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RESEARCH PAPER

Arsenic and Urea in Combination Alters the Hematology, Biochemistry and Protoplasm in Exposed Rahu Fish (*Labeo rohita*) (Hamilton, 1822)

Abdul Ghaffar[2], Riaz Hussain[3], Muhammad Aslam[2], Ghulam Abbas[3], Ahrar Khan[1,*]

[1] University of Agriculture, Pathology, Faisalabad, Pakistan.
[2] The Islamia University of Bahawalpur, Department of Life Sciences (Zoology), and University College of Veterinary and Animal Sciences,- 63100, Pakistan, Bahawalpur, Pakistan.
[3] University of Karachi, Center of Excellence in Marine Biology, 75270, Karachi, Pakistan.

* Corresponding Author: Tel.: 0092.333 6517844; Fax: ;
E-mail: ahrar1122@uaf.edu.pk

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Abstract

This study was conducted to assess hemato-biochemical and different protoplasmic changes, which are indicators of genotoxicity in erythrocytes of *Labeo rohita* exposed to arsenic and urea. For this purpose, 20 fish were kept in glass aquaria for acclimatization (5 days), randomly divided into five equal groups: A (control) and exposed to arsenic (in mg/L) + urea (in g/L) B (8+0.2), C (10+0.4), D (12+0.6) and E (15+0.8), respectively for 9 days. Blood samples with anticoagulant were collected at 3rd, 6th and 9th experiment days for hematology. Serum was separated from blood samples collected without anticoagulants for biochemical studies. Results showed that exposure with arsenic and urea in higher concentrations (groups D and E) significantly decreased ($P<0.05$) erythrocyte counts, hemoglobin concentration, packed cell volume, mean corpuscular hemoglobin concentration, lymphocyte, and monocyte counts, accompanied by significant increase in mean corpuscular volume and leucocyte counts than those in control group (A). Alanine and aspartate amino transaminase activities at 6th and 9th days in high dose (groups D and E) were significantly increased whereas glucose and total protein decreased ($P<0.05$). It is concluded that arsenic has hemato-biochemical and genotoxic effects even at the low dose rate in the presence of nitrogenous chemicals in aquatic ecosystem.

Keywords: Arsenic, Urea, Hemato-biochemical changes, Nuclear changes.

Introduction

With the development and expansion of industrial technology, the extensive use of different synthetic chemicals such as fertilizers, pesticides, insecticides, herbicides and arsenic has led to contamination of many freshwater ecosystems (Mandour *et al.,* 2012; Mashkoor *et al.,* 2013; Ghaffar *et al.,* 2014; Witeska *et al.,* 2014). Different industrial and agricultural processes continuously release various wastes into natural fresh water sources and have adverse effects on aquatic biota (Witeska *et al.,* 2014). Identification, monitoring, and management of these pollutants are crucial in order to minimize their adverse effects on aquatic ecosystems.

Heavy metals are continuously contaminating natural water resources. Their deleterious impacts on different economically important aquatic animals like fishes are manifold (Lima *et al.,* 2010). Although the contamination levels in natural ecosystems are usually well below the limits that can cause mortality in exposed animals, these may be sufficient to impair the normal functioning of tissues. These pollutants induce disturbances in various physiological and biochemical mechanisms of fishes (Abdel-Rahman *et al.,* 2011; Mandour *et al.,* 2012). Freshwater fishes are the best sentinels for determining the health status of an aquatic ecosystem. The early toxic impact of any pollutant is only evident on cellular or tissue levels then it becomes prominent in behavior or morphological changes (Witeska *et al.,* 2014).

Arsenic, being a natural element, is found in several environmental compartments as a result of natural and anthropogenic activities (Abdel-Hameid, 2009; Baldissarelli *et al.,* 2012; Rahman *et al.,* 2012; Aruljothi *et al.,* 2013). Natural sources of arsenic include weathering, volcanic and biological activities (Rehman and Hasegawa, 2012; Zhang *et al*., 2014), while anthropogenic sources include mining activity, use of pesticides and wood preservatives which result in contamination of soil and other related substances (Baldissarelli *et al.,* 2012). Arsenic is found in organic and inorganic forms; the latter being more toxic than the former one and exists in different oxidative???? (Datta *et al.,* 2012; Khan *et al*., 2013; Kousar and Javed, 2014).

Arsenic pollution in natural water resources has become a great challenge throughout the world which poses serious human health problems, being the potent toxic agent in the ecosystem (Aruljothi *et al.,*

2013; Khan *et al*., 2014). Arsenic induces its toxic effects through a variety of mechanisms including impaired cellular respiration, disengagement of oxidative phosphorylation and by inhibition of different mitochondrial enzymes (Mashkoor *et al.*, 2013; Magellan *et al.,* 2014). Arsenic toxic effects results are due to interaction with sulfhydryl groups of various enzymes, proteins and it also substitutes phosphorus in biochemical reactions (Patlolla *et al.*, 2005).

Fertilizers from nitrogen source result in contamination of freshwater ecosystem and target aquatic life due to the changes in the environment. The aquatic organisms exhibit the different degrees of changes in their physical and behavioral pattern when they are exposed to pollutants. Agrochemical fertilizers exert devastating effects on different animals including aquatic biota are available (Bobmanuel *et al.,* 2006; Yadav *et al.,* 2007; Shahzad *et al.,* 2012). Fish are important and a key component of aquatic ecosystems and play a significant role in arsenic mobilization. Even small fish are an important and major part of the aquatic food chain and are a source of proteins for human consumption (Kumar and Banerjee, 2012; Zhang *et al.,* 2013). Some fish are used as biomarkers and bio-indicators of different aquatic contaminants (Bhattacharya *et al.,* 2007). Bioaccumulation of various pollutants in fish in aquatic ecosystem occurs directly through gills, skin and via consumption of prey (Rahman *et al.,* 2012). Therefore, in the present experimental study, we expected to determine the hemato-biochemical and different protoplasmic changes as an indicator of genotoxic potential in erythrocytes of fish exposed to arsenic and urea in combinations even at very low concentrations.

Materials and Methods

In this experimental study, 20 apparently healthy freshwater fish *Labeo rohita* (rohu) of uniform size, weight (250-275 g) and age were obtained from the local fish breeding center and kept in the wet laboratory. Fish were placed in glass aquaria of 100 L volume. The aquaria were cleaned appropriately with chlorinated water before the introduction of fish. Before starting the exposure, the fish were acclimatized in the laboratory for 5 days, in fresh, cleaned water (pH = 7.0; hardness=136 mg/l; conductivity = 1.2 mmoles/cm; temperature = 27.5°C) and a constant photoperiod (12:12 h light: dark cycle). The fish were randomly distributed into five equal groups (A, B, C, D and E) and were exposed to aqueous concentrations of arsenic (in mg/L) + urea (in g/L) B (8+0.2), C (10+0.4), D (12+0.6) and E (15+0.8), respectively for 9 days.

Hematology

The fish were monitored throughout the study for physical and/or behavioral changes. Blood samples (1.50 mL) were taken from the caudal vein of each fish at 3rd, 6th and 9th days of the experiment by using with and without anticoagulant (Ethylenediaminetetraacetic acid; 1 mg/mL). Hematological parameters such as erythrocyte counting, leukocyte counting, hemoglobin concentration and pack cell volume, erythrocyte indices like mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, monocyte, and lymphocyte were determined according to the methods previously ascribed (Ghaffar *et al*., 2014).

Biochemistry

Serum was separated from blood samples collected without anticoagulants and stored at -20ºC till analysis. Commercially available diagnostic kits were used for the estimation of enzymes and glucose spectrophotometrically following the instructions of the manufacturer. For aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and glucose, GOT (ASAT) Reagent Kit # 12021150, GPT (ALAT) Reagent Kit # 12022150, Alkaline Phosphatase Reagent Kit # 12027150 and Glucose Reagent Kit # 10260150, respectively were procured from HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany. Total proteins were determined by Biuret method (Oser, 1976).

Genotoxicity

The duplicate fresh thin blood smears were made from each fish to evaluate any genotoxic/protoplasmic impacts induced by the combination of different arsenic and urea concentrations. Blood smears were dried in air and fixed in absolute methanol. Subsequently, the fixed samples were stained by using Giemsa for four to five minutes. Numerous nuclear and morphological changes in erythrocytes including the frequency of micronuclei or DNA damage were examined with the help of microscope (1000X). A total of two thousand erythrocytes were studied in a fish smear (Hussain *et al*., 2014; Mahboob *et al*., 2014).

Statistical Analysis

The data thus obtained on hematological, serum biochemical and nuclear aberrations were statistically analyzed by using SAS computer program to know the significance among the various parameters.

Results

Hematological and Immunological Findings

The exposure of fish to different concentrations

of arsenic and urea showed the mixed type of effects on different evaluated endpoints (Table 1). The combined exposure with higher concentrations (group D and E) significantly decreased ($P<0.05$) erythrocyte count, hemoglobin concentration, corpuscular hemoglobin contents, packed cell volume, lymphocyte, and monocyte count. Whereas, the mean corpuscular volume and the leucocyte count were significantly increased in respective days in fish of groups D and E as compared to those in the control group.

Serum Biochemical Parameters

The serum activities of alanine transaminase and aspartate transaminase at 6th and 9th days of the experiment in higher exposed fish (groups D and E) were significantly increased as compared to their respective controls (Table 2). Moreover, a significant increase in the levels of both enzymes was also observed on 3rd day. Alkaline phosphate was increased ($P<0.05$) in fish of group E at 3rd and 6th days of experiment and in the fish of groups D and E on the 9th day of the experiment. The blood glucose in groups D and E was decreased ($P<0.05$) throughout the experiment. Serum total protein contents were significantly decreased in the fish of group E at 3rd day and in the fish of groups D-E at 6th and 9th days of the experiment.

Genotoxic/ Protoplasmic Studies

The fish of group E exposed to arsenic and urea showed increased frequency of protoplasmic/nuclear abnormalities in erythrocyte throughout the study. The frequency of cells with lobed nuclei, blebbed nuclei and cells with micronuclei increased significantly in fish exposed to the highest concentration (group E) as compared to control group. Moreover, the frequencies of vacuolated nuclei at 3rd and 6th day of the experiment and vacuolated and notched nuclei at the 9th day of the experiment were significantly higher in fish exposed to two high concentrations (groups D, E) compared to that of control treatment. The occurrence of cells with nuclear remnants, condensed nuclei and fragmented nuclei augmented in groups D and E during the entire period of this study (Table 3). The frequency of various morphological changes in erythrocytes of fish such as dividing erythrocytes, pear shaped erythrocytes, microcytes (Figure 1) and tear shaped erythrocytes (Table 4) increased significantly in

Table 1. Various hematological profile of *L. rohita* treated at different levels of arsenic and urea

	Groups				
Parameter	A	B	C	D	E
Erythrocyte counts (10^6/mm^3)					
3	4.07±0.01	3.94±0.01	3.88±0.00	3.84±0.01*	3.58±0.02*
6	3.98±0.03	3.82±0.01	3.63±0.03	3.25±0.04*	2.76±0.02*
9	4.28±0.23	3.71±0.02	3.66±0.03	2.75±0.13*	2.46±0.04*
Hemoglobin (g/dl)					
3	8.71±0.16	8.19±0.02	7.99±0.01	7.63±0.16*	6.04±0.04*
6	7.54±0.21	7.15±0.01	6.96±0.02	6.35±0.17*	5.69±0.03*
9	7.30±0.01	6.88±0.09	6.57±0.03	5.49±0.05*	4.96±0.03*
Pack cell volume (%)					
3	30.8±0.67	29.9±0.23	28.3±0.23	26.9±0.046*	25.1±0.14*
6	30.65±0.09	28.05±0.11	26.8±0.21	25.07±0.26*	24.4±0.12*
9	30.37±0.41	26.5±0.07	26.1±0.17	24.1±0.07*	23.3±0.08*
Mean corpuscular volume (fl)					
3	21.0±0.20	21.8±0.20	22.7±0.14	22.9±0.11*	25.8±0.20*
6	20.7±0.14	23.2±0.10	23.2±0.13	25.2±0.26*	27.8±0.54*
9	18.3±2.51	23.5±0.07	23.6±0.04	27.6±0.27*	29.4±0.26*
Mean corpuscular hemoglobin concentration (g/dl)					
3	25.7±0.50	24.8±0.29	24.3±0.10	23.2±0.20*	19.1±0.13*
6	26.3±0.35	24.3±0.67	23.4±0.08	22.6±0.28*	17.6±0.20*
9	26.0±0.17	23.4±0.29	22.6±0.23	17.9±0.17*	16.6±0.13*
Leukocyte counts (10^3/mm^3)					
3	16.5±0.33	17.6±0.33	18.2±0.17	19.0±0.19*	24.7±0.48*
6	17.7±0.17	18.1±0.40	19.1±0.17	20.2±0.24*	26.3±0.27*
9	17.9±0.35	19.1±0.27	21.0±0.22	24.4±0.31*	27.2±0.25*
Lymphocytes (%)					
3	19.9±0.21	19.3±0.25	18.8±0.34	17.6±0.08*	14.9±0.12*
6	20.0±0.12	19.1±0.08	18.3±0.14	17.2±0.06*	14.7±0.13*
9	20.0±0.29	18.5±0.09	17.4±0.11	14.6±0.18*	14.1±0.14*
Monocytes (%)					
3	4.12±0.01	4.09±0.00	4.08±0.01	3.98±0.03*	3.92±0.02*
6	4.14±0.01	4.05±0.02	3.97±0.01	3.87±0.01*	3.62±0.03*
9	4.15±0.02	3.98±0.03	3.86±0.05	3.45±0.06*	3.30±0.05*

Table 2. Biochemical profile of *L. rohita* exposed at varying levels of arsenic and urea

	Groups				
Parameter	A	B	C	D	E
Alanine transaminase (U/L)					
3	22.2±0.88	23.1±0.37	24.1±0.29	25.3±0.20	31.6±1.92*
6	23.9±0.19	25.2±0.32	25.3±0.27	32.5±1.30*	41.2±1.47*
9	23.6±0.20	24.9±0.31	26.1±0.16	38.3±1.94*	47.1±1.98*
Aspartate transaminase (U/L)					
3	19.5±0.5	20.5±0.31	21.2±0.10	22.7±0.22	30.0±1.46*
6	19.3±0.42	20.7±0.45	21.9±0.25	23.5±0.08*	33.6±1.46*
9	19.7±0.47	21.8±0.31	23.3±0.23	30.4±0.82*	39.2±1.74*
Alkaline phosphatese (U/L)					
3	37.1±0.37	38.7±0.33	39.5±0.14	40.6±0.34	77.6±5.21*
6	39.8±0.27	42.3±0.21	42.8±0.24	61.8±1.82	104.6±11.6*
9	40.0±0.25	43.1±0.19	43.9±0.26	84.5±4.30*	126.2±10.4*
Glucose (g/dL)					
3	36.6±0.47	36±0.36	35.1±0.21	33.9±0.20*	30.8±0.22*
6	36.4±0.21	35.3±0.18	34±0.20	33.4±0.33*	28.7±0.47*
9	35.6±0.17	33.7±0.23	33.1±0.25	28.9±0.18*	26.3±0.49*
Total protein (g/dL)					
3	3.43±0.02	3.37±0.01	3.34±0.01	3.25±0.08	2.50±0.05*
6	3.42±0.02	3.32±0.02	3.25±0.04	3.05±0.02*	2.45±0.03*
9	3.36±0.03	3.30±0.03	3.01±0.24	2.63±0.02*	2.24±0.03*

Asterisk on the values (mean±SE) in row represent statistical (P≤0.05) difference among treatment groups compared to control.

Table 3. Nuclear changes in erythrocytes of *L. rohita* treated at different levels of arsenic and urea

					Groups
Parameter	A	B	C	D	E
Lobed Nuclei (%)					
3	0.15±0.00	0.15±0.00	0.17±0.00	0.18±0.00	0.25±0.01*
6	0.15±0.00	0.16±0.00	0.17±0.00	0.19±0.00	0.54±0.02*
9	0.16±0.00	0.18±0.00	0.19±0.00	0.36±0.05	0.78±0.05*
Blebbed Nuclei (%)					
3	0.20±0.01	0.20±0.00	0.21±0.00	0.21±0.00	0.43±0.02*
6	0.19±0.00	0.21±0.00	0.22±0.00	0.31±0.03	0.65±0.04*
9	0.20±0.00	0.22±0.00	0.23±0.00	0.40±0.05	0.78±0.04*
Vacuolated Nuclei (%)					
3	0.34±0.00	0.36±0.00	0.37±0.00	0.37±0.00	0.64±0.02*
6	0.35± .01	0.36±0.00	0.38±0.00	0.39±0.00	0.79±0.01*
9	0.36±0.00	0.38±0.00	0.40±0.00	0.67±0.02*	0.93±0.02*
Cells with Micronuclei (%)					
3	0.13±0.00	0.14±0.00	0.15±0.00	0.15±0.00	0.47±0.02*
6	0.14±0.00	0.15±0.00	0.15±0.00	0.16±0.00	0.79±0.04*
9	0.14±0.01	0.15±0.00	0.16±0.00	0.66±0.07	1.05±0.29*
Notched Nuclei (%)					
3	0.23±0.00	0.24±0.00	0.25±0.00	0.26±0.00	0.66±0.03*
6	0.23±0.00	0.25±0.00	0.26±0.00	0.27±0.00	0.87±0.03*
9	0.22±0.00	0.24±0.00	0.26±0.00	0.50±0.02*	1.21±0.08*
Cells with nuclear Remnants (%)					
3	0.14±0.03	0.15±0.03	0.16±0.02	0.16±0.03	0.39±0.1*
6	0.15±0.02	0.16±0.03	0.17±0.03	0.43±0.04*	0.69±0.08*
9	0.15±0.02	0.16±0.02	0.18±0.02	0.57±0.06*	0.95±0.09*
Condensed nuclei (%)					
3	0.12±0.02	0.13±0.02	0.14±0.01	0.14±0.02	0.26±0.01*
6	0.13±0.01	0.15±0.02	0.15±0.01	0.26±0.01*	0.47±0.02*
9	0.13±0.01	0.15±0.02	0.17±0.02	0.43±0.03*	0.71±0.02*
Fragmented nuclei (%)					
3	0.12±0.02	0.14±0.02	0.17±0.01	0.18±0.02*	0.37±0.01*
6	0.13±0.02	0.15±0.01	0.16±0.02	0.25±0.01*	0.45±0.01*
9	0.15±0.01	0.15±0.01	0.18±0.02	0.35±0.01*	0.55±0.02*

Asterisk on the values (mean±SE) in row represent statistical (P≤0.05) difference among treatment groups compared to control.

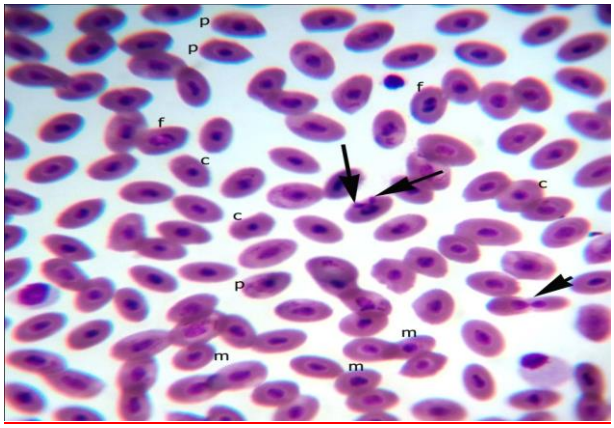

Figure 1. Nuclear and morphological changes in erythrocytes of the blood smear of *L. rohita* treated with arsenic (12 mg/L) and urea (0.6 g/L). Arrow=micronucleus; Arrow heads=dividing erythrocyte; c=condensed nucleus; f=fragmented nucleus; m=microcyte; p=pear shaped; Giemsa stain: 1000x.

Table 4. Morphological changes in erythrocytes of *L. rohita* treated at various levels of arsenic and urea

	Groups				
Parameters	A	B	C	D	E
Pear shape erythrocytes (%)					
3	0.42±0.00	0.43±0.00	0.44±0.00	0.53±0.02*	0.78±0.03*
6	0.45±0.00	0.45±0.00	0.47±0.00	0.60±0.02*	0.92±0.03*
9	0.42±0.00	0.46±0.00	0.47±0.00	0.71±0.07*	1.11±0.10*
Microcyte (%)					
3	0.22±0.03	0.22±0.02	0.23±0.01	0.25±0.02	0.30±0.01*
6	0.23±0.02	0.26±0.01	0.26±0.01	0.38±0.04*	0.59±0.06*
9	0.24±0.02	0.27±0.03	0.28±0.02	0.51±0.03*	0.67±0.07*
Dividing erythrocyte (%)					
3	0.15±0.01	0.16±0.01	0.17±0.02	0.18±0.01*	0.36±0.02*
6	0.14±0.02	0.15±0.02	0.17±0.01	0.28±0.01*	0.48±0.02*
9	0.15±0.02	0.17±0.01	0.18±0.01	0.34±0.02*	0.52±0.01*
Leptocyte (%)					
3	0.24±0.01	0.25±0.02	0.26±0.02	0.26±0.01	0.41±0.02*
6	0.24±0.01	0.26±0.02	0.27±0.01	0.34±0.02*	0.54±0.02*
9	0.25±0.02	0.27±0.01	0.27±0.01	0.47±0.03*	0.65±0.01*
Tear shaped erythrocyte (%)					
3	0.14±0.01	0.15±0.01	0.16±0.02	0.22±0.01*	0.37±0.01*
6	0.16±0.01	0.17±0.02	0.18±0.01	0.26±0.01*	0.43±0.02*
9	16±0.02	17±0.01	18±0.02	39±0.01*	52±0.01*
Stomatocyte (%)					
3	0.17±0.01	0.17±0.01	0.20±0.02	0.20±0.02	0.31±0.01*
6	0.17±0.02	0.18±0.02	0.20±0.01	0.22±0.01*	0.36±0.02*
9	0.17±0.02	0.20±0.01	0.22±0.02	0.32±0.01*	0.42±0.01*

groups D-E throughout the experiment. Moreover, the frequency of stomatocytes in group E at 3rd day and stomatocytes and leptocytes at 6th and 9th days in groups D-E increased significantly ($P<0.05$) as compared to control group.

Discussion

Pathophysiology of arsenic toxicity is dynamic. Arsenic accumulates mainly in liver (Khan *et al*., 2014) where arsenic biomethylation corresponding production of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Buchet and Lauwerys, 1988). MMA and DMA disable many enzymes that are involved in cellular energy production and repair or DNA synthesis. Arsenic antagonizes phosphate during the synthesis of ATP (Haque *et al*., 2011) and also binds with sulfhydryl of many enzymes when used in reduced form, which are required for cellular metabolism (Brouwer *et al.*, 1992). Individuals exposed to arsenic have different nucleotide deletion repair mechanism, thus altering the DNA repair process (Andrew *et al.*, 2006).

The severity and magnitude of risk induced by a

given chemical/pollutant depends on the balance between extent of cytogenetic injury, cell renewal, loss of DNA-repair and apoptosis. Therefore, it is crucial to assess the development and extent of genotoxic effects, assuming the nuclear anomalies as hardly repairable in exposed individuals (Jha *et al.,* 2008; Praveena *et al.,* 2013).

In the present study, all the changes occurred in dose depended manner. Significant decrease in erythrocyte counts, hemoglobin concentration, packed cell volume, mean corpuscular hemoglobin concentration, lymphocyte, monocyte and serum total proteins while increased values of total leukocytes and mean corpuscular hemoglobin were recorded in exposed fish. The lower values of erythrocyte counts and hemoglobin concentration in this study could be due to inability of fish to carry the adequate amount of oxygen to hematopoietic tissue (Hussain *et al.,* 2014). Moreover, decreased values of erythrocytes, hemoglobin concentration, and serum total proteins could also be due to exhaustion and reduced hemopoietic and metabolic activities in fish exposed to arsenic and urea. The hematopoietic system and blood parameters are known to be the best biomarkers to monitor the pathophysiological status of organisms exposed to different toxicants (Kousar and Javed, 2015). Furthermore, increased values of mean corpuscular volume in our study can be related to increased number of immature erythrocytes suggestive of macrocytic anemia (Carvalho and Farnandes, 2006). Decreased values of mean corpuscular hemoglobin concentration observed in this study could be interpreted as an inability of the hematopoietic system to produce hemoglobin, with swelling of the erythrocytes (Kori-Siakpere, 2011). Abnormal values of these blood parameters (MCV and MCHC) are suggestive of anemia, probably due to deleterious effects of arsenic and urea in combination which is supported by decreased values of erythrocyte counts, hemoglobin concentration and packed cell volume. In addition, these hematological changes may be due to the insufficient exchange of gasses by gills and linings of the operculum (Osman *et al.,* 2009; Bhatkar, 2011), while increased leukocytes and decreased lymphocytes could be due to the sensitivity of the immune system to the stress induced by arsenic and urea.

In the present experiment, arsenic and urea significantly increased alanine transaminase, aspartate transaminase, and alkaline phosphatase activities. Serum ALT and AST are best indicators of overall health status of an individual especially hepatocyte injury and related stress (Ahmad *et al*., 2011). Increased aminotransferases are associated with liver injury (Manna *et al*., 2003). The increased levels of these enzymes also suggestive of adverse effects of arsenic and urea on the hepatocytes and abnormal cellular metabolism leading to cell death (Humtsoe *et al.,* 2007; Sharma *et al.,* 2007; Haque and Roy, 2012). ALP is membrane bound enzyme which is found on all cell membranes, where active transport occurs. Serum ALP activity increases in case of damage to hepatic cells (El-Demerdash *et al*., 2003).

The most frequently observed nuclear abnormalities in erythrocytes of fishes exposed to arsenic and urea were: lobed nuclei, blebbed nuclei, vacuolated nuclei, micronuclei, notched nuclei, nuclear remnants, condensed nuclei and fragmented nuclei. So far as our knowledge is concerned, no information is available about such nuclear alterations in erythrocytes of fish exposed to arsenic and urea. The formation of lobed nuclei, nuclear remnants, fragmented nuclei and micronuclei in erythrocytes in the present study might be due to increased generation of caspase-activated DNase, resulting in cleavage of cytoskeleton (fodrin, vimentin and gelsolin) and nuclear proteins, leading to mitochondrial damage (Hussain *et al.,* 2014). Different morphological changes in erythrocytes (Table 4) of fish exposed to arsenic and urea could have occurred due to increased generation of lipid peroxidation. Moreover, toxicants with oxidative stress potential might assault DNA leading to damage at molecular level (Jha, 2008; Hussain *et al.,* 2014). Frequencies of nuclear and morphological anomalies in erythrocytes of fish such as irregular nucleus shape, poikilocytosis, binuclear, and micronuclei indicate genotoxic effects which could have taken place due to arsenic intoxication (Ergene *et al.,* 2007; Strunjak-Perovic *et al.,* 2010). A high proportion of abnormal shapes erythrocytes could also be due to increased erythropoiesis to compensate the impaired function and to eliminate the damaged cells (Witeska *et al.,* 2014). It was concluded from the findings of the present study that arsenic exerts deleterious effects in freshwater fish in the form of various hemato-biochemical changes and genotoxicity. Presence of nitrogenous compounds in aquatic ecosystem enhances the toxicity of arsenic.

Competing Interests

The authors declare that they have no competing interests.

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