Chinese Journal of Oceanology and Limnology
Vol. 34 No. 1, P. 109-117, 2016
http://dx.doi.org/10.1007/s00343-015-4392-3

Effects of a low-radiotoxicity uranium salt (uranyl acetate) on biochemical and hematological parameters of the catfish, *Clarias gariepinus**

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Received Dec. 24, 2014; accepted in principle Feb. 25, 2015; accepted for publication Mar. 22, 2015

Abstract Specimens of *Clarias gariepinus* were treated with lethal (70, 75, 80, 85, 90, and 95 mg/L) and sub-lethal concentrations (8, 12 and 16 mg/L) of uranyl acetate, a low-radiotoxicity uranium salt. The LC_{50} value was registered as 81.45 mg/L. The protein and glycogen concentrations in liver and muscles were decreased in the fish exposed to sub-lethal concentrations. The red blood cell (RBC) and white blood cell (WBC) counts, haemoglobin (Hb) concentration and haematocrit (Hct) values were decreased. Different blood indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were negatively affected. Level of plasma glucose was elevated whereas protein was decreased. The level of calcium concentration (Ca) was declined in the blood of exposed fish whereas magnesium (Mg) remains unchanged. The activity level of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) was elevated in exposed fish. These effects were more pronounced in the last period of exposure and in higher concentrations. Results of the present study indicate that uranyl acetate has adverse effects on *Clarias gariepinus* and causes changes in the biochemical and hematological parameters of the fish.

Keyword: *Clarias gariepinus*; uranyl acetate; sub-lethal concentration; biochemical and haematological parameters

1 INTRODUCTION

In recent years environmentalists have raised concerns about the accumulation of radioactive substances in the bodies of animals and its consequences in the atmosphere and water. Testing of atomic weapons adds anthropogenic radioactivity to the naturally present radioactivity. The use of atomic energy for peaceful purposes, such as generation of power, is being encouraged because of the curtailment in the supply of fossil fuels and the prevailing energy crisis. Realistically, environmentalists anticipate an increase in the output of radioactive wastes. Hence, strong effort should be made to monitor the environmental implications and to furnish advice for effective safeguards in the interest of public health. Strontium, cesium, radium, plutonium and uranium are common radioactive elements that enter the environment through waste disposal or fallout, and influence the aquatic fauna. Strontium and uranium enter the fish body through the intestine, gills and skin, and accumulate chiefly in the bones and to a smaller extent in the viscera, gills and muscles (Nikolsky, 1963; Goulet et al., 2011). These radionuclides interfere in the calcium metabolism in the bones. Rice et al. (1965) studied the dynamics of strontium accumulation in the gold fish. Accumulation of radioactive elements (uranium, radium, plutonium, cesium) and their effects on biological processes of fish and shellfish have also been documented by Cambray and Bakins (1980); Hamilton (1980); Shekhanova (1980); Guéguen et al. (2006); Darolles et al. (2010). Several studies have assessed the effect of uranium salts on fish and other animals, and their possible consequences on human health (Stearns et

* Supported by the Deanship of Scientific Research at King Saud University (No. PRG-1436-011)
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al., 2005; Periyakaruppan et al., 2007; Zymmerman et al., 2007; Priyamvada et al., 2010; Vicente-Vicente et al., 2010; CCME, 2011; WWSA, 2011; Amer and Alwachi, 2012; Daraie et al., 2012; Ahmad, 2014). Many investigators, in the past, focused on the effect of uranyl acetate on the biochemical, hematological and immunological parameters of fish and other laboratory animals (Domingo, 2001; Abou-Donia, 2002; Yazzie, et al., 2003; Goldman et al., 2006; Hartsock et al., 2007; Ahmad, 2014). Accumulation of depleted uranium and its effect on oxidative stress in fish tissues has been reported by Barillet et al. (2007) and by D'Ilio et al. (2007). Simon et al. (2014) have studied the reproductive development in *Danio rerio* under the influence of low (20 μg/L) and moderate (250 μg/L) doses of uranium. Depleted uranium contamination might be pathogenic by suppressing defense mechanism or inducing hypersensitivity in zebra fish, as reported by Gagnaire et al. (2014). Fish are an important human food source; so it is likely that when uranium or its breakdown products are accumulated in fish tissue, these radioactive isotopes will enter the human body. The high tolerance of fish to uranium salts and its long half-life (7×10^8 years) raise concerns about this alarming situation. These contaminants in fish persist for relatively long periods and this is one reason why humans are particularly vulnerable to radioactive compounds. The physiological change in animals caused by toxicant will be reflected in the values of one or more hematological parameters (Van Vuren, 1986). In fish, exposure to chemical pollutants can induce either increase or decrease in the level of hematological parameters. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological alterations as well as disease in fishery management and aquaculture (Mulcahy, 1975). Furthermore, the investigations pertaining to the tracking down biochemical and hematological abnormalities in the fish can provide indication of exposure to pollutants before any gross signs become apparent. Hence they serve as reliable indices of fish health. Furthermore, biochemical and hematological responses provide cues regarding the type and level of pollutants in the environment (Rao, 2010). The objective of present study was to evaluate the changes in the commercially important fish, *Clarias gariepinus*, exposed to uranyl acetate. Effects on plasma protein, glucose, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) levels, red blood cell (RBC) and whit blood cell (WBC) counts, hemoglobin concentration and hematocrit values were investigated. Levels of protein and glycogen in the liver and muscle of the fish were measured. Concentrations of Ca and Mg in the plasma of fish were also measured.

2 MATERIAL AND METHOD

Specimens of *Clarias gariepinus* (Total length 20–23 cm and total body weight 55–60 g) were obtained from fish farm located at Mozahmiah, north-west of Riyadh. The fish were acclimatized to laboratory conditions for three weeks, during which period they were fed a commercial fish food twice daily to satiety. After preliminary trials, ten fish specimens were kept in each of six aquaria and subjected to different concentrations (70, 75, 80, 85, 90, 95 mg/L) of uranyl acetate dihydrate [UO_2 $(CH_3COO)_2$ $2H_2O$] obtained from Sigma-Aldrich, England, for 96 hours. The normal commercial stocks of uranyl acetate prepared from depleted uranium have a typical radioactivity of 0.37–0.51 microcuries/g. This is a very mild level and is not sufficient to be harmful, while material remains external to body (Wikipedia-en.wikipedia.org/wiki/uranyl_acetate). The necessary safety measures were adopted during the experiment. A parallel control was performed with uranium-free water. Mortality of fish in each concentration was observed after every 24 hours and the number of dead fish was recorded. The medium in the aquaria was renewed daily. The experiment was run in triplicate. The LC_{50} value for 96 hours was deduced from the graph made between probit of kill and $\log_{10}$ concentration of uranyl acetate as suggested by Finney (1971). The mean temperature, pH, dissolved oxygen, and hardness of water determined weekly, were 22.5±0.5°C, 7.8±0.7, 7.2±0.6 mg/L and 230.5±5.23 mg/L, respectively.

In another set of experiment fish were exposed to three different sub-lethal concentrations selected as 10%, 15% and 20 % of the LC_{50} (8, 12 and 16 mg/L) of uranyl acetate for 3 weeks. At the end of every week, 3 fish from each treatment and the control were removed, and their blood samples were collected by excising the caudal peduncle. This is an easy method to collect the blood from small and medium sized fishes. Heparinized vials were used for the collection of blood samples. In case of insufficient quantity, the blood of two or more fish was pooled. Cyano-methemoglobin method (Blaxhall and Daisley, 1973) was used to estimate the hemoglobin. Hematocrit values were determined by using a micro-hematocrit

Table 1 Effects of uranyl acetate on the hematological profile of *Clarias gariepinus*

Parameters	Time (weeks)	Concentrations (mg/L)				
		Control	8	12	16	$P<0.05$
Erythrocytes (cell×10^6/mm^3)	1	1.68±0.18	1.59±0.06	1.56±0.05	1.56±0.04	
	2	1.65±0.08	1.53±0.05	1.43±0.06	1.42±0.07	*
	3	1.67±0.06	1.52±0.06	1.45±0.04	1.42±0.05	*
Leucocytes (cell×10^3/mm^3)	1	34.31±0.51	32.25±0.45	32.21±0.61	31.21±0.85	
	2	35.52±0.43	31.83±0.67	30.65±0.56	29.12±0.52	*
	3	34.91±0.65	30.21±0.53	29.35±0.69	28.25±0.51	*
Hematocrit (%)	1	34.76±0.76	32.66±0.52	30.94±0.95	29.41±1.02	
	2	35.68±085	31.65±1210	30.41±0.85	29.21±1.01	*
	3	33.95±0.95	30.75±0.86*	29.48±1.22	28.41±1.10	*
Hemoglobin (g/dl)	1	6.25±0.10	4.92±0.22	4.35±0.10	4.12±0.11	*
	2	6.94±0.14	4.88±0.15	4.22±0.19	3.54±0.19	*
	3	6.34±0.09	4.39±0.08	4.04±0.12	3.65±0.11	*
MCV (fl/cell)	1	206.96±3.91	205.06±4.51	194.66±4.58	188.55±3.18	*
	2	216.25±4.35	206.74±3.26	212.47±4.21	205.47±4.21	
	3	203.90±4.35	202.27±4.55	203.13±5.25	200.13±6.65	
MCH (Pg/cell)	1	37.34±1.72	30.95±1.35	27.89±2.21	26.49±2.01	
	2	42.03±2.52	31.36±2.05	29.57±1.45	24.87±1.35	*
	3	37.85±2.42	28.74±1.55	27.79±2.25	25.90±2.25	*
MCHC (%)	1	17.98±1.15	15.09±1.26	14.09±1.05	14.29±1.35	
	2	19.40±1.25	15.44±1.36	13.47±1.05	12.17±0.81	*
	3	18.48±1.25	14.27±1.25	13.94±1.55	12.44±1.45	*

Values are mean±standard error.

centrifuge. The WBC and RBC counts were made using a Neubar hemocytometer after diluting the blood with Turk's solution and Dace's solution, respectively. The indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined by methods used by Ahmad (2012). The blood was centrifuged at 6 000 r/min for 10 minutes at 4°C and the collected plasma was stored at -20°C until analyzed for different constituents. Glucose, total protein, Mg, Ca, GOT and GPT were analyzed using their respective kits (bioMérieux, France).

The livers were immediately dissected out and weighed. The white-muscle samples were excised from a fixed location below the site of origin of dorsal fin. Glycogen in the liver and muscle was extracted as described by Ashman and Seed (1973) and determined by the method of Montgomery (1957) on wet weight basis. Protein contents were measured in dry, fat free samples. The dry fat-free samples were prepared using the technique of Webb and Levy (1955). The method of Lowry et al. (1951) was followed for the determination of protein.

One way analysis of variance (ANOVA) was applied to test the significance of the differences between the values. P values less than 0.05 were considered statistically significant.

3 RESULT

It was observed that the uranyle acetate is moderately toxic to fish and its 96-hour LC_{50} was recorded as 81.45 mg/L. The red blood cell count, hemoglobin concentrations and hematocrit values declined in the fish exposed to different concentrations of uranyl acetate (Table 1). Reduction in the leucocyte count was also evident in exposed fish. The fall was more distinct in the fishes exposed to higher dose. Changes in the values of different indices like MCV, MCH and MCHC were apparent in uranium-exposed fish.

A significant ($P<0.05$) increase in glucose level (Table 2) was obtained in exposed fish. Hypoproteinemia was also recorded in the plasma of fish exposed to uranyl acetate (Table 2). The activity

Table 2 Effects of uranyl acetate exposure on plasma biochemical composition of *Clarias gariepinus*

Parameters	Time (weeks)	Concentrations (mg/L)				
		Control	8	12	16	$P<0.05$
Total protein (g/dl)	1	30.65±1.78	27.38±1.37	26.15±2.12	24.15±1.42	*
	2	29.68±1.48	25.62±1.02	24.95±1.35	23.65±1.25	*
	3	30.05±1.28	24.81±1.25	23.21±1.56	22.31±1.51	*
Glucose (mg/dl)	1	48.23±3.25	69.22±4.63	72.21±4.57	79.15±5.56	*
	2	50.65±4.68	70.61±5.25	76.51±5.75	85.44±4.25	*
	3	47.52±5.88	73.25±5.54	92.28±6.25	98.25±6.85	*
Ca (mg/dl)	1	185.45±11.3	165.25±12.2	150.45±10.2	148.45±10.2	*
	2	180.25±12.2	163.45±10.3	148.25±12.6	141.25±8.6	*
	3	192.65±10.1	151.35±9.6	146.65±8.9	133.65±6.9	*
Mg (mg/dl)	1	39.25±4.11	37.03±2.55	36.98±4.65	37.35±4.05	
	2	37.23±5.61	36.41±5.31	34.68±5.53	36.25±4.63	
	3	36.95±5.51	37.56±3.45	36.48±4.84	35.45±5.64	
PGOT (IU/l)	1	82.25±11.2	98.36±10.5	110.26±11.4	119.26±9.4	*
	2	86.25±12.2	108.35±11.3	111.45±11.5	125.45±13.5	*
	3	84.25±11.2	119.25±12.2	125.35±11.2	131.35±11.2	*
PGPT (IU/l)	1	68.12±6.66	89.22±5.11	95.21±5.84	108.21±6.64	*
	2	66.65±11.3	88.25±9.3	98.25±12.2	110.25±10.2	*
	3	67.25±10.2	91.48±10.1	106.25±10.1	121.25±9.1	*

Values are mean±standard error.

of GOT and GPT was significantly ($P<0.05$) increased in all exposed groups of fish (Table 2). The level of Ca was reduced in the plasma of exposed fish, whereas the concentration of Mg was unchanged.

4 DISCUSSION

The 96-hour LC_{50} value for *Clarias gariepinus* computed from the graph (Fig.1) was deduced as 81.45 mg/L in hard water (hardness 230 mg $CaCO_3$). The LC_{50} values documented in the present investigation are higher than the values (0.37–3.46 mg/L) reported by Bywater et al. (1991) for different species of fish. Bywater et al. (1991) and Hinck et al. (2010) have tabulated the 96-hour LC_{50} of uranium compounds for fish varying from 0.73–135 mg/L. Parkhurst et al. (1984) have reported 96 h LC_{50} of uranyl sulfate for brook trout as 5.5 mg/L in soft water (35 mg/L hardness) and 23 mg/L in hard water (208 mg/L hardness). Hamilton (1995) has reported 96-hour LC_{50} of uranyl nitrate as 46 mg/L in water of hardness of 197 mg $CaCO_3$. These differences can be attributed to multiple factors like age and size of fish (Skidmore, 1965; Farmer et al., 1979; McKim, 1985) and hardness of water (Black et al., 1979). The disparity in the toxic potential of uranyl acetate can also be related to the differences in susceptibility and tolerance related to its accumulation, biotransformation and excretion. Discrepancies in metabolic pathways among species may result in varied patterns of biotransformation, leading to more or less toxic metabolites (Johnson and Toledo, 1993). The magnitude of toxic effects of pollutants also depends on length and weight, corporal surface/body weight

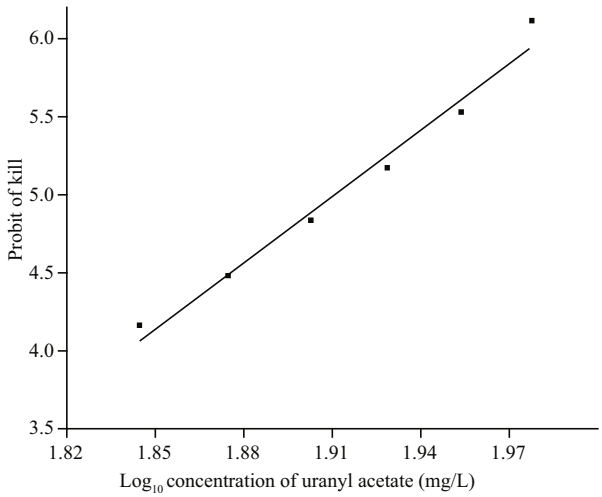

Fig.1 Relationship between probit of kill and Log_{10} concentration of uranyl acetate

ratio and breathing rate (Murty, 1986). Cheng et al. (2010) suggested that the uranium toxicity appears to be a function of exposure water quality and feeding regime.

The hematological parameters play an important role in evaluating the effects of pollutants (Roche and Bogé, 1996). Exposure of toxic chemicals adversely affects the internal and external environment of animals and causes reduction in different blood constituents. Conforming to aforesaid view a decrease in the number of RBC count, hemoglobin concentration and hematocrit values in toxicant exposed fish was reported by Banaee et al. (2011); Ahmad (2012). These authors have ascribed this reduction to the destruction of cells and/or decrease in size of cells induced by pollutants. Similar results have been presented by Zaki et al. (2009). Lead nitrate exposure has reduced the hemoglobin level, RBC count and hematocrit values in *C. gariepinus* (Adeyemo, 2007). In contrast to this Gilman et al. (1998) have reported an elevated level of hemoglobin and RBC count in fish exposed to uranyl nitrate. Undoubtedly, pollutants would exert adverse effects on the hematopoietic system of fish, thus, affecting the production or subsequent rapid destruction of blood constituents. The above mentioned factors may be responsible for the reduced level of the hematological parameters recorded in the present investigation.

The dwindled leukocyte count (Table 2) recorded in the present investigation can be attributed to malfunctioning of the hematopoietic organs caused by uranyl acetate. The fall in the leucocyte count may be due to fall in the delivery of these cells to the blood stream because of reduced production or alternatively more rapid destruction of cells. This line of reasoning was presented by Alkahemal-Balawi et al. (2011). Changes in the leukocyte system manifest in the form of leukocytosis with heterophilia and lymphopenia, which are characteristics of leukocytic response in animals under stress. Al-Kahem (1995) reported a significant decline in the WBC count in fish exposed to chromium and has ascribed it to the reduction in the number of lymphocytes and thrombocytes. Jaffer Ali and Rani (2009) have reported decreased leukocyte count in carp exposed to toxicant.

The indices, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC), are sensitive to changes in environmental conditions especially alterations caused by the addition of chemical pollutants. Alterations in the values of MCV, MCH and MCHC reflect the variations in the RBC counts, hemoglobin concentrations and hematocrit values. Rao (2010) reported an increase in these indices in common carp exposed to acute toxic level of pesticide. A decrease in MCH value was reported by Gilman et al. (1998) in fish exposed to uranyl nitrate and extends support to the present findings.

The significant (P<0.05) elevation of glucose level (Table 2) in exposed fish may be due to the mobilization of glycogen into glucose to meet the increased demand of energy. The rapid secretion of the hormones including glucocorticoids and catecholamines, from adrenal tissue due to stress stimuli (Schaedler, 1981) are known to produce hyperglycemia. Enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their increased energy demands may be other reason for hyperglycemia (Winkaler et al., 2007). The elevated level of glucose observed in the present study may also be attributed to increased production of these hormones, thus causing glycolysis in the fish exposed to uranium salts. The present result agrees with the findings of Gilman et al. (1998), Alkahemal-Balawi et al. (2011) and Ahmad (2012). The toxicants may change the functioning of vital organs including the liver and the kidney, disrupting the homeostatic condition of the body, which may in turn alter the concentrations of metals. Alterations in the metal concentration in the carp, *Cyprinus carpio*, have been reported by Al-Akel et al. (2010) after exposure to dietary copper, and give support to the present investigation.

The activity levels of GOT and GPT are also used as important diagnostic indices of the toxic potential of various toxicants (Nelson and Cox, 2000). The activity of these enzymes increases after the exposure of various concentrations of metals (McKim et al., 1970). Jeney et al. (1991) reported elevated levels of both the enzymes, GOT and GPT, in the serum of ammonia-exposed fish. Their conception was that serum glutamopyruvate transferase (SGPT) is extremely sensitive to changes in the environment. Zaki et al. (2009) have documented an elevated level of ALT activity in the fish exposed to malathion and extract of *Porkia biglosa* pods. The increased activity of this enzyme in the exposed fish is suggestive of damages to hepatic tissues leading to their leakage into circulation (Mousa et al., 2008) and /or increased synthesis of enzyme in the liver. A significant increase in alkaline phosphatase (ALP), alanine transaminase

Table 3 Changes in protein (dry W.) and glycogen (wet W.) concentrations in the liver and white muscle of *Clarias gariepinus* after the exposure to uranyl acetate

Parameters	Concentrations (mg/L)	Exposure time (weeks)					
		Liver			Muscle		
		1	2	3	1	2	3
Protein (mg/100 mg)	Control	69.23±1.31	68.69±1.89	66.57±1.72	57.37±1.05	55.41±1.13	56.21±1.09
	8	56.13±1.19	55.13±1.03	56.29±1.26	48.23±1.03	47.71±1.03	45.25±1.21
	12	54.36±1.29	53.51±1.15	52.42±1.04	47.26±1.02	46.71±0.69	45.05±1.08
	16	54.26±1.39	53.24±1.25	52.42±1.04	44.26±1.07	45.36±1.09	43.05±1.13
	$P<0.05$	**	**	**	**	**	**
Glycogen (mg/g)	Control	10.22±0.22	9.98±0.20	9.50±0.19	3.85±0.11	3.95±0.26	4.05±0.16
	8	6.15±0.19	6.05±0.17	6.28±0.27	2.45±0.08	2.32±0.09	2.30±0.06
	12	5.86±0.19	5.75±0.18	5.45±0.19	2.04±0.05	2.02±0.08	2.02±0.06
	16	5.16±0.25	5.01±0.18	5.15±0.15	1.95±0.06	1.76±0.08	1.46±0.09
	$P<0.05$	**	**	**	**	**	**

Values are mean±standard error.

(ALT) and aspartate transaminase (AST) levels in mice after treatment by uranyl acetate was reported by Ozmen and Yurekli (1998). Agrahari et al. (2007) have reported an elevated activity level of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) in *C. punctatus* after the exposure to monocrotophos. Palanivelu et al. (2005) opined that liver is rich in SGOT and SGPT, and damage caused by toxicants to hepatic tissues could result in liberation of large quantities of these enzymes into the circulation. Hence, an increase in the activity of these enzymes (PGOT and PGPT) is a sensitive indicator of cellular damage (Palanivelu et al., 2005; Alkahemal-Balawi et al., 2011). Therefore, the increase in the activity of these enzymes in the present investigation may be ascribed to damage caused to liver by uranyl acetate.

The exposure of *C. gariepinus* to uranyl acetate affected the metabolic processes and altered the concentrations of protein and glycogen in different tissues of fish (Table 3). Possibly, protein biosynthesis pathway was disrupted, especially by inhibiting the activities of enzymes, checking the epigenetic origin of this macromolecule, or it can stimulate catabolism of the pre-synthesized quantities. Previous studies by Mazeaud et al. (1977) and Strange et al. (1977) have demonstrated that the output of adrenocorticotrophic hormone (ACTH) is increased due to the stresses imposed on animals by toxicants. ACTH stimulates the adrenal gland to increase the production of corticosteroids, augmenting the enzymatic conversion of glycogen, protein and fat to glucose. Hypoproteinemia was recorded in fish exposed to different pollutants (Omoniyi et al., 2002). The reduction in the protein level may be attributed to cell destruction or necrosis, with subsequent impairment of protein synthesis machinery (Bradbury et al., 1987) or to excessive loss of proteins due to pathological changes in kidney (Salah El-Deen et al., 1996). This result has demonstrated the toxicity of uranyl acetate in *C. gariepinus* in terms of its effects on the primary biochemical machinery. Briefly, adverse effects caused by uranyl acetate imposed high stresses on fish, resulting in retarded growth and impaired function of the liver, kidney and other organs. The bio-kinetics of uranium have been extensively studied, and the kidney has been identified as the most affected organ (FPTCDW, 2001). Cooley et al. (2000) suggested that long-term exposure causes both kidney and liver damage in white fish, *Coregonus clupeaformis*. At cellular level, toxicity may cause from the binding of uranium to enzymes (Khangarot, 1991), which would subsequently disrupt or inactivate enzyme function. More comprehensive assessments of the physiological effects of pollutants on fish are required.

5 CONCLUSION

The results of this study demonstrate the chemical toxicity of uranyl acetate in fish. Although, its toxicity to fish is relatively low, uranyl acetate has sufficient toxic potential to alter the biochemical composition of liver and muscle tissue and hematological parameters in fish. It is hypothesized that the toxicant

either suppresses the activity of the enzymes responsible for the synthesis of these macromolecules or enhances the catabolism of the compounds. The presence of uranium salt (Uranyle acetate) in the environment is dangerous and poses a health hazard both to the animals inhabiting the environment and, of course, to humans.

5 ACKNOWLEDGEMENT

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this research through the Research Group Project No. PRG-1436-011.

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