

Biochemical and Haematological Changes Induced by Low-Radiotoxicity Uranium Salt (Uranyl acetate) in *Heteropneustes fossilis*

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Heteropneustes fossilis were treated with sub-lethal concentrations (4.0 and 8.0 mg/l) of a low-radiotoxicity uranium salt (uranyl acetate) for a period of 30 days. It has been observed that protein and RNA concentrations were decreased, and the DNA increased. The RNA/DNA ratio was significantly decreased in both the liver and the muscle of fish. The decrease in RNA/DNA ratio was comparatively higher in liver than muscles. Sub-lethal uranium exposure has decreased significantly ($P < 0.05$) the level of glycogen in Liver and muscle. The uranyl acetate has adversely affected the growth and condition of the fish which were worked out as condition (CF) factor and hepatosomatic index (HSI) values. It has caused a decrease in red blood cell (RBC) and white blood cell (WBC) counts, haemoglobin (Hb) concentration and haematocrit (Hct) values. Blood indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also adversely affected. Plasma glucose level was elevated whereas plasma protein was decreased. The level of calcium (Ca) was also reduced in the blood of this fish whereas magnesium (Mg) remains unchanged. The activity of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) was increased in the fish exposed to uranium salt of low radiotoxicity. It has been concluded that these effects were more pronounced with increase in concentration and in the time of exposure.

Key words: Uranyl acetate, Sub-lethal concentration, Biochemical and Haematological parameters, *Heteropneustes fossilis*.

With the increasing use of nuclear energy, environmentalists have raised concerns about the consequences of radioactivity in the atmosphere and water and about the accumulation of radioactive substances in the bodies of animals. There is no doubt that testing of atomic weapons adds anthropogenic radioactivity to the radioactivity that present naturally. World opinion favors the control of nuclear energy, but 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 prevailing energy crisis. Realistically, an increase in the output of radioactive wastes must be anticipated and strong effort should be made to monitor the environmental implications and to furnish advice about effective safeguards in the interest of public health. Some of the radioactive elements that commonly enter the environment through fallout or wastes disposal and influence the aquatic fauna are strontium, cesium, radium, plutonium and uranium. Nicolsky (1963) has reported that strontium enters the fish body

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through the intestine, gills and skin, and accumulates chiefly in the bones and to a smaller extent in the viscera, gills and muscle. This radionuclide disrupts the calcium metabolism in the bones. Rice et al. (1965) studied the dynamics of strontium accumulation in the gold fish. Accumulation of radioactive elements such as uranium, radium, plutonium, cesium in the fish and shellfish, and the resultant effects on biological processes, have also been documented by Cambray and Bakins (1980); Hamilton (1980); Shekhanova (1980). The RNA/DNA ratio is positively correlated with growth of some fishes (Bullow et al., 1981; Tripathi et al., 2002; Smith and Buckley, 2003; Mukherjee and Jana, 2007; Raksheskar, 2012). Several studies have assessed the effect of uranium salts on the fish and possible consequences of radioactive contamination on human health (Priyamvada et al., 2010; Vicente-Vicente et al., 2010; WWSA, 2011; CCME, 2011). Because fish are an important human food source, it is conceivable that when uranium or its breakdown products are deposited in the tissue of fish, these radioactive isotopes will enter the human body. The high tolerance of fish to uranium salts and long half-life of this nuclide (7×10^8 years) raise concerns about this situation because the fish and contaminant both persist for relatively long periods, and humans are therefore vulnerable to radioactive exposure. The present study sought to evaluate the changes in the commercially important cat fish, *Heteropneustes fossilis*, exposed to uranium salt. Changes in protein, nucleic acids (RNA and DNA) content and the ratios of nucleic acids were measured. Effects of uranyl acetate on plasma protein, glucose, GOT, GPT, RBC and WBC count, haemoglobin concentration and haematocrit values were also investigated. Previous studies have shown that when the water quality is affected by toxicants, any physiological change will be reflected in the values of one or more of the haematological parameters (Van Vuren, 1986). Undoubtedly, blood cell responses are important indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. The fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and

pathological alterations and disease manifestations of fishery management and aquaculture (Mulkahy, 1975). Furthermore, the studies pertaining to the detection of abnormalities in the fish (biochemical and haematological) could provide indication of exposure to pollutants before any gross sign become apparent and hence used as reliable indices of fish health. It is also worth to mention that biochemical and haematological responses are said to provide cues regarding the type of pollutants and level of pollution in the environment (Rao, 2010).

MATERIALS AND METHODS

Specimens of *Heteropneustes fossilis* (Total length 21-24 cm and total body weight 50-65 g) were obtained from fish market at Aligarh. The fish were acclimatized to laboratory conditions for two weeks, during which they were fed a minced meat twice daily to satiety. After preliminary trials, two sub-lethal doses (4.0 and 8.0 mg/l) of uranyl acetate dihydrate [$\text{UO}_2(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$] were selected for use in the fish exposure experiments. It had a molecular weight of 424.15 and was 98% ex-U (CAS number is 6159-44-0). A parallel control was performed with uranium-free water. All experiments were run in triplicates. The water of aquarium was renewed after 24 hours. 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.

After 10, 20 and 30 days, 3 fish from each treatment and the control were removed from aquaria, and their total length and body weight were recorded. Blood samples were collected by excising the caudal peduncle to allow the blood from dorsal aorta to flow out. This is an easy method and increasingly used in recent years for small and medium sized fishes. Heparinized vials were used for the collection of blood samples. Haemoglobin was estimated by the cyano-methemoglobin method (Blaxhall and Daisley, 1973). Hematocrit values were determined by using a micro-hematocrit centrifuge. The red blood cell (RBC) and white blood cell (WBC) count was made using Neubauer haemocytometer after diluting the blood with Dace's solution and Turk's solution, respectively. The different indices like MCV, MCH

and MCHC were determined using the methods outlined by Ghai (1986). For biochemical analysis, blood was centrifuged at 6000 rpm for 10 minutes at 4°C and the collected plasma was stored at -20°C till analyzed. Glucose, total protein, Mg, Ca, GOT and GPT were analyzed using their respective kits (BIOMERIEUX, FRANCE).

The livers were immediately dissected out, placed on absorbent paper to absorb excess liquid and weighed. A white muscle sample was excised from a fixed location below the site of origin of dorsal fin and above the lateral line. Glycogen in the liver and muscle was extracted as described by Ashman and Seed (1973) and determined as described by Montgomery (1957) on wet weight basis. Protein, RNA and DNA contents were measured in dry, fat free samples. The technique of Webb and Levy (1955) was followed for the preparation of dry fat-free samples. The method of Lowry et al. (1951) was used for the determination of protein in dry fat-free samples. The technique of Schneider (1957) was employed for the extraction and quantification of RNA. DNA was extracted

from dry fat-free samples as described by Webb and Levy (1955) and measured as described by Ashwell (1957). These are simple and easy methods for the estimation of protein and nucleic acids (RNA and DNA) and undoubtedly give reliable results.

For statistical analysis, 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.

RESULTS

The present findings indicated that sub-lethal exposure of uranium salt has altered the blood parameters of the *H. fossilis*. Reduction in the red blood cell count, haemoglobin concentrations and haematocrit values was evident in the fish exposed to different concentrations of uranyl acetate (Table 1). Decline in the leucocyte count was also registered in the uranyl acetate exposed fish. The fall was more distinct in the

Table 1. Effects of exposure of uranyl acetate on the hematological profile of *Heteropneustes fossilis*. Values are mean \pm standard error

| Parameters | Time (Days) | Concentrations of uranyl acetate (mg/l) | | | |
|--|----------------|---|-------------------|-------------------|--------|
| | | Control | 4.0 | 8.0 | P<0.05 |
| Erythrocytes(Cellx10 ⁶ /mm ³) | 10 | 1.69 \pm 0.15 | 1.54 \pm 0.06 | 1.53 \pm 0.05 | |
| | 20 | 1.68 \pm 0.08 | 1.58 \pm 0.05 | 1.45 \pm 0.06 | * |
| | 30 | 1.66 \pm 0.06 | 1.53 \pm 0.06 | 1.41 \pm 0.04 | * |
| Leucocytes(Cellx10 ³ /mm ³) | 10 | 36.51 \pm 0.51 | 34.23 \pm 0.65 | 34.21 \pm 0.71 | |
| | 20 | 37.56 \pm 0.44 | 32.89 \pm 0.57 | 30.12 \pm 0.50 | * |
| | 30 | 36.95 \pm 0.61 | 31.01 \pm 0.43 | 30.25 \pm 0.59 | * |
| Hematocrit(%) | 10 | 33.86 \pm 0.74 | 32.66 \pm 0.50 | 31.44 \pm 1.05 | |
| | 20 | 34.65 \pm 0.93 | 32.35 \pm 1.10 | 31.41 \pm 1.05 | * |
| | 30 | 33.45 \pm 0.95 | 31.85 \pm 0.96* | 30.42 \pm 1.20 | * |
| Hemoglobin(g/dl) | 10 | 5.85 \pm 0.09 | 4.92 \pm 0.12 | 4.45 \pm 0.10 | * |
| | 20 | 6.54 \pm 0.14 | 4.68 \pm 0.13 | 4.26 \pm 0.09 | |
| | 30 | 5.94 \pm 0.09 | 4.59 \pm 0.08 | 4.14 \pm 0.12 | * |
| MCV(fl/cell) | 10 | 200.36 \pm 3.75 | 213.06 \pm 4.21 | 206.00 \pm 3.58 | * |
| | 20 | 206.25 \pm 4.35 | 204.74 \pm 3.56 | 216.47 \pm 5.21 | |
| | 30 | 201.90 \pm 4.33 | 207.17 \pm 4.25 | 215.13 \pm 5.65 | |
| MCH(Pg/cell) | 10 | 34.84 \pm 1.75 | 31.65 \pm 1.65 | 29.19 \pm 2.11 | |
| | 20 | 38.73 \pm 2.55 | 29.36 \pm 2.45 | 29.87 \pm 1.75 | * |
| | 30 | 35.35 \pm 2.32 | 30.04 \pm 1.45 | 29.39 \pm 2.45 | * |
| MCHC(%) | 10 | 17.29 \pm 1.35 | 15.29 \pm 1.46 | 14.79 \pm 1.25 | |
| | 20 | 18.90 \pm 1.15 | 14.84 \pm 1.26 | 13.47 \pm 0.85 | * |
| | 30 | 17.48 \pm 1.15 | 14.47 \pm 1.45 | 13.94 \pm 1.65 | * |

fishes exposed to higher dose. A slight change in the value of different indices like MCV, MCH and MCHC was apparent in uranium exposed fish.

A significant ($P < 0.05$) elevation of glucose level (Table 2) in the blood of exposed fish was perceived. Hypoproteinaemia was also recorded in the plasma of fish exposed to uranyl acetate especially in the final period of exposure and at higher concentration (Table 2). The uranyl acetate exposure significantly ($P < 0.05$) increased the activity of GOT and GPT enzymes in all exposed groups (Table 2). Reduction in the level of Ca was evident in the plasma of exposed fish but the concentration of Mg was not affected.

Considerable depletion in the level of protein in liver and muscle of the exposed fish was observed (Table 3). The decrease was more pronounced in higher concentration of uranyl acetate and in the later periods of exposure. Similarly, RNA concentration in both the liver and white muscle was lower in exposed fish. In contrast, the DNA concentration in both the tissues was increased after the exposure of the uranium salt. The RNA/DNA ratio of the treated fish was significantly ($P < 0.05$) lower than that of the untreated fish, particularly during the last period of exposure (see Table 3). The amount of

glycogen was significantly lower in the liver and white muscle of exposed fish than those of the control fish. The condition factor (CF) and hepatosomatic index (HSI) of the fish were negatively affected by exposure to uranyl acetate (Fig. 1).

DISCUSSION

It is an accepted fact that blood parameters play an important role in evaluating the effects of chemicals (Roche and Boge, 1996). Generally, toxicants exposure to animals adversely affects and causes reduction in different blood constituents. Conforming to aforesaid view a decrease in the number of RBC count, hemoglobin concentration and haematocrit values in diazinon exposed fish was reported by Banaee *et al.* (2008, 2011); Ahmad (2011, 2012) and they have ascribed it to destruction of cells and/or decrease in size of cells due to adverse effects induced by pesticide. Concordant results have been presented by Zaki *et al.* (2009). Adeyemo (2007) reported decreased haemoglobin level, RBC count and haematocrit values in metal (lead nitrate) exposed *H. fossilis*. Undoubtedly, toxicants would exert detrimental effects on the haematopoietic system, thus

Table 2. Effects of uranyl acetate exposure on plasma biochemical composition of *Heteropneustes fossilis*. Values are mean \pm standard error

| Parameters | Time (Days) | Concentrations of uranyl acetate (mg/l) | | | |
|---------------------|----------------|---|-------------------|-------------------|--------|
| | | Control | 4.0 | 8.0 | P<0.05 |
| Total Protein(g/dl) | 10 | 28.75 \pm 1.58 | 26.35 \pm 1.57 | 25.65 \pm 2.09 | * |
| | 20 | 27.98 \pm 1.68 | 24.65 \pm 1.12 | 24.05 \pm 1.65 | * |
| | 30 | 28.05 \pm 1.48 | 23.85 \pm 1.35 | 22.25 \pm 1.46 | * |
| Glucose(mg/dl) | 10 | 45.25 \pm 7.25 | 65.25 \pm 8.68 | 70.25 \pm 7.56 | * |
| | 20 | 48.35 \pm 6.68 | 64.65 \pm 8.25 | 78.54 \pm 6.75 | * |
| | 30 | 46.52 \pm 6.88 | 72.25 \pm 7.54 | 90.25 \pm 8.25 | * |
| Ca(mg/dl) | 10 | 180.45 \pm 12.3 | 160.25 \pm 10.2 | 155.45 \pm 11.2 | * |
| | 20 | 195.25 \pm 11.2 | 161.45 \pm 12.3 | 150.25 \pm 10.6 | * |
| | 30 | 205.65 \pm 13.1 | 155.35 \pm 09.6 | 145.65 \pm 08.9 | * |
| Mg(mg/dl) | 10 | 37.25 \pm 3.12 | 36.03 \pm 2.15 | 36.32 \pm 4.25 | |
| | 20 | 36.24 \pm 5.31 | 37.45 \pm 5.21 | 34.45 \pm 5.23 | |
| | 30 | 34.23 \pm 5.51 | 36.56 \pm 3.45 | 35.45 \pm 4.24 | |
| PGOT(IU/l) | 10 | 80.25 \pm 15.2 | 95.32 \pm 13.5 | 105.26 \pm 12.4 | * |
| | 20 | 87.25 \pm 16.2 | 115.35 \pm 14.3 | 110.45 \pm 10.5 | * |
| | 30 | 85.25 \pm 14.2 | 120.25 \pm 16.2 | 122.35 \pm 14.2 | * |
| PGPT(IU/l) | 10 | 65.12 \pm 5.66 | 84.22 \pm 8.11 | 98.21 \pm 6.84 | * |
| | 20 | 64.25 \pm 11.3 | 86.25 \pm 10.3 | 93.25 \pm 11.2 | * |
| | 30 | 66.25 \pm 11.2 | 88.45 \pm 12.1 | 105.25 \pm 11.1 | * |

Table 3. Concentrations of nucleic acids, protein and glycogen in the liver and white muscle of *Heteropneustes fossilis* after the exposure to uranyl acetate. Values are mean \pm standard error

| parameters | Concentrations (mg/l) | Exposure Time (Days) | | | | | | | | |
|----------------------|--------------------------|----------------------|----------------------|----------------------|---------------------|---------------------|---------------------|--------|----|----|
| | | Liver | | | Muscle | | | Muscle | | |
| | | 10 | 20 | 30 | 10 | 20 | 30 | 10 | 20 | 30 |
| Protein(mg/100 mg) | Control | 67.03 \pm 1.91 | 66.99 \pm 1.99 | 65.87 \pm 1.78 | 56.47 \pm 1.10 | 56.81 \pm 1.19 | 57.31 \pm 0.89 | | | |
| | 4.0 | 58.33 \pm 2.19 | 58.43 \pm 2.03 | 57.89 \pm 1.86 | 50.53 \pm 1.23 | 49.73 \pm 1.33 | 49.45 \pm 1.25 | | | |
| | 8.0 | 55.86 \pm 1.79 | 55.54 \pm 1.65 | 54.82 \pm 1.24 | 49.36 \pm 1.07 | 48.76 \pm 0.89 | 48.00 \pm 1.03 | | | |
| | P<0.05 | ** | ** | ** | ** | ** | ** | | | |
| RNA(μ g/100 mg) | Control | 5762.49 \pm 89.96 | 5720.78 \pm 108.81 | 5650.03 \pm 101.89 | 1077.17 \pm 44.06 | 1065.17 \pm 28.90 | 1080.17 \pm 30.87 | | | |
| | 4.0 | 4629.92 \pm 118.41 | 4586.26 \pm 137.62 | 4503.87 \pm 189.24 | 823.52 \pm 55.46 | 809.56 \pm 57.40 | 799.86 \pm 43.94 | | | |
| | 8.0 | 4617.50 \pm 145.03 | 4558.84 \pm 150.50 | 4507.27 \pm 126.40 | 817.68 \pm 52.71 | 805.65 \pm 52.15 | 796.94 \pm 42.07 | | | |
| | P<0.05 | ** | ** | ** | ** | ** | ** | | | |
| DNA(μ g/100 mg) | Control | 536.33 \pm 9.35 | 549.65 \pm 11.65 | 560.35 \pm 19.25 | 176.12 \pm 17.29 | 180.13 \pm 15.65 | 185.63 \pm 18.25 | | | |
| | 4.0 | 652.89 \pm 13.11 | 695.36 \pm 15.62 | 715.26 \pm 20.12 | 228.76 \pm 18.68 | 250.62 \pm 15.95 | 265.25 \pm 19.25 | | | |
| | 8.0 | 668.63 \pm 15.88 | 702.13 \pm 18.35 | 718.35 \pm 19.35 | 240.67 \pm 18.89 | 260.25 \pm 19.65 | 268.26 \pm 16.39 | | | |
| | P<0.05 | ** | ** | ** | ** | ** | ** | | | |
| RNA/DNARatio | Control | 10.74 \pm 1.05 | 10.41 \pm 0.95 | 10.08 \pm 1.12 | 6.12 \pm 0.42 | 5.91 \pm 0.51 | 5.82 \pm 0.35 | | | |
| | 4.0 | 7.39 \pm 0.87 | 6.60 \pm 0.98 | 6.30 \pm 1.01 | 3.60 \pm 0.35 | 3.23 \pm 0.25 | 3.02 \pm 0.23 | | | |
| | 8.0 | 6.89 \pm 0.89 | 6.48 \pm 1.01 | 6.26 \pm 0.87 | 3.11 \pm 0.12 | 3.08 \pm 0.21 | 2.99 \pm 0.35 | | | |
| | P<0.05 | ** | ** | ** | ** | ** | ** | | | |
| Glycogen(mg/g) | Control | 9.12 \pm 0.12 | 9.92 \pm 0.21 | 9.90 \pm 0.17 | 3.45 \pm 0.08 | 3.35 \pm 0.06 | 3.35 \pm 0.06 | | | |
| | 4.0 | 7.25 \pm 0.16* | 7.25 \pm 0.19* | 7.32 \pm 0.17* | 2.15 \pm 0.06* | 2.12 \pm 0.05* | 2.10 \pm 0.05* | | | |
| | 8.0 | 7.36 \pm 0.15* | 7.25 \pm 0.16* | 7.35 \pm 0.15* | 2.05 \pm 0.05 | 2.06 \pm 0.05 | 2.06 \pm 0.06* | | | |
| | P<0.05 | ** | ** | ** | ** | ** | ** | | | |

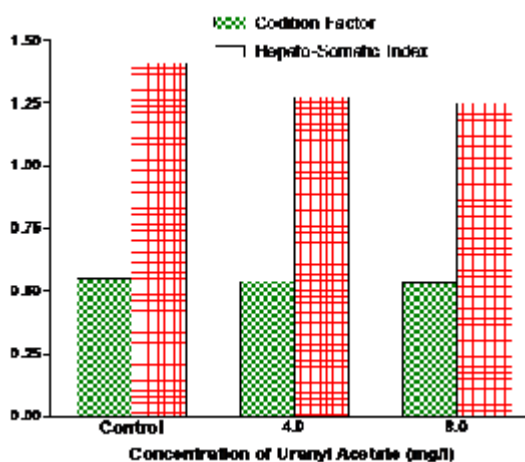


Fig. 1. Hepato-somatic index and condition factor of control and uranyl acetate treated fish

checking the production or subsequent rapid destruction of blood constituents. The results obtained in the present investigation are similar to those mentioned above and can be attributed to aforesaid factors.

The decreased leukocyte count (Table 2) registered in the present investigation would be attributed to malfunctioning of the haematopoietic system caused by exposure of uranyl acetate. The reduction in the number of leucocyte count may also be attributed to fall in the delivery of these cells to the circulation because of reduced production or alternatively an increased rate of removal from circulation and subsequent rapid destruction of cells. This line of reasoning was presented by Al-Kahem *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 exhibiting stress. Significant decline in the number of lymphocytes and thrombocytes may be the main reason for the reduction of WBC count in the fish exposed to chromium was stated by Al-Kahem (1995). Jaffar Ali and Rani (2009) have reported decreased leukocyte count in carp exposed to diazinon- based pesticide.

Blood cell indices like Mean corpuscular hemoglobin (MCH), Mean corpuscular volume (MCV) and Mean corpuscular hemoglobin concentration (MCHC) seem to be changes which are more sensitive. Fluctuations in the values of MCV, MCH and MCHC correspond with values of RBC count, hemoglobin concentration and

haematocrit values. Rao (2010) reported an increase in these indices in common carp exposed to acute toxic level of pesticide.

The glucose level (Table 2) in exposed fish was significantly ($P < 0.05$) elevated which may be due to the mobilization of glycogen into glucose to meet the increased demand for energy. Stress stimuli elicit rapid secretion of glucocorticoids and catecholamines hormones from adrenal tissue of the fish (Pickering, 1981) which are known to produce hyperglycemia in animals. Such elevation may also be due to enhanced gluconeogenesis response of stressed fish in their attempt to satisfy their increased energy demands (Winkaler *et al.*, 2007). The hyperglycemic condition in present study may also be attributed to increased secretion of these hormones which causes glycolysis in the fish exposed to uranium. The present result agrees with the findings of Abalaka *et al.* (2011), Ahmad (2011, 2012); Alkahem-Al-Balawi *et al.* (2011); Al-Ghanim (2012). The toxicant may changes the functions of vital organs like liver and kidney, disrupting the homeostatic condition of the body which may alters the concentrations of metals. Similar observations have been reported by Al-Akel *et al.* (2010) in the carp, *Cyprinus carpio*, after the exposure of dietary copper and support to present investigation.

The enzymes GOT and GPT are also used as important diagnostic tools to detect the toxic effects of various pollutants (Nelson and Cox, 2000). Metals exposure at various concentrations increases the activity of these enzymes (McKim *et al.*, 1970). Jeney *et al.* (1991) also reported an elevated level of both these enzymes (GOT, GPT) in the serum of ammonia exposed fish. Their conception was that SGPT is highly sensitive to any change in the environment. An elevated level of ALT activity in the fish exposed to malathion and extract of *Porkia biglosa* pods was documented by Zaki *et al.* (2009) and Abalaka *et al.* (2011), respectively. These authors believe that the increased activity of this enzyme in the exposed fish is suggestive of hepatic damages leading to their leakage into circulation (Mousa *et al.*, 2008) and /or increased synthesis of enzyme in liver. Similarly, significantly higher values of glutamic – oxaloacetic acid transaminase (SGOT) activities were recorded by Lemair *et al.* (1991) in the fish fed diet without docosahexaenoic acid but the activity of

SGPT did not show any change. They found that hepatic parenchyma develop into generalized massive steatosis, exhibiting necrosis centers with docosahexaenoic acid free diet. Agrahari *et al.* (2007) have reported an increased activity of SGOT and SGPT in *C. punctatus* after the exposure of mercuric chloride and monocrotophos. Vaglio and Landriscina (1999) and Palanivelu *et al.* (2005) suggested that liver is rich in SGOT and SGPT, and damage caused by toxicants to it could result in liberation of large quantities of these enzymes into the blood. Hence, an increase in the activity of these enzymes (PGOT and PGPT) is a sensitive indicator of cellular damage (Van-der *et al.*, 2003; Palanivelu *et al.*, 2005; Ahmad, 2011; Alkahem Al-Balawi *et al.*, 2011). Therefore, eminent 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 *Heteropneustes fossilis* to uranium salt influenced the metabolic processes and altered the tissue concentrations of protein, glycogen and nucleic acids (Table-3). Possibly, uranium disrupts RNA and protein biosynthesis pathway, especially by inhibiting the activities of polymerases, checking the epigenetic origin of these macromolecules, or else it can promote catabolism of the preformed quantities. Studies by Mazeaud *et al.* (1977) and Strange *et al.* (1977) have clearly demonstrated that toxins impose stresses on the body, leading to an increased output of adrenocorticotrophic hormone (ACTH). This pituitary hormone stimulates the adrenal gland to produce larger quantities of corticosteroids, augmenting the enzymatic conversion of glycogen, protein and fat to glucose. Significant hypoproteinaemia was recorded in the fish exposed to different pollutants (Omoniyi *et al.*, 2002; Shalaby, 2009). This reduction was attributed to the cell destruction or necrosis, with subsequent impairment of protein synthesis machinery (Bradbury *et al.*, 1987) or to pathological alterations in kidney, leading to excessive loss of proteins (Salah El-Deen *et al.*, 1996). In contrast, hyperproteinaemia was reported by Al-Attar (2005), Omitoyin (2007) and Abalaka *et al.* (2011) in animals exposed to various toxicants. RNA/DNA ratio was lower in the fish exposed to uranyl acetate than the control. This result demonstrated the extreme toxicity of uranyle acetate in *Heteropneustes*

fossilis in terms of its effects on the primary biochemical machinery. According to Kearns and Atchison (1979), Gwak and Tanaka (2001), Adham (2002) and Joanna *et al.* (2010) the RNA/DNA ratio is an integrative indicator of contaminant stress and of overall effects on fish growth. Briefly, the contamination of the water by uranyl acetate caused adverse effects and imposed high stresses on fish, resulting in retarded growth and impaired function of the liver, kidney and other organs. Mukharjee and Jana (2007), Priyamvada *et al.* (2010); Raksheskar, (2012) observed decreased level of RNA/DNA ratios in the fish treated with different pollutants. The biokinetics (Absorption, distribution, transformation and elimination) of uranium have been extensively studied, and the kidney has been identified as target organ (ATSDR-1999; FPTCDW, 2001). The study of the white fish, *Coregonus clupeaformis*, suggested that long-term exposure causes both kidney damage and liver damage (Cooley *et al.*, 2000). At cellular level, toxicity may result from the binding of uranium to enzymes (Khangarot, 1991), which would subsequently inactivate or disrupt enzyme function. More comprehensive assessments of the physiological effects of pollutants on fish is required.

In light of the proven and well documented (West and Todd, 1963; Mustafa and Mittal, 1982) metabolic stability of DNA, the increased DNA content found in the uranium-exposed fish in this study appears to be paradoxical. The need to determine the so-called 'cause-and-effect' relationship, so important in the realm of molecular biology, is critical in such a situation. It is plausible that DNA can't be synthesized by the fish under the stressful conditions produced by uranium exposure. It is evident that depletion of cellular constituents, primarily proteins, in the experimental fish, reduces the weight of individual cell. In fact, a larger number of cells that individually weigh less can form a mass of tissue equal to that formed by a smaller number of cells with intact macromolecule reserves. This greater number of cells per unit weight of sample can explain the higher DNA concentration in the fish exposed to uranium. That DNA content is related to cell number is already well established (Mustafa and Mittal, 1982; Mustafa and Shams, 1982; Mustafa and Zofair, 1984). The condition factor (CF) has

been used to compare growth conditions of fish. A high condition factor reflects good environmental quality, while a low condition factor reflects poor environmental quality. Hepatosomatic index (HSI) provides an indication on status of energy reserve in an animal. In a poor environment, fish usually have a smaller liver (with less energy reserved in the liver). HSI has been reported to decrease in fish exposed to different pollutants.

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 haematological parameters in fish. It is hypothesized that the toxicant either suppresses the activity of the enzymes responsible for the synthesis of these macromolecules or enhanced 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.

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