 0.19	21.32 ±1.98	18..88** ± 1.25	33.64*** ± 1.98	10..32* ±1.152	11.63 ± 0.146	15.66** ± 1.25
8	1.94 ± 0.02	2.12 ± 0.18	2.94 ±0.27	19.45 ±1.90	20.34 ±1.43	34.42*** ± 0.87	11.23 ±1.33	10.6 ± 0.35	21.82 ±1.086
9	2.67 ± 0.09	2.47 ±0.38	1.91** ±0.13	19.01 ±1.43	19.23* ± 1.54	32.23*** ±2.01	10.45 ±2.11	10.12 ±0.79	15.6** ±1.22

Data are represented as means ± S.E

* Significant at P<0.05 **Significant at P<0.01

*** Significant at P<0.001

may probably affect the enzyme mediated biodefence mechanisms of the fish. Further studies are required to elucidate the impact of chromium on detoxifying enzymes for assessing the fish health.

Addition of adsorbents reduced the toxic effect of Cr. Biochemical parameter was improved in fish exposed to Cr with different chelating agents. These results are in agreement with those of Verma *et al.* (1981) who stated that EDTA reduced Cr from liver, kidney, gills and brain of freshwater fish, Notopterus *notopterus.*

The average value of AST activity in plasma of control group was 46.34 ±1.33, 67.05 ± 1.42 and 78.98 ± 3.74 (IU/l) after 7, 30 and 75 days, respectively (Table 5). It could be seen also from data in the table that the mean value of AST was significantly increased in fish of treatments 2 and 3 among the three periods. These values also increased significantly in fish exposed to mixture of 1/4 LC_{50} with 0.3 mg of charcoal or super phosphate/l for 7 and 30 days. In the same trend, the AST values in liver and muscle were increased significantly in fish exposed to all doses of Cr alone at all periods. The increase of AST may reflect the degeneration and necrotic changes observed during the histopathological examination. On the other hand, these values were insignificantly varied in fishes treated with mixture of Cr with EDTA or activated charcoal and or super phosphate (Table 5). The increase of AST levels indicate the damage of the liver that noticed microscopically in this study.

Table (5): The changes of aspartate amino transeferase (AST) in plasma, liver and muscle of blue tilapia *O. aureus* after exposure to chromium and addition chelating agents .

Parameters	Plasma			Liver			Muscle		
Days / Group	7 days	30 days	75days	7 days	30 days	75days	7 days	30 days	75days
1	46.34 ±1.33	67.05 ±1.42	78.94 ±3.74	88.12 ±1.54	98.13 ±1.43	90.08 ±2.759	61.95 ±1.59	55.733 ± 3.1	69.67 ± 2.14
2	86.85*** ±1.31	180.96***8 ±7.55	105.88*** ±6.62	105.20*** ±2.41	120.59* ±1.46	108.98* ± 1.454	76.75* ±1.58	82.6*** ±0.84	140.33*** ± 3.17
3	93.39*** ±5.01	174.88*** ±8.90	103.84*** ±2.97	107.66*** ± 2.24	144.85* ±1.41	118.36** ±2.22	110.35*** ± 3.65	82.86*** ±1.53	164.66*** ±3.57
4	49.64 ±1.84	63..31 ±2.54	64.73 ±4.96	83.85 ±2.10	98.1 ±1.91	100.67* ±1.37	67.3 ±1.89	65.18 ±1.24	64.07 ± 2.32
5	59.98*** ±2.01	84.85** ±4.24	88.87 ±3.02	90.34 ± 2.18	94.51 ±1.49	101.21* ±2.21	61.43 ±2.34	73.42** ±2.01	78.66 ±3.44
6	50.45 ±4.10	66.29 ±2.58	82.83 ±1.81	107.09*** ±2.31	102.53 ±1.98	86.74 ±1.52	57.63 ±3.83	47.83 ±1.22	73.72 ± 2.24
7	54..91** ±1.71	63.99 ±1.45	94..91* ±4.60	98.94* ±3.67	94.88 ±1.44	97.63 ±2.35	64.26 ±239	48.23 ±2.04	79.4 ± 2.16
8	58.62*** ±2.52	62.88 ±1.00	68.57 ±2.90	85.23 ±1.34	100.81 ±1.94	86.58 ±2.21	55.8 ±2.24	56.45 ±1.98	68.9 ± 2.22
9	61.23*** ±1.12	104.25** ±2.14	72.45 ±3.23	92.54 ±1.32	96.43 ±2.44	101.35* ±1.80	66.54 ±2.11	63.89 ±2.22	75.16 ±1.94

Data are represented as means ± S.E

* Significant at P<0.05 **Significant at P<0.01

*** Significant at P<0.001

Evidence for acute Cr nephrotoxicity was provided by distended fish gall bladders indicating distrupted osmoregulation (i.e water retention) as reported by Acharya *et al*. (2001). The increase of AST enzyme activity may be attributed to the effect of the Cr on tissue and their content leakage due to cellular damage (Mathur and Gupta, 1999).

The average value of ALT activity in plasma of control group was 39.52 ±1.02, 43.10 ± 1.25 and 44.30 ± 2.34 (IU/l) after 7, 30 and 75 days of treatment, respectively. It could be seen from data in table (6) that the mean value of ALT was significantly increased in fish treated with 1/8 & 1/4 LC_{50} of Cr among the three periods. These values also increased significantly in fish exposed to mixture of high dose of Cr with all adsorbants substance for 75 days. In the same trend, the ALT in liver and muscle were increased significantly in fish exposed to all doses of Cr alone due to its toxic effects. On the other hand, addition of EDTA , activated charcoal and super phosphate enhancement the changes which occurred in the ALT of fish toxicfied by chromium (Table 6).

Table (6): The changes of alanine amino transeferase (ALT) in plasma, liver and muscle of blue tilapia (*O. aueus*) after exposure to chromium and addition chelating agents .

Parameters	Liver			Muscle			Plasma		
Days / Group	7 days	30 days	75days	7 days	30 da	75days	7 days	30 days	75days
1	39.52 ±1.02	43.1 ±1.25	44.3 ±2.34	55.65 ± 2.03	ND	72.97 ± 3.43	51.56 ± 2.35	63.66 ±1.55	69.01 ±2.44
2	36.0* ±1.50	57.8** ±0.88	93.93** ±2.09	156.8*** ± 3.9	ND	196.13*** ± 5.19	88.82** ±1.80	78.86*** ±1.32	78.33** ±0.918
3	63.87*** ±1.04	64..38*** ±1.25	144.6 *** ±3.95	141.95*** ± 2.77	ND	236.1*** ±4.58	96.25*** ±0.302	89.78*** ±2.64	88.43*** ±1.64
4	38.9 ±1.68	52..35** ±3.42	48.32 ±2.208	59.65 ±3.9	ND	76.3 ±3.38	54.34 ±2.43	59.895 ±1.316	59.89 ±3.316
5	38.62 ±2.52	56.37** ±9.493	67.99** ±1.41	52.4 ±2.34	ND	94.16** ±2.50	60.46 ±1.34	72.0* 2.64	73.64 ±2.64
6	40.32 ±1.33	43.44 ±0.912	40.23 ±3.57	57.63 ±3.83	ND	88.13 ± 3.98	55.67 ±2.39	57.34 ±1.64	67.53 ±2.64
7	39.68 ±1.96	46.57 ±2,99	68.53* ±1.87	54.26 ±1.39	ND	109.4*** ± 2.16	64.87 ±1.96	69.89 ±2.316	59.895 ±3.316
8	38.22 ±1.68	39.15 ±2.37	72.81** ±1.78	45.8 ±2.03	ND	77.43 ± 3.74	49.98 ±2.87	58.87 ±3.2	74.39 ±2.45
9	50.45** ± 2.10	53.1* ±2.27	82.46*** ±0.902	57.76 ±3.01	ND	75.16 ±1.94	63.72 ±2.43	71.67* ±2.3	77.34* ±2.30

Data are represented as means ± S.E

* Significant at P<0.05 **Significant at P<0.01*

** Significant at P<0.001 ND= non detected

In conclusion, the changes in hematological and enzymatic activities with Cr treatement were prevented by addition of EDTA, super phosphate and charcoal being the most effective. These results indicated that EDTA, super phosphate and charcoal were not toxic for liver and kidney of tilapia and the treatment with EDTA is more effective than super phpsphate or charcoal in protecting tilapia from acute hepatic or renal toxicity caused by chromium.

Reproductive changes:

Results of fecundity, gonadosomatic index and 17beta-Estradiol of female *O. aureus* exposed to Cr with or without adsorbent substances are shown in table (7). The fecundity was significantly decreased in female fish expose to sublethal doses of Cr. Also, in the same trend, GSI and 17 beta-Estradiol (levels were significantly decreased to 2.91 ±0.31and 2.35 ±0.46 ng/ml in female fish exposed to Cr toxicity

when compared to the control fish group (4.15 ±0.31 ng/ml). These results agreed with the findings of Al-Hamood *et al*. (1998) who found that fertility was reduced in female offspring mice exposed to either trivalent or hexvalent chromium compound, the body weight and weight of ovaries were reduced after toxicity with Cr. The present results indicate that under our experimental condition, the exposure of female blue tilapia to sublethal doses of Cr impaired reproductive function and fecundity of adults.

Table (7) Changes of fecundity, gonado somatic index and 17β-Estradiol (ng/ml) in female *O. aureus* after exposure to 1/8 and 1/4 LC_{50} of chromium alone and addition chelating agents

Groups / Parameters	1	2	3	4	5	6	7	8	9
Fecu¨ndity	576.5	320.57***	277.76***	542.23	524.45	530.32	519.12	532.22	523.01.5
Fecundity	±21.133	±17.31	±13.41	±15.67	±14.76	±16.7931	±15.65	±15.89	±20.5
GSI	1.24	**0.91**	**0.62****	**1.19**	**1.11**	**1.17**	**1.05**	**1.16**	**1.04**
GSI	±0.131	**±0.11**	**±0.08**	**±0.61**	**±0.31**	**±0.16**	**±0.21**	**±0.11**	**±0.12**
β –Esradiol	4.15	**2.91***	**2.35****	**3.84**	**3.41**	**3.33**	**3.11**	**3.88**	**3.52**
β- Esradiol	±0.311	**±0.31**	**±0.46**	**±0.68**	**±0.59**	**±0.77**	**±0.31**	**±0.67**	**±0.71**

Data are represented as means ± S.E

* Significant at P<0.05 **Significant at P<0.01

*** Significant at P<0.001

Results of sperm count, gonadosomatic index and testosterone levels in male of O*. aureus* exposed to Cr with or without adsorbent substances are shown in table (8). The sperm count was significantly decreased in fish exposed to sublethal dose of Cr. Also, the same trend was observed in the GSI and testosterone levels which were significantly decreased in male fish exposed to Cr toxicity, as

Table (8) Changes of Sperm count (106/cell/ml) , gonado somatic index and testosterone (ng/ml) in male *O. aureus* after exposure to 1/8 and 1/4 LC_{50} of chromium alone and addition chelating agents

Groups / Parameters	1	2	3	4	5	6	7	8	9
Sperm	11.45 ±0.6331	**8.54****	**6.13*****	**8.98**	**8.47****	**9.89**	**8.36****	**9.99**	**9.75**
Count		**±0.60**	**±0.41**	**±0.95**	**±0.53**	**±0.13**	**±0.43**	**±0.35**	**±0.67**
GSI	1.24 ±0.131	**0.81***	**0.56****	**1.16**	**1.0**	**1.09**	**0.90***	**1.16**	**1.01**
		±0.11	**±0.09**	**±0.21**	**±0.09**	**±0.16**	**±0.01**	**±0.21**	**±0.10**
Testosterone	5.27 ±0.511	**3.22****	**2.98*****	**3.84**	**3.82**	**4.12**	**3.59**	**4.15**	**3.99**
		±0.31	**±0.36**	**±0.68**	**±0.53**	**±0.77**	**±041**	**±0.67**	**±0.22**

Data are represented as means ± S.E

* Significant at P<0.05

**Significant at P<0.01

*** Significant at P<0.001

reported also by Al-Hamood et al. (1998) in male mice exposed to chromium compound. Under our experimental condition, the exposure of male blue tilapia to sublethal doses of Cr impaired reproductive function and fertility in adult fishes.

Histopathological findings

The microscopic examination of the gills and internal organs of the control group revealed no marked pathological changes with normal tissue architecture and cellular details (Figs 1-4).

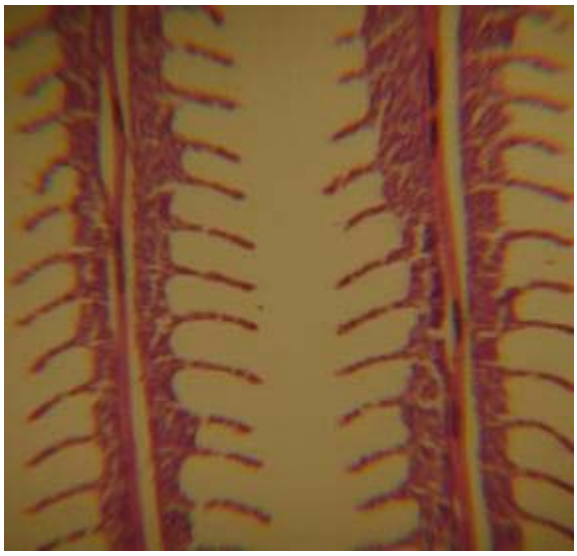

Fig. 1: Section in gill of control fish group showing normal primary and secondary lamellae.

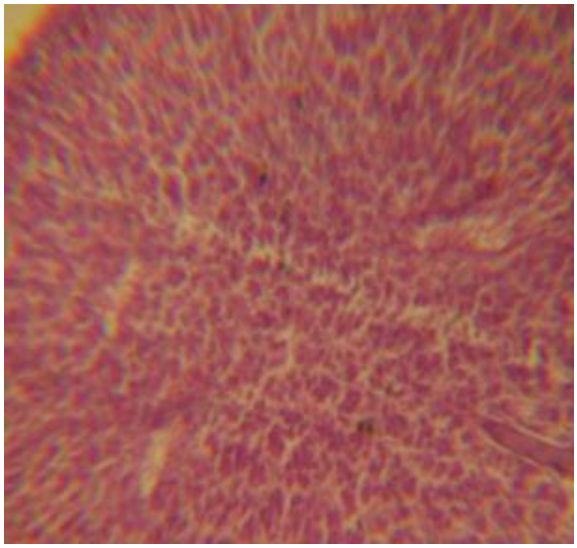

Fig. 2: Section in liver of control fish group showing normal hepatic parenchyma and non dilated blood vessels.

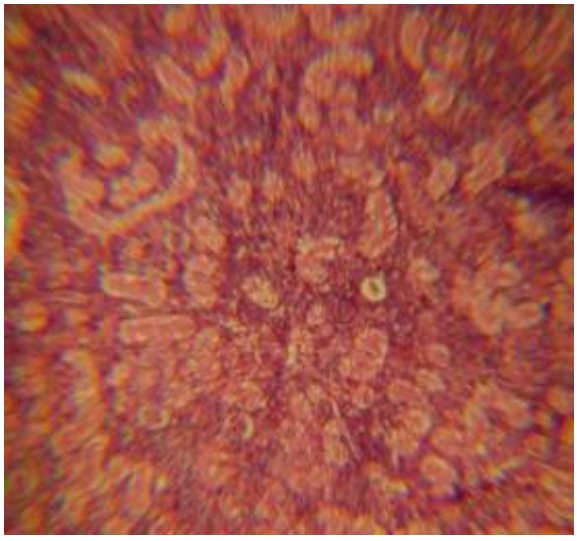

Fig. 3: Section in spleen of control fish group showing both white and red pulp and melanomacrophage center.

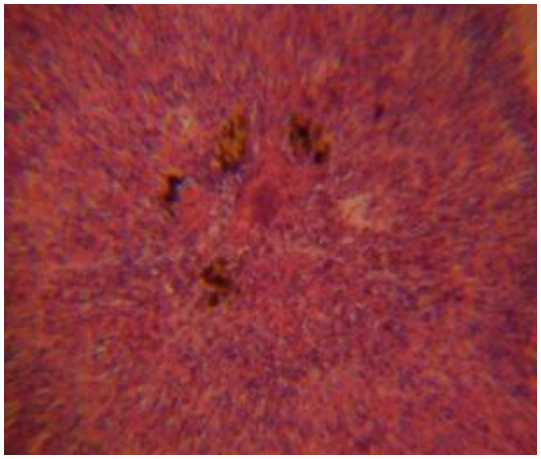

Fig. 4: Section in kidneys of control fish group showing normal renal parenchyma and hematopoietic tissue.

Groups treated with 1/8 LC50 chromium (4.925 mg/l):

After 7 days of exposure, the gills showed sloughing in the epithelial of the secondary lamellae (Fig. 5) with mild edema in the muscles and tubular nephrosis in the kidneys (Fig. 6). Addition of charcoal resulted in hyperplasia of the secondary lamellae

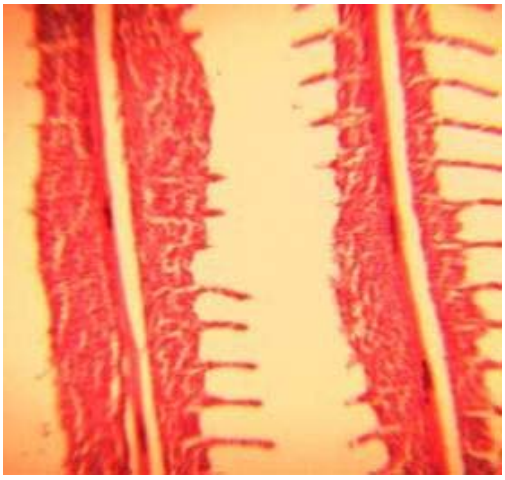

Fig. 5: Section in gill, at 7th day of exposure, showing sloughing in the epithelial of the secondary lamellae.

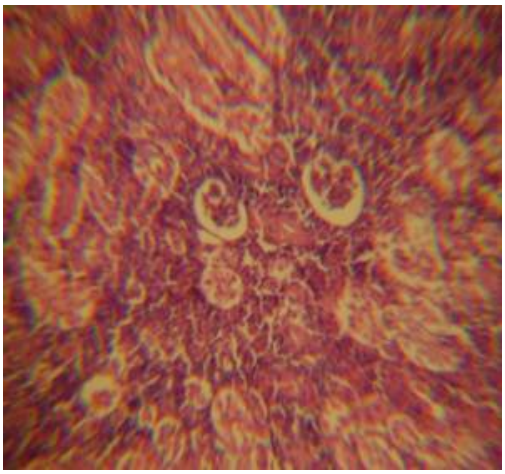

Fig. 6: Section in kidney, at 7th day of exposure, showing tubular nephrosis.

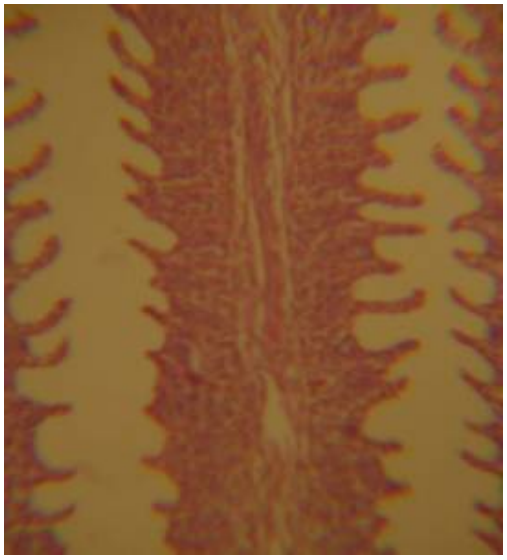

Fig. 7: Section in gill, at 7th day of exposure and treated with EDTA resulted showing excessive hyperplasia in the secondary lamellae.

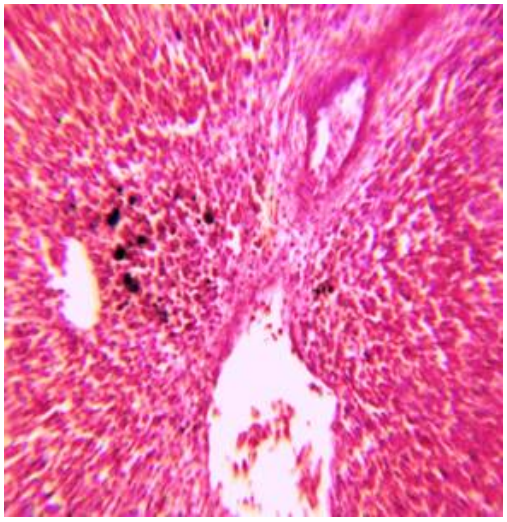

Fig. 8: Section in spleen, at 7th day of exposure and treated with EDTA, showing hyperplasia in melanomacrophages in the spleen.

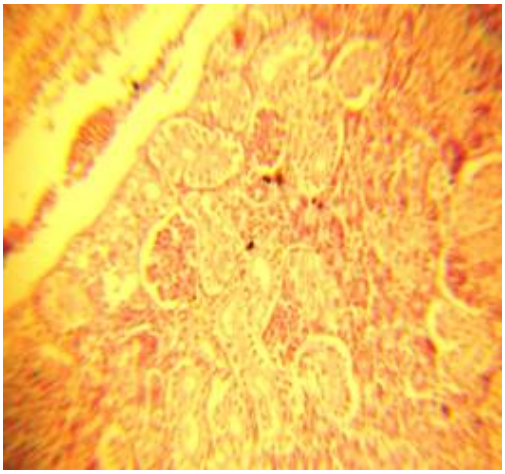

Fig. 9: Section in kidney, at 7th day of exposure and treated with superphosphate, showing mild tubular degeneration in the kidney.

with degeneration in the kidney architecture. Addition of EDTA resulted in congestion and excessive hyperplasia in the secondary lamellae (Fig. 7) with hyperplasia in melanomacrophages in the spleen (Fig. 8). Addition of super-phosphate, induced mild hyperplasia of the secondary lamellae with mild degeneration of the kidney tubules (Fig. 9).

After 30 days of exposure, congestion and epithelial desquamation of the secondary lamellae (Fig. 10) were evident with congestion and hemorrhage in the gill arch. Marked degenerative changes in the liver, kidneys and muscles were also seen.. Addition of charcoal resulted in hyperplasia of the secondary lamellae with mild degeneration in the internal organs. Addition of EDTA revealed mild congestion in the gill arch and lamellae with normal secondary lamellae with mild hyperplasia (Fig. 11). Addition of super-phosphate revealed mild congestion of the gill lamellae and its vacuolation in the liver.

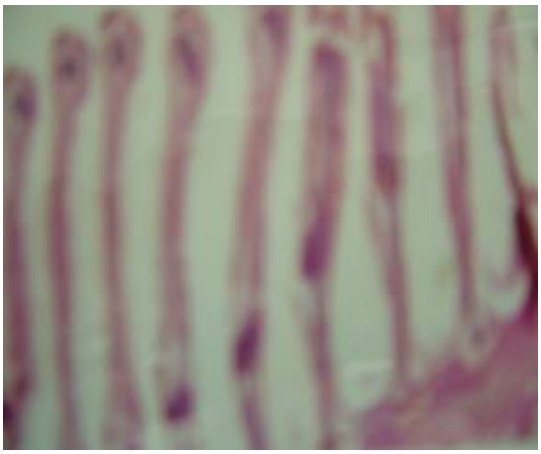

Fig. 10: Section in gill, at 30th days of exposure, showing epithelial desquamation of the secondary lamellae.

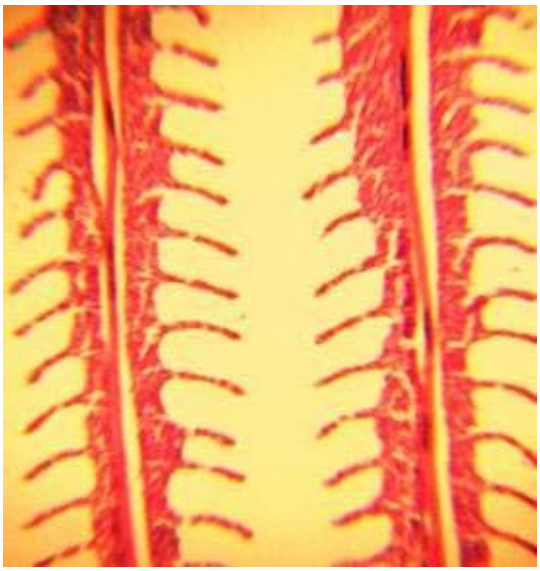

Fig. 11: Section in gill, at 30th days of exposure and treated with EDTA, showing normal secondary lamellae with mild hyperplasia.

After 75 days of exposure, sloughing in the epithelial of the gill lamellae were evident with edema, hemorrhage and mononuclear cells in the gill arch was observed. The kidney showed tubular nephroisis and depletion of hematopoietic tissue (Fig. 12). Addition of charcoal resulted in hyperplasia of the secondary lamellae with focal fusion of their cells. Addition of EDTA resulted in congestion and focal hyperplasia in the secondary lamellae with mild degeneration in the parenchymatous organs. Addition of super-phosphate revealed normal gill lamellae and mild vacuolation in the liver (Fig. 13).

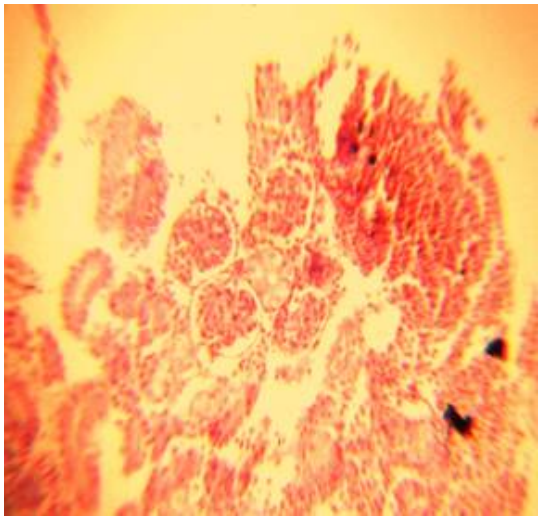

Fig. 12: Section in kidney, at 75th days of exposure, showing tubular nephroisis and depletion of hematopoietic tissue.

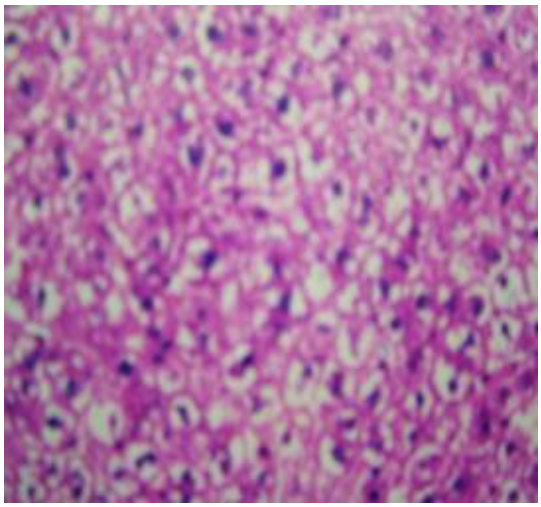

Fig. 13: Section in liver, at 75th days of exposure, showing mild vacuolation.

Groups treated with 1/4 LC_{50} chromium (9.85 mg/l):

After 7 days of exposure, the gills showed degeneration in the epithelium of the secondary lamellae (Fig. 14) with hyaline degeneration in the muscles (Fig. 16) and activation of melanoma-crophages. Addition of charcoal resulted in hyperplasia of the secondary lamellae with focal sloughing (Fig. 15) mild tubular nephrosis and depletion of hemetopoietic tissue. Addition of EDTA revealed mild congestion and hemorrhage in the gill arch and gill lamellae with focal desquamation of the secondary lamellae. Addition of super-phosphate caused congestion and mild proliferation of the secondary lamellae with mild edema in the muscles.

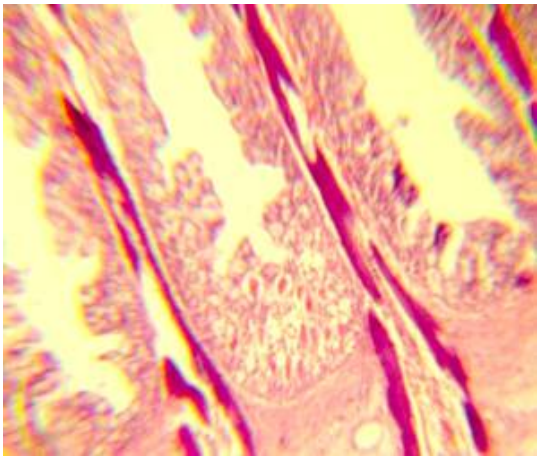

Fig. 14: Section in gill, at 7th day of exposure, showing degeneration in the epithelial of the secondary lamellae.

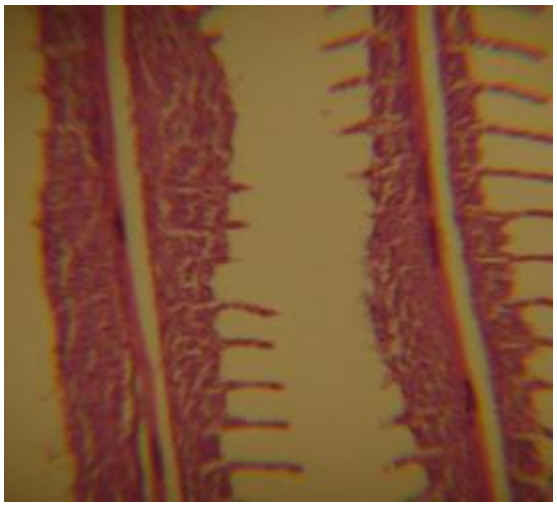

Fig. 15: Section in gill, at 7th day of exposure treated with charcoal showing hyperplasia of the secondary lamellae with focal sloughing.

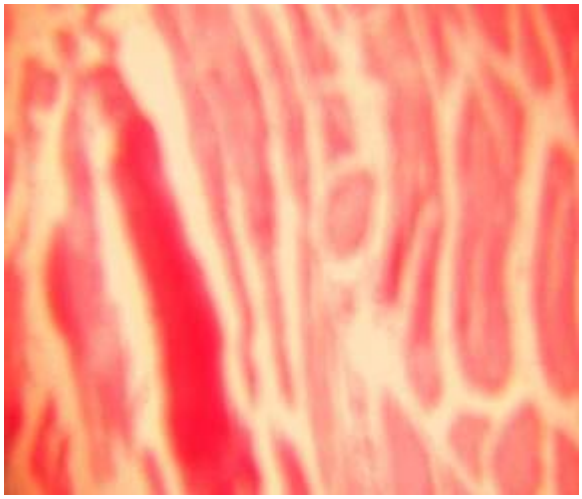

Fig. 16: Section in muscle, at 7th day of exposure, showing hyaline degeneration.

After 30 days of exposure, congestion and hyperplasia in the gill lamellae were evident with edema and eosinophilic granular calls in the gill arch. Addition of charcoal resulted in edema in the gill arch, hyperplasia of the secondary lamellae, mild tubular nephrosis, atrophy of melanomacrophages and focal depletion of hemetopoietic tissue. Addition of EDTA revealed mild congestion, hemorrhage and leukocytic infiltration in the gill arch and lamellae with focal desquamation of the lamellar epithelium. Addition of super-phosphate showed hyperplasia of the secondary gill lamellae and edema as well as hemorrhage in the gill arch.

After 75 days of exposure, the gill revealed hyperplasia in the secondary lamellae and mononuclear cells infiltrations with degenerative and necrotic changes in the parenchymatous organs including the liver (Fig. 17). Addition of charcoal resulted in congestion and hyperplasia in the tip of the primary lamellae with marked degeneration in the liver. Addition of EDTA showed congestion and hyperplasia in the gill lamellae with focal sloughing of secondary lamellae and nuclear pyknosis of hepetocytes. Addition of super-phosphate revealed hyperplasia in the gill lamellae with mild tubular nephrosis and focal depletion of hematopoietic tissue (Fig. 18).

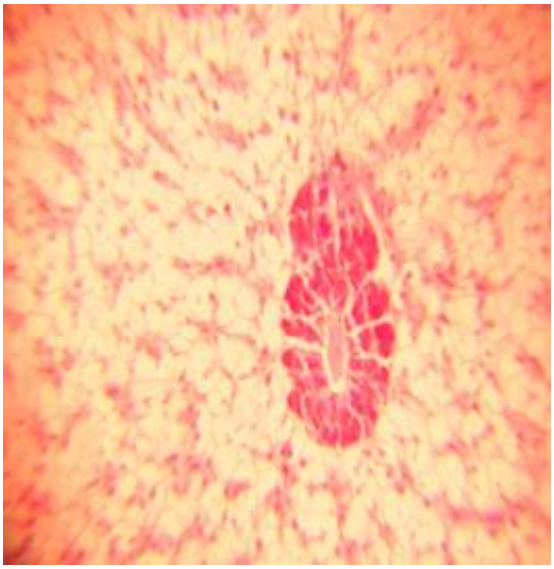

Fig. 17: Section in liver, at 75th days of exposure, showing degenerative and necrotic changes including the liver.

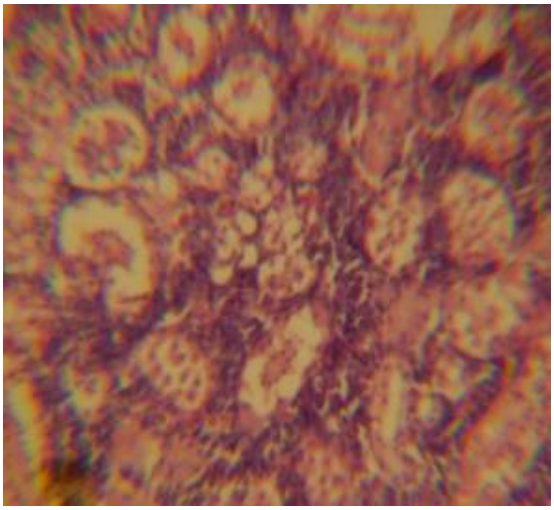

Fig. 18: Section in kidney, at 75th days of exposure, showing mild tubular nephrosis and focal depletion of hematopoietic tissue.

However, the histopathological findings of chromium exposed fish compared to Wong *et al***.** (1982) were more or less similar dependent upon dose and period of exposure. The improvement of this picture by using the chelating agent support the concept of Hilmy *et al*. (1986).

CONCLUSION:

It could be concluded that water pollution with chromium included significant negative impact in the hematological and biochemical parameters together with marked histopathological alteration and lowering reproductive performance of fish. The addition some chelating agents (EDTA, super phosohate and charcoal) could decrease this degree of toxic effects of chromium and improve the fish condition.

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ألاستجابة التكاثرية والفسيولوجية المرضية لاسماك البطى الأوريا المعرضة لعنصر الكرميوم منفردا أو مختلطا مع المواد المد مصة.

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1-قسم التفريخ وفسيولوجيا الأسماك

2- قسم امراض الأسماك بالمعمل المركزي لبحوث الثروة السمكية, العباسة, أبوحماد, شرقية.

3- المركز الدولى للاسماك. العباسة, أبوحماد, شرقية

اجرى هذا البحث لدراسة تأثير عنصر الكرميوم ومعالجته باضافة بعد المواد المدمصة لها قدرة على امتصاص العناصر فى الوسط المائى (أديتا, سوبر فوسفات, والفحم النشط) على بعض التغيرات الفسيولوجية وا البيوكيميائية والتغيرات المرضية فى الأنسجة وكذلك التغيرات المحدثة فى الأداء التكاثرى فى الأسماك البلطى الأوريا (40- 45جم).

وتم توزيع الأسماك فى تسعة معاملات حيث تركت المجموعة الأولى (المجموعة الضابطة) للمقارنة بينما تعرضت المجموعة الثانية والثالثة الى ثمن وربع الجرعة المميتة لعنصر الكرميوم (529و4, 58و9 مجم كرميوم/ لتر فقط على التوالى وكذلك تعرضت المجموعتين والرابعة والخامسة الى مخلوط من نفس تركيز الكرميوم (529و4, 58و9 مجم/لتر) مع 3و جم الأديتا/ لتر. كما تعرضت المجموعات السادسة والسابعة الى مخلوط من نفس تركيز الكرميوم (529و4, 58و9 مجم/لتر) مع 3و جم سوبر فوسفات. وكذلك تعرضت المجموعتين الثامنة والتاسعة إلى مخلوط من نفس تركيز الكرميوم (529و4, 58و9 مجم/لتر) مع 3و جم الفحم النشط على التوالي سوبر فوسفات عت مختلفة من الأديتا (1و، 2و، 3و جرام /لتر) على التوالى لمدة 7، 30 ,75 يوما.وأسفرت النتائج عن الأتى:

1- حدوث نقص معنوى فى معدلات الدم (كرات الدم الحمراء والهيموجلوبين والهياتوكريت فى البلطى الأوريا بعد تعرضة للكرميوم فقط.بينما اضافة 3و جم /لتر من الأديتا او سوبر فوسفات او الفحم النشط الى هذا الوسط الملوث بالكرميوم ادى الى تحسن معدلات الدم السابقة فى هذة المجموعات خلال 7 و30, 75 يوما.

2- حدث زيادة معنوية فى جلوكوز الدم و الأنزيمات الناقلة لمجموعات الأمينى فى بلازما الدم والكبد وعضلات الأسماك التى تعرضت للكرميوم فقط. . وقد عادت هذة القياسات الى المستوى الطبيعى كما فى المجموعة الضابطة فى الأسماك التى تعرضت الى مخلوط منالكرميوم والمواد الممدصة.

3-حدث انخفاض معنوى فى المحتوى البروتينى والدهنى بالكبد والعضلا ت والبلازما الأسماك التى تعرضت لعنصر الكرميوم منفرد. بينما حدث تحسين معنوى للمحتوى الكيمائى فى الأسماك التى تعرض الى مخلوط من الكرميوم مع الأديتا او مع السوبر فوسفات او مع الفحم.

4-حدث اختزال معنوى فى خصوبة الأسماك التى تعرضت للكرميوم فقط بينما اضافة هذة المواد اللمدصة لعنصر الكرميوم ادت الى تحسين الخصوبة ومعامل المناسل الجسدى وزيادة مستوى هرمون الأستروديول فى اناث البلطى الأوريا وذيادة عدد الحيونات المنوية ومستوى الهرمون الذكرى (التستستيرون) فى ذكور البلطى الأوريا خلال 75 يوما

واوضحت النتائج حدوث تحسن ملحوظ فى التغيرات الفسولوجية والبيوكيميائية التى حدثت فى مكونات الدم ونسبة الجلوكوز والنشاط الأنزيمى والمحتوى البروتينى بالكبد والعضلات وتحسين خصوبة الأسماك والحالة المرضية بأنسجة الأسماك عندما تم اضافة جرعة 3و جم من الأديتا او االسوبلا فوسفات او الفحم النشط الى الوسط المائى الملوث بالكرميوم. مما سبق نستخلص ان اضافة جرعات محددة (3وجم /لتر) من الأديتا او السوبر فوسفات او الفحم النشط الى الوسط المائى الملوث بالكرميوم يؤدى الى ازالة هذا الملوث من المياة حيث يتم تكوين مركب معقد من الكرميوم لايمكن امتصاصة عبر انسجة الكائن الحى مثل الأسماك مما يؤدى الى عدم تعرض هذة الأسماك لسمية عنصر الكرميوم وبذلك تكون هذة الأسماك امنة صحيا. ولذا نوصى باضفة 3و جرام اديتا او الفحم النشط /لترماء بالمياة الصرف الزراعى المستخدمة لتربية الأسمااك لحمايتها من التلوث

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