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Research Article
Protective Role of Vitamin C and Thyme Extract (*Thymus vulgaris*) on Chromium-Induced Toxicity in Catfish (*Clarias gariepinus*)

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Abstract

Background and Objective: Chromium toxicity in aquatic environment may occur through industrial pollutions. The impact of its toxicity may represent health hazards for aquatic environments and public health. Therefore, *Clarias gariepinus* were used to investigate the chromium toxicity and assess the ameliorative effect of thyme and vitamin C. **Materials and Methods:** A total of 90 fish were divided into sixequal groups (n = 15). Group 1 (control negative) was fed on basal diet without chromium in water. Group 2 (control positive) was fed on basal diet with Potassium Dichromate (PD) at dose 3.6 mg L^{-1} dissolved in water. Group 3 was fed on diet with vitamin C at dose 200 mg kg^{-1} of diet without PD in water. Group 4 was fed on vitamin C diet with PD in water. Group 5 was fed on diet supplemented with Thyme extract at dose 10 g kg^{-1} dry food without PD in water. Group 6 was fed on thyme diet with PC in water. The experiment was done for a period of 30 days. Analyses of blood, antioxidant in tissues, micronucleus test, chromium tissue residues, comet assay and histopathology were done. **Results:** There was a significant decrease ($p<0.001$) in RBCs count, Hb content, PCV% and WBCs count with significant increase ($p<0.001$) in liver and kidney function. There were increased activities of SOD, CAT, GSH and MDA and chromium tissue residues. DNA damage and histopathological changes occurred in groups with chromium when compared to control negative group. Vitamin C and thyme extract decreased the toxic effect of chromium and improved biochemical parameters, with reduction of chromium residues in gills and muscles. **Conclusion:** Therefore, vitamin C and thyme extract can be used as ameliorative factors against chromium toxicity in aquaculture.

Key words: Catfish, chromium toxicity, oxidative damage, biochemical changes, vitamin C and thyme extract

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish are delicious foods that provide humans with high quality protein, various minerals, poly unsaturated fatty acids and vitamins. In addition, Fish are considered as one of the valuable bioindicators for pollution in the aquatic habitats as a result of their lower detoxification enzymes than those in mammals and thereby allowing a higher bioaccumulation for toxicants[1,2]. The hazardous compounds that accumulate in their tissues are directly or indirectly consumed by human and can transform xenobiotic compounds into carcinogenic and mutagenic metabolites[3]. The Nile in Egypt facing major environmental problems associated with the dispersal or disposal of agricultural, industrial and urban wastes generated by human activities[4]. These pollutants cause serious damage to aquatic life[5]. Toxic metals are widely found in aquatic environments. These compounds have been found to be highly toxic, genotoxic, mutagenic and carcinogenic. Among them, water pollution due to chromium (Cr) is regarded as one of the main and most severe environmental problems[6].

Chromium is considered as a heavy metal and environmental pollutant as well as an essential micronutrient[7]. In nature chromium does not exist as elementary form (Cr (0)) but exists in Earth Crust in many oxidation states. Chromium oxidation states range from divalent (Cr(II)) to hexavalent (Cr (VI)) but is most frequently found in the environment in the trivalent (Cr (III))and hexavalent (Cr (VI))oxidation states. The trivalent and hexavalent forms are the most important because other forms are unstable and are rapidly converted to trivalent chromium, which in turn is oxidized to hexavalent chromium[8].

In aquatic environment chromium is found as Cr (III) and Cr (VI) as water soluble complex anions. Cr has truly unique toxicological characteristics. Toxicological impact can result from the action of Cr (VI) itself as an oxidizing agent[9]. Also, Cr (VI) is more toxic due higher solubility and mobility, ability to penetrate the cell membrane and strong oxidizing ability. The hexavalent form of chromium exists in the form of chromate, hydro chromate or dichromate in dissolved condition as a component of a complex anion[10]. Potassium dichromate ($K_2Cr_2O_7$) is an inorganic soluble hexavalent chromium compound that is widely used in several industries[11]. Fish upon exposure to pollutants (Cr) can elicit the production of Reactive Oxygen Species (ROS) like superoxide, hydrogen peroxide and hydroxyl radical as the ROS levels increase, the biological system develops a first line defense mechanism by modulating the activities of antioxidants such as catalase (CAT), Superoxide Dismutase (SOD) and glutathione related enzymes[12]. The genotoxicity of Cr(VI) has been reported in human and animals[13,14]. There are many methods like micronucleus test and DNA damage assays as Comet assay have been used for assessing genotoxicity of various chemicals in different animals[15,16].

Because of the hazardous effect of Cr in aquaculture, several studies were designed to find out protective agents. Vitamin C (Ascorbic acid) is an important antioxidant vitamin that is essential nutrient for optimum growth and maintenance[17]. Another beneficial effect of vitamin C, which has been established in several animal species, including fish, is its role in enhancing the non-specific immune response[18]. It also prevents oxidative renal and brain damage induced by stress and secures the body tissues against toxic effects of heavy metals by efficiently metabolizing these toxicants. Ascorbic acid can reduce chromium-induced toxicity on liver and kidney[19].

Thyme (*Thymus vulgaris*) is herbaceous plant of the platoon species, grows in mountainous areas, used as a beverage instead of or with tea and added to some food to give it an acceptable flavor. the plant is used in folk medicine frequently where it is prescribed to treat mouth infections, stomach, intestine and airways, coughing and gastroenteritis and expel intestinal worms as well as to strengthen the heartcontractility[20]. Fresh Thyme herb has one of the highest antioxidant levels among herbs. It is packed with minerals and vitamins that are essential for optimum health. Its leaves are one of the richest sources of potassium, iron, calcium, manganese, magnesium and selenium[21]. Extracts from Thyme have been used in traditional medicine for the treatment of several respiratory diseases like asthma and bronchitis and for the treatment of other pathologies thanks to several properties such as antiseptic, antispasmodic, antitussive antimicrobial, antifungal, antioxidative and antiviral[22].

Because Cr exposure is more likely to occur in aquaculture due to heavy industrial pollution, it was very crucial to investigate some of natural protective agents that reduce it deleterious effects. Therefore, the present study aimed to evaluate the toxic effect of potassium dichromate in catfish and amelioration and antagonizing this toxic effect by using vitamin C and thyme extract.

MATERIALS AND METHODS

Study area and location: *Clarias gariepinus*, commonly known as African catfish was selected as an experimental model. The fish within average weight 150±20 g were collected from River Nile (El Rayah El Tewfik) of Qalubia, Egypt and transported alive to the laboratory of Physiology Department of Faculty of Veterinary Medicine, Benha

University where the experimental procedures were done from August 1-31, 2018. The fish were placed in large glass tanks disinfected with potassium permanganate to prevent fungal infection and washed thoroughly prior to introduction of fish[23]. Fish were distributed equally to six groups and kept in glass tanks with tap water with a photoperiod 12 hrs light and 12 hrs dark was maintained during the experiment[24].The fish were acclimated to the laboratory conditions and water parameters for two weeks prior to the experiment. The tank water was partially changed every day with aerated tap water. The water was continuously aerated. All groups were fed on basal diet twice a day at a daily rate of 4% body weight thorough out the acclimatization period. The experiment was conducted according to the rules of the Ethical Committee of Faculty of Veterinary Medicine, Benha University.

Experimental design: The experiment was done on 90 fish of Claris *gariepinus*, fish were divided into 6 groups (15 fish per group) as following: Group 1 represented as control group that fed only on basal diet. (Control negative), group 2 (control positive) was fed on basal diet and exposed to PD (Cr VI) (Al Gomhorria Company, Egypt) dissolved in water at dose 3.6 mg L^{-1} (1|10th of LC_{50}) according to the median lethal concentration dose[25]. Group 3 was fed on diet supplemented with vitamin C (Al Kahera Pharm Company) at dose 200 mg kg^{-1} of diet as previously recorded[26]. Group 4 was exposed to both vitamin C and Potassium dichromate at the same doses described above, group 5 was fed on diet supplemented with Thyme extract (*Thymus vulgaris*) at dose 10 g kg^{-1} dry food[27]. Group 6 was exposed to both thyme extract and potassium dichromate at the same doses. The overall period of experiment was 30 days.

Preparation of the plant extracts (aqueous and alcoholic): *Thymus vulgaris* extract was prepared as previously reported[28]. The thyme extract was added to the fish diet as 10 g kg^{-1} dry food.

Blood and tissue sampling: The blood samples were collected at the end of the experiment (30 days), about 1 mL of blood was collected from the caudal vein of fish by caudal venous puncture using sterile syringes[29]. Apart of the collected blood placed in tubes with EDTA used as anticoagulant while the other part of blood placed in sterile centrifuge tubes for serum separation by centrifugation at 3500 rpm for 15 min. The whole blood was collected and immediately processed for Micronucleus test. On the final day of the experimental period, fish were sacrificed and the tissues dissected for various analysis as follows, Liver and kidney tissues samples were collected and preserved in -80°C for determination of antioxidant parameters and Comet assay. Muscles and gills samples collected and preserved in deep freeze for determination of chromium residues. Tissue sample slices from liver, kidney and gills were kept in 10% formalin for histopathological examination.

Hematological and biochemical analysis: Hematological parameters were measured and analyzed including Red Blood Cells (RBCs) count, white blood cells (WBCs) count, Hemoglobin (Hb) and Packed cell volume (PCV%) Using the routine methodology of fish hematology[30,31]. Biochemical parameters were measured in the collected serum samples including alanine aminotransferase (ALT), aspartate aminotransferase (AST)[32], alkaline phosphatase (ALP), total protein, albumin and cholesterol[33] and urea, creatinine and uric acid[34]. Antioxidant parameters were measured in liver and kidney tissues including SOD[35], CAT activity[36], Reduced Glutathione (GSH)[37] and Malondialdehyde (MDA)[24]. The hematological and biochemical analysis were done at the Central Lab of Faculty of Veterinary Medicine, Benha University.

Micronucleus test: The peripheral blood samples were smeared onto the clean glass slides from each group. The slides were air dried for 24 hrs after fixation in pure ethanol for 20 min. The smeared slide was then stained with 6% Giemsa solution for 25 min, then washed and dried. The slides were examined under microscope using oil immersion lens (100× magnification). A total of 2000 erythrocyte cells were scored from each slide for presence or absence of micronuclei. Small, extra-nuclear, circular or ovoid bodies were scored as micronuclei[6]. Micronucleus test was done at veterinary Teaching Lab of the Faculty of Veterinary Medicine, Benha University.

Chromium tissue residues: The samples were taken from tissues of muscles and gills. About 5 g of tissue samples were wrapped in aluminum foil and stored on ice until transfer to freezer and later on analyzed using atomic absorption spectrophotometer in Soil, Water and Environment Research Institution (SWERI) as recorded[38] for detection of Cr residues concentration in tissues.

Comet assay: Comet assay was performed in liver tissues[39] for detection of DNA damage induced by hexavalent Cr. Tail Length (TL) and Tail Moment (TM) were analyzed and documented. An increase in TL/TM on the slides is indicative for the occurrence of direct DNA damage.

Histopathology: Different parts of liver, kidney and gills were taken from the different groups. The specimens were fixed in Bouin's solution. After fixation, the specimens were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Paraffin sections were stained as previously reported[40].

Statistical analysis: Statistical analysis of data was performed and expressed by Means±SE using one way analysis of variance (ANOVA) to determine the significant differences between the control and experimental groups. The differences were considered significant when $p<0.001$.

RESULTS

Clinical findings: After the end of the experiment, fish were examined for any abnormal appearance. During the examination of fish, it was observed that there were many changes on the external morphological appearance of the examined cat fish, these changes were the presence of many erosions in fins and fin rays in fish that exposed to potassium dichromate.

Hematological findings: The data presented in Table 1 showed that exposure to chromium toxicity in catfish decreased the mean values of RBCs (10^6 mm^{-3}), Hb (g dL^{-1}), PCV% and WBCs (10^6 mm^{-3}) count from 3.7, 7.66, 31.66 and 37.77 to 2.6, 6.1, 28.6, 32.93, respectively. Addition of vitamin C increased mean values of RBCs, Hb, PCV% and WBCs count compared to group 2 exposed to chromium. Addition of thyme extract increased RBCs, Hb, PCV% and WBCs count values compared to group 2 exposed to chromium group.

Biochemical analysis: Data presented in Table 2 showed that exposure to chromium in catfish (group 2) increased values of liver function [AST (µ L^{-1}), ALT(µ L^{-1}), ALP(µ L^{-1}) and cholesterol (mg dL^{-1})] and kidney function [urea, uric acid and creatinine (mg dL^{-1})] from 61.44, 23.696, 32.69, 136.016, 13.87, 3.28, 0.196 to 133.3, 40.31, 97.71, 293.33, 24.276, 4.69 and 0.563, respectively. Addition of vitamin C significantly decreased these values . The albumin, total protein IgA and IgG decreased in group 2 chromium exposed catfish. Whereas addition of vitamin C to chromium exposed group significantly elevated the mean values of these parameters. Also, Addition of thyme extract significantly increased the mean values of these parameters.

Antioxidant assays: The data presented in Table 3 showed that exposure of catfish to chromium (group 2) increased values of liver SOD (U g^{-1}), CAT(U g^{-1}), GSH(mg g^{-1}) and MDA (nmol g^{-1}) from 84.0±2.31, 4.35±0.030, 12.60±0.07, 164.53±5.67 to 99.72±7.35, 4.95±0.03, 20.05±0.89, 465.3±2.86, respectively. Addition of vitamin C to chromium exposed group reduced these values to 98.48±0.37,

Table 1: Hematological findings of control and examined groups of catfish

Parameters	G_1 (n = 15)	G_2 (n = 15)	G_3 (n = 15)	G_4 (n = 15)	G_5 (n = 15)	G_6 (n = 15)
RBCs count (10^6 mm^{-3})	3.7 ± 0.057^{a}	2.6 ± 0.057^{d}	3.5 ± 0.115^{b}	2.9 ± 0.15^{cd}	3.7 ± 0.057^{c}	3.0 ± 0.057^{d}
Hb (g dL^{-1})	7.66 ± 0.33^{b}	6.1 ± 0.057^{d}	8.26 ± 0.375^{a}	7.26 ± 0.128^{ba}	7.7 ± 0.288^{ba}	7.1 ± 0.346^{ba}
PCV (%)	31.66 ± 0.837^{ab}	28.6 ± 0.208^{d}	33.8 ± 1.732^{a}	30.00 ± 1.558^{b}	30.56 ± 0.088^{b}	29.86 ± 0.260^{b}
WBCs count (106 mm^{-13})	37.77 ± 5.46^{b}	32.93 ± 2.97^{d}	37.9 ± 2.07^{a}	34.8 ± 0.866^{c}	36.166 ± 1.18^{b}	35.10 ± 1.44^{b}

G_1 (control negative): Basal diet without chromium in water, G_2 (control positive): Basal diet with PD, G_3: Diet with vitamin C without PD in water, G_4: Diet with Vitamin C with PD in water, G_5: Diet with Thyme extract without PD in water, G_6: Diet with vitamin Thyme extract with PD in water, means with different superscript letters in the same row are significantly different at $p<0.001$

Table 2: Biochemical results of control and examined groups of catfish

Parameters	G_1 (n = 15)	G_2 (n = 15)	G_3 (n = 15)	G_4 (n = 15)	G_5 (n = 15)	G_6 (n = 15)
AST (u L^{-1})	61.44 ± 9.33^{d}	133.3 ± 4.9^{a}	78.59 ± 5.55^{cd}	92.04 ± 3.02^{bc}	75.66 ± 10.39^{b}	109.00 ± 5.04^{bcd}
ALT (u L^{-1})	23.696 ± 1.95^{b}	40.31 ± 3.92^{a}	24.46 ± 1.84^{b}	28.39 ± 3.12^{b}	22.66 ± 2.02^{b}	32.74 ± 5.87^{ab}
ALP (u L^{-1})	32.69 ± 3.83^{c}	97.71 ± 17.31^{a}	54.16 ± 3.06^{bc}	98.66 ± 18.52^{a}	38.66 ± 0.88^{c}	75.60 ± 6.35^{ab}
Chol (mg dL^{-1})	136.016 ± 8.14^{b}	293.33 ± 26.034^{a}	155.74 ± 13.21^{b}	244.66 ± 2.0^{a}	173.22 ± 8.90^{b}	176.00 ± 8.04^{b}
Alb (mg mL^{-1})	30.33 ± 1.039^{a}	13.58 ± 1.44^{c}	20.19 ± 1.605^{b}	21.29 ± 0.35^{b}	15.14 ± 1.47^{c}	32.69±2.21a
TP (mg mL^{-1})	50.55±5.164a	31.03 ± 4.36^{b}	45.51 ± 2.28^{ab}	37.04 ± 6.69^{ab}	43.88 ± 4.02^{ab}	51.77 ± 3.91^{a}
Urea (mg dL^{-1})	13.87 ± 0.278^{b}	24.276 ± 2.94^{a}	12.74 ± 0.855^{b}	14.92 ± 2.61^{b}	14.55 ± 2.25^{b}	16.56 ± 2.80^{b}
Uric acid (mg dL^{-1})	3.28 ± 0.275^{b}	4.69 ± 0.514^{a}	2.823 ± 0.08^{bc}	3.29 ± 0.162^{b}	2.21 ± 0.22^{c}	3.503 ± 0.26^{b}
Creatinine (mg dL^{-1})	0.196 ± 0.023^{b}	0.563 ± 0.044^{a}	0.15 ± 0.047^{b}	0.158 ± 0.04^{b}	0.13 ± 0.026^{b}	0.276 ± 0.09^{b}
IgA (mg mL^{-1})	4.65 ± 0.475^{a}	2.856 ± 0.401^{b}	4.186 ± 0.210^{ab}	3.40 ± 0.61^{ab}	4.046 ± 0.36^{ab}	3.763 ± 0.35^{ab}
IgG (mg mL^{-1})	3.06 ± 0.313^{a}	1.876 ± 0.265^{b}	2.753 ± 0.140^{ab}	1.99 ± 0.48^{b}	3.133 ± 0.23^{a}	2.66 ± 0.24^{ab}

Means with different superscript letters in the same row are significantly different at $p<0.001$

Table 3: Antioxidant results of liver of control and examined groups of catfish

Parameters	G_1 (n = 15)	G_2 (n = 15)	G_3 (n = 15)	G_4 (n = 15)	G_5 (n = 15)	G_6 (n = 15)
SOD (U g^{-1})	84.00±2.31^{b}	99.72±7.35^{a}	103.68±2.49^{b}	98.48±0.37^{b}	84.0±2.31^{b}	86.00±1.15^{b}
CAT (U g^{-1})	4.35±0.030^{bc}	4.95±0.03^{a}	4.78±0.05^{ab}	3.39±0.27^{d}	4.01±0.17^{bc}	3.73±0.12^{cd}
GSH (mg g^{-1})	12.60±0.07^{b}	20.05±0.89^{a}	8.78±0.19^{c}	12.52±0.28^{b}	11.59±0.66^{b}	12.61±0.46^{b}
MDA (nmol g^{-1})	164.53±5.67^{d}	465.3±2.86^{a}	125.82±7.01^{e}	268.89±1.78^{b}	123.72±1.71^{d}	226.38±8.54^{c}

Means with different superscript letters in the same row are significantly different at p<0.001

Table 4: Antioxidant results of kidneys of control and examined groups of catfish

Parameters	G_1 (n = 15)	G_2 (n = 15)	G_3 (n = 15)	G_4 (n = 15)	G_5 (n = 15)	G_6 (n = 15)
SOD (U g^{-1})	38.00±3.46^{c}	88.00±2.3^{a}	70.00±1.15^{b}	56.00±9.24^{b}	26.00±3.46^{c}	62.50±0.88^{b}
CAT (U g^{-1})	3.53±0.14^{cb}	4.93±0.53^{a}	3.84±0.09^{bc}	4.07±0.22^{ba}	4.28±0.18^{ba}	2.83±0.51^{c}
GSH (mg g^{-1})	31.27±0.18^{a}	32.52±0.29^{a}	28.47±2.08^{a}	12.35±2.93^{b}	22.53±6.06^{a}	25.98±3.21^{a}
MDA (nmol g^{-1})	127.00±11.67^{b}	370.60±50.14^{a}	188.29±7.12^{b}	212.31±23.56^{b}	190.57±4.1^{a}	316.50±36.27^{b}

Means with different superscript letters in the same row are significantly different at p<0.001

Table 5: Chromium residues in muscles and gills of control and examined catfish

Parameters	G_2	G_4	G_6
Muscle (mg kg^{-1})	533.0±14.17^{b}	189.1±8.180^{a}	184.0±4.50^{c}
Gills (mg kg^{-1})	3021.0±01.59^{a}	2494.0±2.05^{b}	1066.0±76.02^{c}

Means with different superscript letters in the same row are significantly different at p<0.001

3.39±0.27, 12.52±0.28, 268.89±1.78, respectively. Addition of thyme extract to chromium exposed group decreased these values to 86.0±1.15, 3.73±0.12, 12.61±0.46 and 226.38±8.54, respectively.

The data presented in Table 4 showed that exposure to chromium in catfish (group 2) increased mean values of kidney SOD (U g^{-1}), CAT (U g^{-1}), GSH (mg g^{-1}) and MDA (nmol g^{-1}) to 88.0, 4.93, 32.52, 370.60, respectively. Addition of vitamin C to chromium exposed group reduced these values to 56.0, 4.07, 12.35 and 212.31, respectively. Addition of thyme extract to chromium exposed group decreased the mean values of these parameters.

Micronucleus test: The micronucleus was demonstrated in RBCs as shown in Fig. 1a and b. The occurrence of micronucleus was increased from 2.0±0.288 to 23.0±1.58 after exposure to chromium. Addition of vitamin C to chromium exposed group reduced the micronucleus to 12.66±1.45. Addition of thyme extract to chromium exposed group decreased the micronucleus to 13.0±1.154.

Chromium residues: The data presented in Table 5 showed that addition of vitamin C and thyme extract to chromium exposed catfish reduced the chromium residues in muscle from and in gills.

Comet assay: The data presented in Table 6 showed that cat fish exposed to chromium had increase in tail length, DNA%, Tail moment and olive moment from. Addition of vitamin C to chromium exposed catfish reduced these values. Also, addition of thyme extract to chromium exposed catfish reduced the mean values of these measurements.

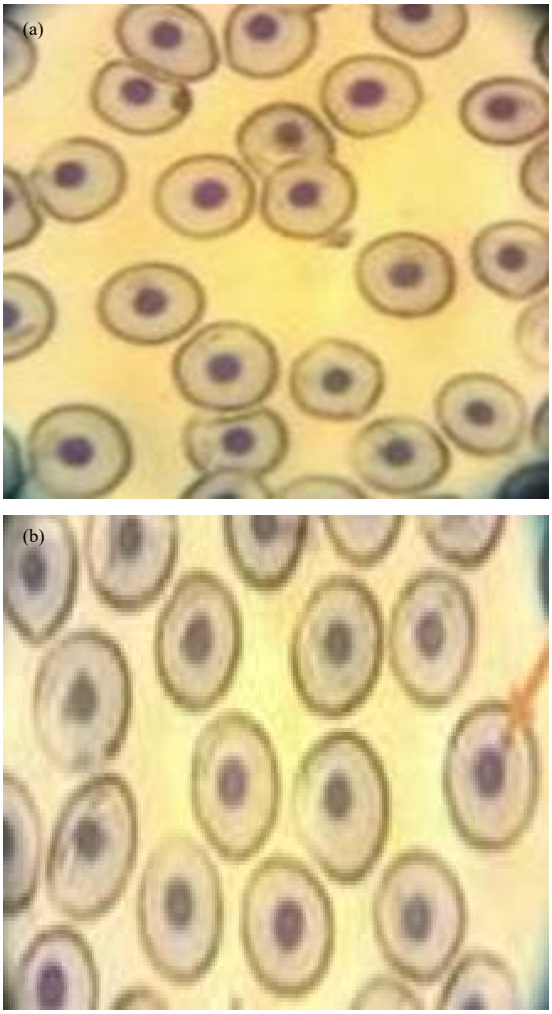

Fig. 1(a-b): (a) Normal RBCs without micronucleus and (b) RBCs with micronucleus (arrow)

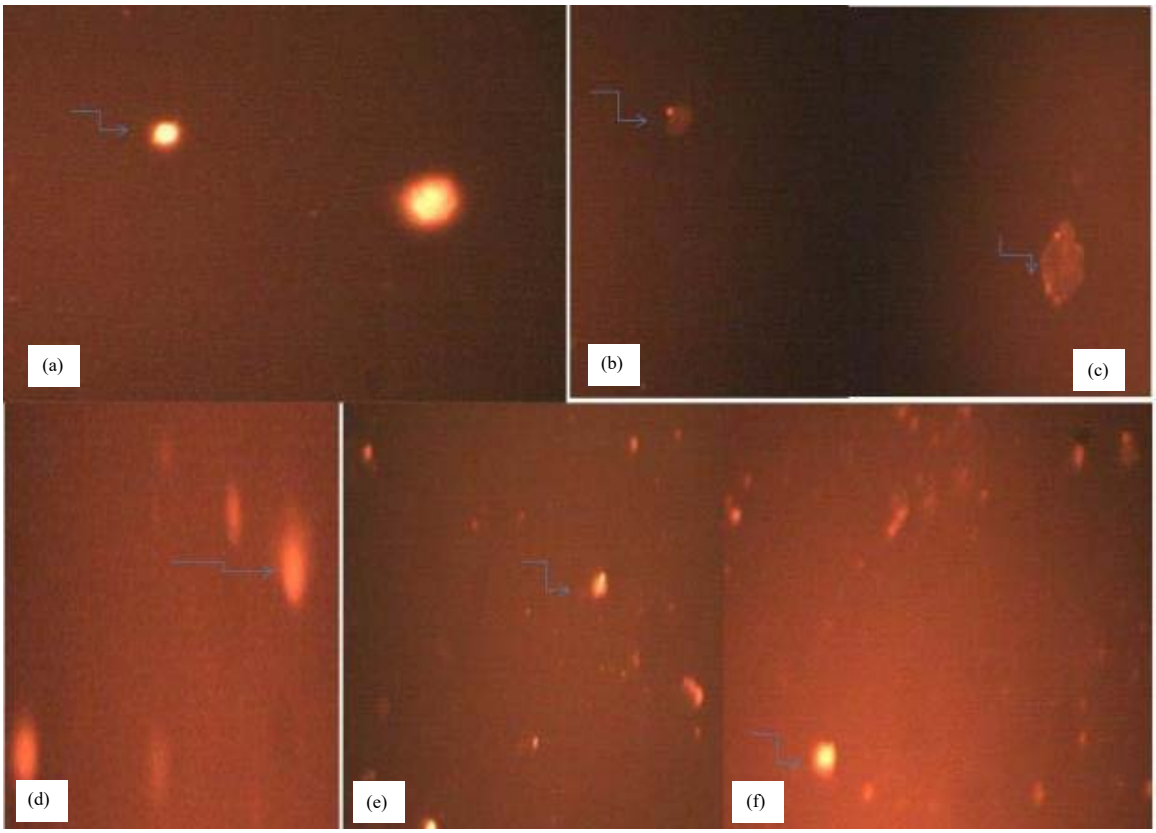

Fig. 2(a-e): Ethidium bromide images of comet assay

(a) G_1 (Control) showing normal intact cells, (b) G_2 (PD-treated) showing DNA damage in PD groups, (c) G_2 (PD-treated) showing DNA damage with high magnification, (d) G_4 (vitamin C-PD group) showing slightly damaged DNA, (e and f) G_6 (thyme-PD group) showing slightly damaged DNA

Table 6: Comet assay finding of liver of control and examined catfish

Parameters	G_1 (n = 15)	G_2 (n = 15)	G_3 (n = 15)	G_4 (n = 15)	G_5 (n = 15)	G_6 (n = 15)
Tail length	6.75±0.09^{a}	4.03±1.16^{a}	6.32±0.65^{a}	4.56±0.24^{a}	7.23±1.13^{a}	4.53±0.54^{a}
DNA (%)	6.25±1.19^{b}	5.94±0.67^{b}	6.45±0.25^{b}	4.72±0.53^{b}	9.06±0.56^{a}	5.03±0.45^{b}
Tail moment	0.57±0.214^{ba}	0.26±0.061^{cb}	0.34±0.014^{ba}	0.19±0.040^{c}	0.76±0.074^{a}	0.30±0.046^{cb}
Olive moment	0.81±0.061^{b}	0.67±0.0896^{b}	0.87±0.003^{b}	0.46±0.0200^{c}	1.62±0.118^{a}	0.73±0.338^{b}

Means with different superscript letters in the same row are significantly different at p<0.001

In comet assay, the slides were stained with ethidium bromide and examined under fluorescent microscope and demonstrated the presence of damage in DNA (Fig. 2). This damage differs in different groups. Group 1 (control) showed normal intact cells and no damage of DNA reported (Fig. 2a). While group 2 (PD-treated) showed presence of damage in DNA appeared in form of tail length (Fig. 2b and c, low and high magnification, respectively). While group 4 (Fig. 2d and 6 (Fig. 2e and f) showed slight damage in DNA.

Histopathological findings: Figure 3 showed the histopathological changes in liver tissues. In group1 (control), liver tissue consisted of central vein which lined by simple squamous cells, from the central vein numerous radiating hepatic cords which appeared as polygonal in shape with deep basophilic centrally located nuclei and faint acidophilic cytoplasm (Fig. 3a). While in group 2, liver tissue was characterized by thickened wall central vein, the hepatocytes showed disruption with pyknotic nuclei and disintegrated cytoplasm. The hepatic blood vessels showed vascular changes include hemorrhage and edema (Fig. 3b). Liver tissue in group 3 and 5 appeared as normal structure and normal architecture as in control group (Fig. 3c and e). Group 4 showed thick wall and hemorrhage in the central vein (Fig. 3d). Group 6 showed thick wall central vein and increased connective tissue fibers between hepatic cords (Fig. 6f).

Histopathological findings of kidney tissues were shown in Fig. 4. In group 1, renal tissue consisted of numerous nephrons which consisted of glomeruli, proximal convoluted tubules, distal convoluted tubules and collecting tubules, in

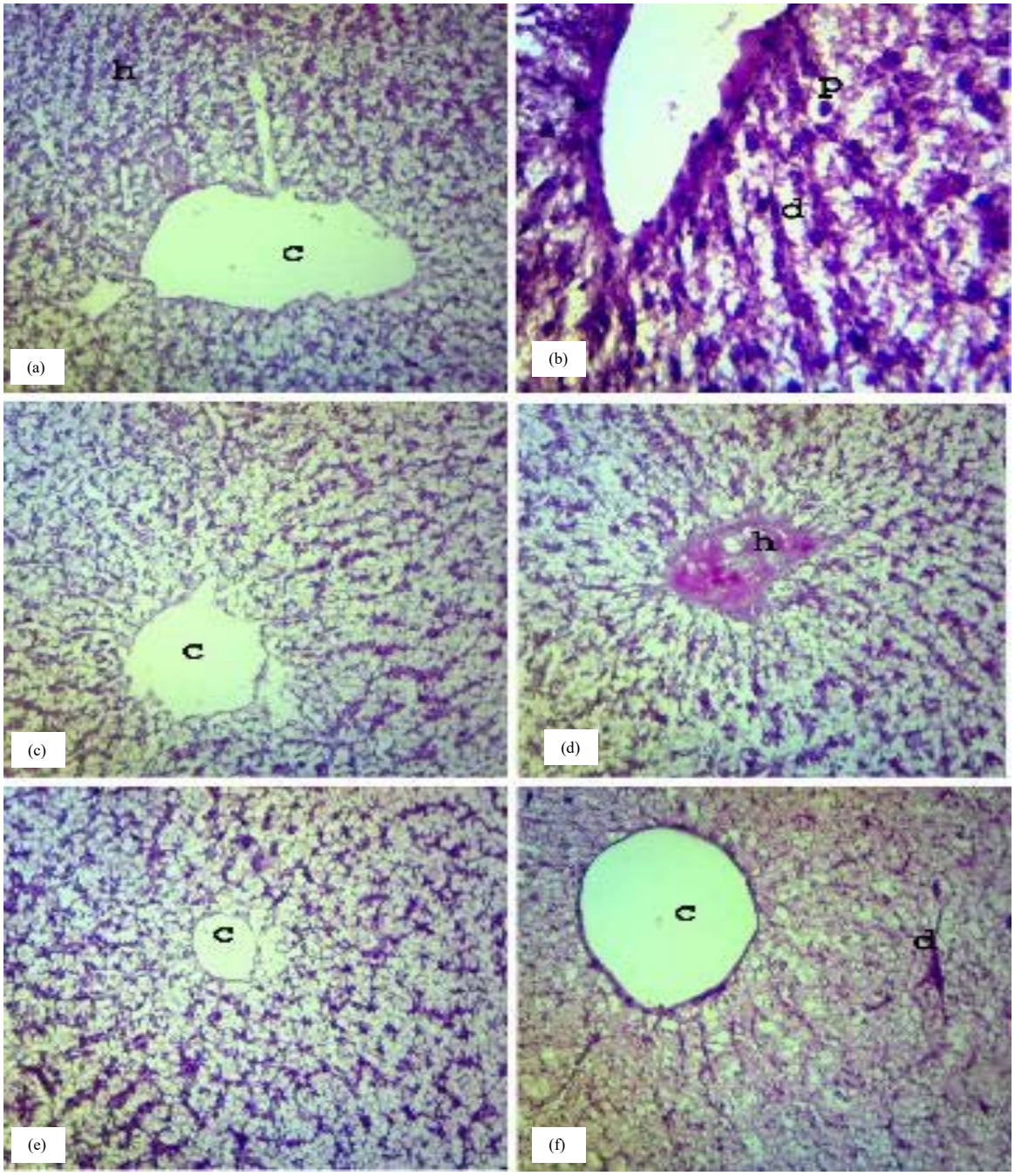

Fig. 3(a-f): Photomicrograph of liver tissue

(a) Normal hepatic tissue with central vein (C), (b) Pyknotic nuclei (p) and disintegrated cytoplasm (d), (c) Normal central vein (c) (d) Hemorrhage in central wall (h), (e) Normal central vein C and (f) Increased CT between hepatic cords (d). H and E ×10 all while No.2 ×40

between them the haemopoietic tissue was located (Fig. 4a). While in group 2, kidney showed destruction and necrosis in renal tubules and spread of patches of eosinophilic exudate between renal tissues (Fig. 4b). Renal tissue in group 3 and 5 consisted of normal structure as in control group with prominent haemopoietic tissue (Fig. 4c and e). Kidney in group 4 showed desquamation of some epithelial tissue and pyknosis of some nuclei while the other renal tissues appeared normal (Fig. 4d). Group 6 showed desquamation of epithelial tissue of renal tubules with prominent haemopoietic tissue distributed between them (Fig. 4f).

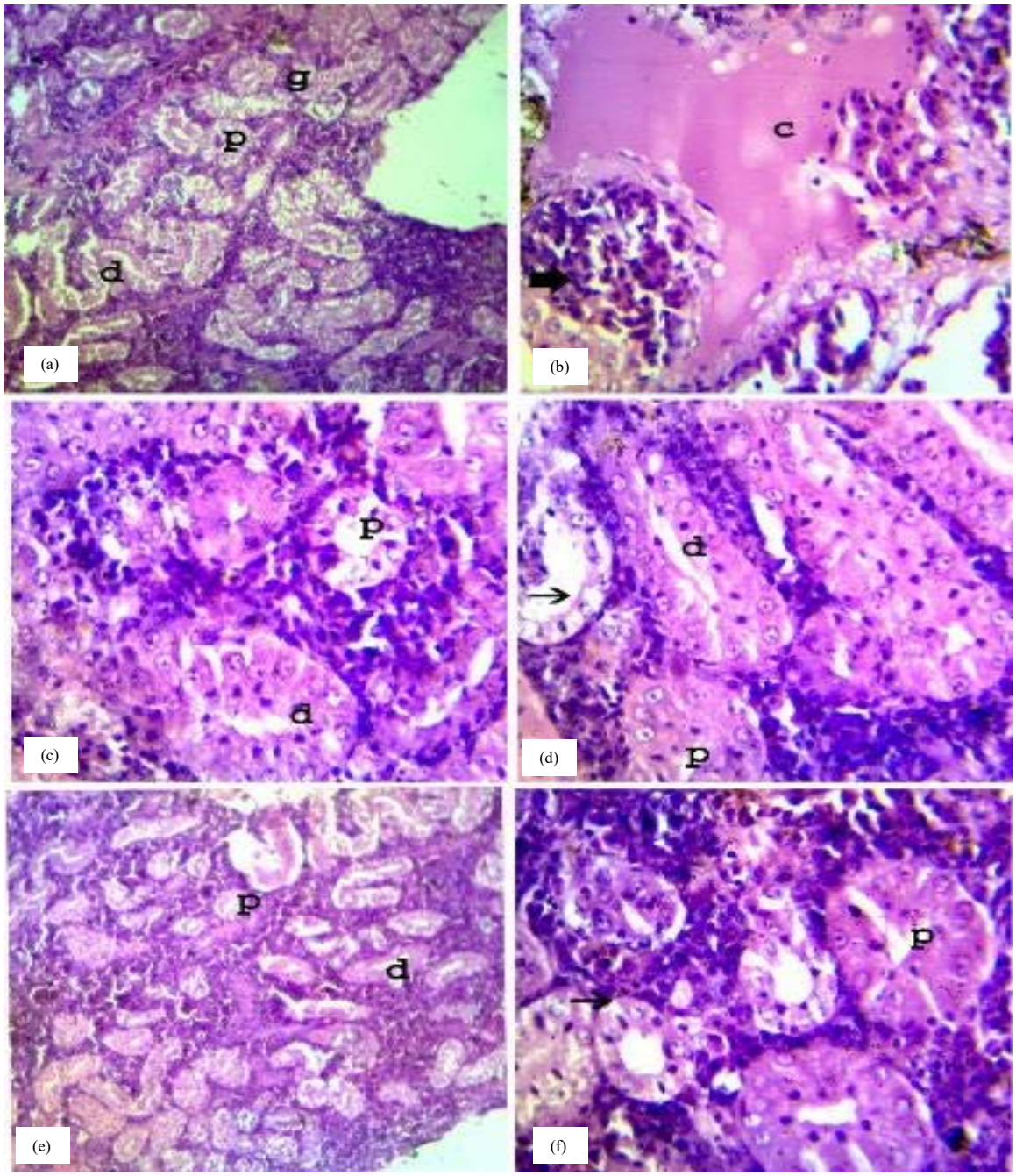

Fig. 4(a-f): Photomicrograph of renal tissue

(a) Normal renal tissues consisted of glomeruli (g), proximal convoluted tubules (p) and distal convoluted tubules (d), (b) Eosinophilic exudate between renal tissues (C), (c) Normal structure consisted of glomeruli (g), (d) Proximal convoluted tubules (p) and distal convoluted tubules (d), (e) Desquamation of some renal tissues (d) and pyknotic nuclei (p) and (f) Prominent haemopoietic tissues (arrow). H and E×20

Histopathological results of gills were shown in Fig. 5. Gills in group 1 showed gill arch, primary and secondary lamellae, the secondary lamellae lined by mucous cells and pillar cells (Fig. 5a). While in group 2, gills showed desquamation the epithelial cells, thickening in the wall (hyperplasia) of secondary lamellae and circumscribed area of lymphocytic infiltration was located between the secondary lamellae which destruct the underlying structures (Fig. 5b, c). In group 3 and 5 gills appeared as normal structure as in control group with numerous goblet cells which gave positive

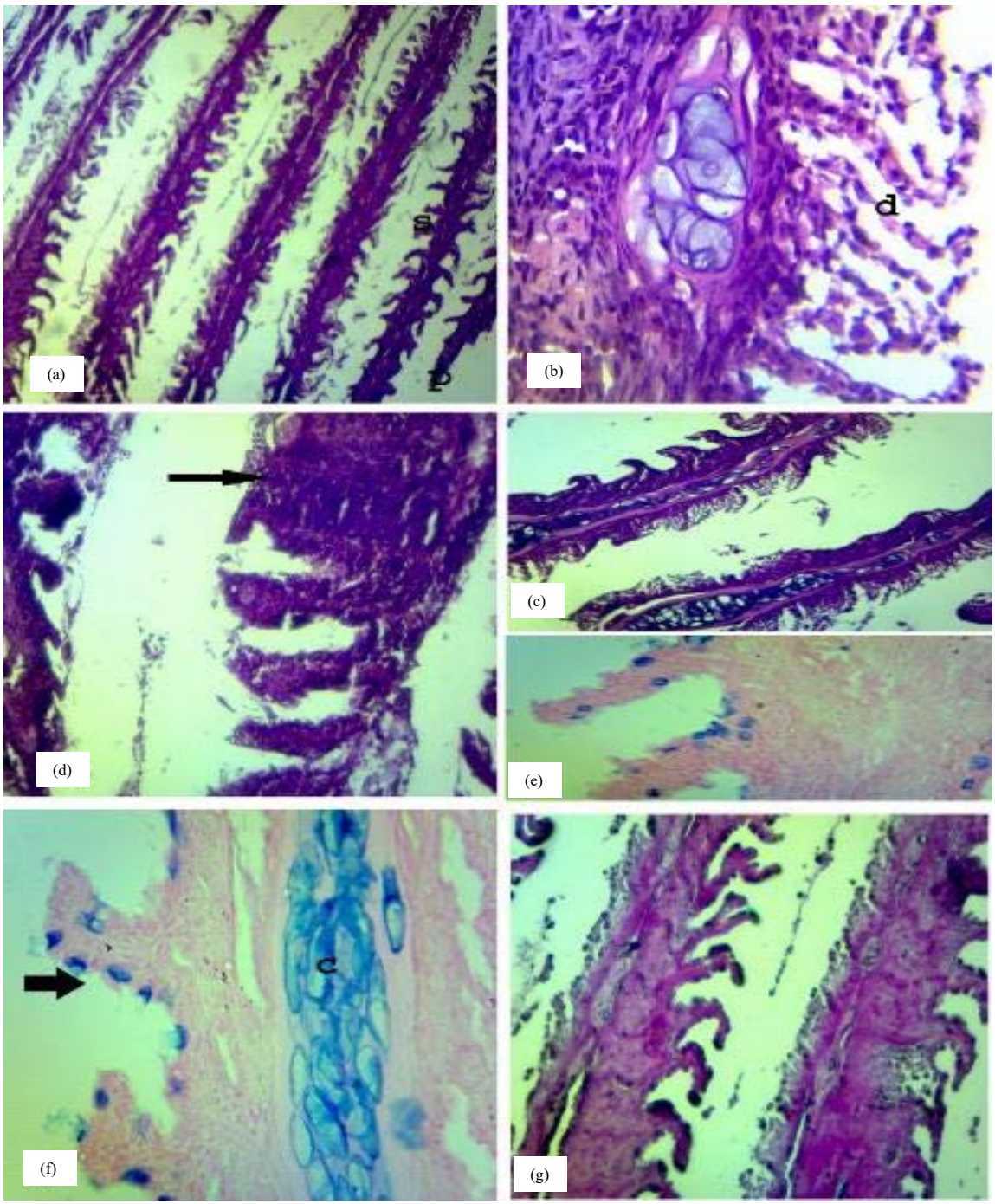

Fig. 5(a-g): Photomicrograph of gill tissue

(a) Gill consisted of gill arch, primary (p) and secondary lamellae (s) H and E×10, (b) Desquamation of epithelial cells (d) H and E ×20, (c) Thickening in the wall (arrow) H and E×20, (d) Gills appear to be in normal structure, H and E×10, (e) Decrease number of goblet cells and wall of the gills, Alcian blue ×10, (f) Gills appear to be in normal structure, Alcan blue (c) ×20 and (g) Some foci of lymphocytic infiltration located between secondary lamellae. H and E×20

Alcian blue(Fig. 5d). Gills in group 4 showed decrease in the thickness of wall of secondary lamellae with decrease of number of mucous goblet cells (Fig. 5e). While in group 6, gills appeared normal while some foci of lymphocytic infiltration still located between the secondary lamellae (Fig. 5f). While some foci of lymphocytic infiltration located between secondary lamellae. H and E ×20 (Fig. 5g).

DISCUSSION

Chromium is one of the important pollutants that affect aquatic environment. It exerts its adverse effect directly or in directly on human health. The more toxic form of chromium is known as hexavalent chromium[10]. Catfish was chosen as a model for the experimental study because it can tolerate and survive in many of experimental conditions, it can also be distributed in wide areas of water[41]. In this study chromium exposure for 30 days leads to changes in fish appearance, morphology, blood analysis, cytology and histology[42]. Presence of erosions in fins and fin rays was the predominant clinical signs appeared on fish exposed to chromium. These symptoms are supported by the different previous studies[10].

Blood analysis of fish differs according to fish species, sex, age and condition of health[43]. The hematological and biochemical studies in fish are considered as important parameters for evaluation of health status of fish exposed to metallic stress[44]. The chromium induced toxicity in this study results characterized by decrease in RBCs, Hb, PCV and WBCs. These results agreed with other different studies[45-48]. They added that the decrease in hematological parameters may be attributed to the toxic effect of chromium that affect the metabolic and hematopoietic system. The liver and kidney function tests showed different changes due to the toxicity induced by chromium. The increase in AST and ALT in the present study is augmented by previous study[47]. The increase in cholesterol may be attributed to prolonged exposure of chromium that cause difficult adaptation and weakness because of metallic stress[44]. The decrease in protein and albumin could be attributed to the stress of toxin causing liver hypofunction or impairment in protein synthesis[48,49,10]. The use of vitamin C and thyme extract in our study decrease the toxicity of chromium by enhancing the fish hematology[26] and improving the liver and kidney function[50]. The protein and albumin were increased after adding of vitamin C and thyme extract in diet and this due to serum protein are sensitive to nutritional influences[51,52].

The immunity of fish in this study was affected by long exposure to chromium and manifested in form of decreasing in immunoglobulin and this result is agreed with other studies[42,53]. The decrease in immunity may be attributed to toxin stress that lead to production of oxidative effect which in turn causing reduction or suppression of primary antibody response. While vitamin C and thyme extract in our study elevated the immunoglobulin levels and improve immune response of catfish[54-57].

The present study revealed that the exposure to chromium for 30 days caused an increase in metallic stress in catfish and this stress lead to production of ROS the body of the fish cause a defense mechanism against this oxidative stress via production of antioxidant enzymes which work to remove the damage effect of this stress, so this explained the increase of SOD, CAT, GSH and MDA in our study which showed agreement with[58,12,59]. Vitamin C and thyme extract when given in this study act as antioxidant by increasing antioxidant activities and decreasing the oxidative stress in form of decrease MDA which agreed with studies[41,60].

The micronucleus test was used for detection of fish genotoxicity in polluted aquatic area[61]. In the present study, there was significant increase in micronuclei in fish erythrocytes and this result coincided with previous studies[6,62]. Accumulation of chromium was recorded by detection of chromium residues in fish tissues. In this study, the highest concentration of chromium residues in fish gills while the lowest levels of residues were founded in fish muscles. This result is augmented by the other studies[8,9]. Vitamin C and thyme extract in current results decreased chromium residues by acting as reducing agent, decrease accumulation and persistence of chromium in tissues as previously explained[63,64]. Comet assay was used for detection of DNA damage[65]. In the present study, there was damage in DNA appeared in chromium group and this genotoxic damage was similar to that previously reported[66,24]. They added that the chromium induced metallic stress which causing induction of relative oxygen species which attack DNA and enhancing DNA damage. Vitamin C and thyme extract decreased oxidative stress and decrease DNA damage in tissues and this is supported by the findings of other studies[67,68].

Histopathological examination of organs was used as a biomarker for evaluation of the toxicity of different pollutants[69,70]. Chromium in this study induced marked changes and damage in liver, kidney and gills. These changes in form of degeneration and necrosis of tissues may be due to accumulation of inflammatory cells associated with chromium toxicity. These results are lined with previous studies[71,72,73].The addition of vitamin C and thyme extract had ameliorative effect since it minimized the hazard effect of chromium on the tissues.

CONCLUSION

Chromium exposure in catfish induced clinical, hematological, biochemical and histopathological changes, especially in liver, kidney and gills. The use of vitamin C and thyme extract have a protective and ameliorative effect against toxicity induced by potassium dichromate in catfish.

SIGNIFICANCE STATEMENT

This study discovered the addition of vitamin C or thyme extract can be beneficial for protection of catfish from chromium toxicity by potentiating the liver and kidney function and decreasing the oxidant stress. This study will help the researchers to uncover the critical areas where chromium toxicity may occur that many researchers were not able to explore. Thus a new theory on DNA adducts and micronucleus changes in cat fish caused by chromium exposure in industrial area may be arrived at.

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