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CLINICO-HEMATOLOGICAL AND MUTAGENIC CHANGES INDUCED BY ARSENIC AND COPPER SULPHATE IN ADULT POULTRY MALES

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ABSTRACT

The present experimental study was conducted to investigate the clinico-hematological and mutagenic changes induced by concurrent oral administration of arsenic and copper sulphate in adult poultry males. After acclimatization, a total of 28birds were randomly divided into seven equal groups. All the experimental birds received arsenic and copper sulphate alone and in combinations at different doses for 30 days in the feed. Blood samples were collected from each bird at days 10, 20 and 30 of the experiment. Various clinical signs like decreased feed intake, body weight, ruffled feather, depression, dullness, ocular discharge, open mouth breathing, diarrhea and pale comb were observed at higher levels of arsenic and copper sulphate. In treated birds,the values of total erythrocytes counts, total leukocyte counts, hemoglobin concentration and mean corpuscular hemoglobin concentration were significantly decreased, while packed cell volume and mean corpuscular volume increased.Moreover, frequency of erythrocytes with micronuclei, blabbed, lobed, notched and cells with nuclear remnants were significantly increased compared to controls. From the results of this study it can be concluded that arsenic and copper sulphate alone at higher levels and in combination even at lower levels have severe clinico-hematological and mutagenic effects in adult male birds.

Key words: Birds, Copper sulphate, Arsenic, Hematology, Micronuclei, Nuclear remnants.

INTRODUCTION

Arsenic from both natural and anthropogenic sources has universal impacts (Rahman and Hasegawa, 2012) and different aquatic systems such as fresh waters are the key repositories for this metal (Mashkoor *et al*., 2013; Naz and Javed, 2013; Magellan *et al*., 2014).Arsenic toxicity depends on various interacting factors including its source, method of application, bioavailability, environmental factors, resistance of the exposed organisms and detoxifyingmechanisms (Rahman and Hasegawa, 2012). Biological activity also plays important role in arsenic distribution, speciation and cycling in freshwater (Rahman *et al*., 2012). Different animals take arsenic through direct ingestion, absorption and inhalation or indirectly throughfood chain (Khan *et al*., 2014). In the body arsenic combines with sulfhydryl group and disrupts the cellular metabolic activities (Manna *et al.,* 2008; Kousar and Javed, 2014). It can induce chromosomal aberrations, inhibit DNA repair and disrupts gene expression (Banerjee *et al.,* 2008).The clinical signs of arsenic toxicity include decreased feed intake, weight gain, dullness, salivation and skin lesions (Jun *et al.,* 2008). In avian species, depletion of lymphocytes, hemorrhages in spleen and cystic spaces in bursa of Fabricius have been reported (Kalavathi *et al.,* 2011). Low levels of arsenic exposure may cause gastrointestinal irritation, decreased production of red and white blood cells (Abernathy *et al*., 2003). One of the most powerful toxic metalloid (Silbergeld *et al.,* 2008) occurs in Pakistan (especially in southern areas of Sindh province), Bangladesh and India (Islam *et al*., 2009; Wadhwa *et al*., 2011). In southern areas of Pakistan, the level of arsenic in drinking water is 3-30 folds higher than the permissible level (Baig *et al*., 2011; 2012). Millions of the people are exposed to inorganic forms of arsenic through food and water (Orloff *et al*., 2009). Higher concentration of arsenic has been found in different tissues of many aquatic organisms such as fish, wild and sea birds.

In Pakistan, the poultry industry is the most important component of livestock and has been rapidly expanding for the last decade (Islam *et al*., 2013; Rasool *et al.,* 2013; Saleemi *et al*., 2014). In poultry sector, farmers frequently use copper sulphate in broiler feed as growth promoter and to remove the bacterial, parasitic and fungal infections (Shahzad *et al*., 2012). Copper is an important component of several metalo-enzymes, proteins and some naturally occurring pigments. It is essential for hemoglobin synthesis, bone formation, mitochondrial functions, cellular metabolism, signal transduction, blood clotting and in several other processes (Kim *et al.,* 2008). However, adverse effects such as poor feed intake, decrease in body weight and hemato-biochemicals changes at higher doses of copper have been reported (Song *et al*., 2011; Shahzad *et al*., 2012; Rasool *et al*., 2013).

Previously, in Pakistan different reports are available about the arsenic and copper sulphate toxicity in poultry; however, in published literature no report is available regarding the clinico-hematological and mutagenic effects in concurrently arsenic and copper sulphate intoxicated birds. Therefore, the present experimental research describes the mutagenic effects of arsenic and copper sulphate in adult male birds.

MATERIALS AND METHODS

Birds and management: A total of 28 adult males of Lohmann Selected Leghorn of 17-18 weeks age and free from any clinical ailments were purchased from a local poultry farm. All the birds were kept in wire cages for 7 days under similar laboratory conditions of temperature (25-35°C) and humidity (65-70%) for acclimatization purpose. After acclimatization, the experimental birds were allocated randomly into 7 groups (A-G) each having four birds.The arsenic and copper sulphate were orally given to birds of different groups as follow: A (Control), B (Copper sulphate@ 150mg/kg), C (Arsenic @10 mg/kg + Copper sulphate @50 mg/kg), D (Arsenic @ 15mg/kg + Copper sulphate @75 mg/kg), E (Arsenic @ 20mg/kg + Copper sulphate@ 100mg/kg), F (Arsenic @25mg/ kg + Copper sulphate @100 mg/kg) and G (Arsenic @35mg/kg). These treatments were given to birds of respective group daily for 30 days.

Physical parameters and blood collection: All the birds were observed for any clinical and behavioral alterations twice daily for 30 days. Blood samples about 2-3 ml with anticoagulant (EDTA; 1 mg/ml) were collected from the wing vein of each bird on days 10, 20 and 30 of the experiment. All the blood samples were analyzed for different parameters including hemoglobin concentration, erythrocyte count, erythrocyte indices, leukocyte count, differential leukocyte count, and hematocrit (Ahmad *et al*., 2013).

Micronuclei and Nuclear changes: For mutagenic, nuclear and morphological changes in erythrocytes, duplicate thin smears of fresh blood were made using glass slides separately at the time of sampling from each bird. Thin smears were air dried, fixed in methanol for 2-3 min andstained with Giemsastain for 4-5 min. The frequency of micronuclei, different nuclear and morphological changes wasexamined under oil immersion lens (1000x) from each bird using light microscope. A total of 1500 erythrocytes/smear/bird was observed (Mahboob *et al*., 2014).

Statistical analysis: The data obtained in this study were using ANOVA under completely randomized design. Mean±SE values for different blood parameters were computed and different group means were compared by Tukey's test with $P \leq 0.05$.

RESULTS

Clinical sign and behavioral disparities: All the birds in control group remained healthy, active and were responsive to any stimulus at the time of feeding and watering throughout the experiment.Similarly, birds in groups C, D and E given various concentrations of arsenic and copper sulphate did not indicate any apparent clinical and behavioral signs. However, the birds given copper sulphate @ 150 mg/kg (group B) and arsenic@35 mg/kg (group G) revealed different clinical and behavioral signs such as depression, dullness, ocular discharge, salivation, dysponea, watery droppings and emaciation after day 20 of the experiment. Similar mild to moderate clinical and behavioral alterations were also observed in birds of group F (given arsenic @ 25mg/kg + Copper sulphate @100 mg/kg) at days 20 and 30 of experiment. Feed intake and body weight were significantly ($P<0.05$) reduced in birds of groups B, F and G throughout the experiment when compared to the birds of control group (Table 1).

Hematological parameters: It shows that total erythrocyte counts, hemoglobin concentration, pack cell volume and mean corpuscular hemoglobin concentration (MCHC) decreased significantly ($P<0.05$) throughout the experiment in birds of groups E-G and at days 20 and 30 of the experimentin birds of group B when compared to control group (Table 2). Mean corpuscular volume (MCV) was significantly increased in birds of groups E- G at day 10 while in birds of groups B, E-G at day 20 and 30 of experiment. The values of total leukocyte counts showed significant increase in birds of groups F-G throughout the experiment, while in group B at day 20 and 30 and in group E at day30 of experiment. No significant changes were observed in values of lymphocytes in any treated groups throughout the experiment (Table 2).

Mutagenic and morphological studies: The frequencies of erythrocytes with micronucleus (Fig. 1), notched nuclei, erythrocytes with nuclear remnants, blebbed nuclei, condensed nuclei and binucleated erythrocytes revealed significant increase in different groups as compared to control group (Table 3). The frequencies of erythrocyte with micronuclei, notched nuclei, cells with nuclear remnants, blebbed nuclei and binucleated erythrocytes were significantly ($P<0.05$) higher in birds of groups F-G throughout the experiment. The frequency of erythrocytes having condensed nuclei was significantly increased in groups E-G throughout the experiment. The frequencies of various morphological alterations observed in erythrocytes of birds of treated and control groups are presented in Table 4. It reveals that the frequency of erythrocytes with pear shape and leptocyte in groups E-G while the frequency of microcyte and lobed nuclei was significantly higher in birds of groups F-G throughout the experiment as compared to control group.

Table 1. Feed intake and body weight of male birds administered different concentrations of arsenic and copper sulphate

Parameters/day	Groups						
	A	B	C	D	E	F	G
Feed intake (g)							
10	61.4±1.9	54.3±1.6*	58.9±2.2	57.5±2.6	56.2±1.1	54.0±0.3*	53.5±0.7*
20	65.5±1.3	52.2±2.4*	60.9±1.4	60.8±1.9	53.0±1.8*	51.0±1.6*	52.3±0.9*
30	68.3±2.6	51.5±1.2*	60.9±1.7	61.2±1.4	52.5±2.4*	50.2±2.3*	51.2±0.3*
Body weight (g)							
10	1406.7±5.1	1275.5±1.8*	1354.2±4.8	1344±3.1	1333.7±12.8	1284±5.78*	1275±1.8*
20	1414.6±9.7	1270.2±4.2*	1366.2±4.1	1355.7±1.8	1341.5±18.4	1268.7±5.2*	1253±1.8*
30	1422.5±8.1	1256.5±3.4*	1373.2±2.9	1439.7±7.7	1332.5±26.0	1235±5.3*	1228±4.4*

Values (mean±SE) in a row bearing asterisk are significantly (P<0.05) different from control group.

Table 2. Various hematological profile of male birds administered different concentrations of arsenic and copper sulphate.

Parameters/day	Groups						
	A	B	C	D	E	F	G
Total erythrocyte count (10^6/µl)							
10	3.29±0.06	2.74±0.05	2.96±0.01	2.94±0.01	2.61±0.01*	2.50±0.01*	2.64±0.01*
20	3.24±0.02	2.65±0.01*	2.85±0.02	2.82±0.01	2.58±0.01*	2.45±0.02*	2.57±0.00*
30	3.25±0.01	2.61±0.01*	2.83±0.01	2.71±0.01	2.56±0.01*	2.41±0.02*	2.49±0.00*
Hemoglobin (g/dl)							
10	14.1±0.16	11.8±0.21	12.8±0.01	12.4±0.05	10.4±0.23*	9.81±0.08*	9.97±0.00*
20	14.1±0.04	10.4±0.24*	12.6±0.02	12.3±0.04	10.1±0.03*	9.78±0.04*	9.79±0.02*
30	14.1±0.03	9.72±0.14*	11.9±0.01	11.5±0.09	10.0±0.06*	9.68±0.03*	9.74±0.01*
Packed cell volume (%)							
10	37.7±0.47	30.4±0.28	34.2±0.40	31.1±0.40	28.7±0.27*	28.2±0.19*	29.1±0.13*
20	37.1±1.51	29.1±0.50*	32.9±0.30	31±0.43	27.7±0.22*	27.2±0.11*	28.0±0.16*
30	37.1±0.30	28.1±0.44*	31.9±0.41	30.3±0.26	27.1±0.11*	26.6±0.11*	27.1±0.26*
Mean corpuscular volume (fl)							
10	124.6±0.40	128.0±0.60	126.4±0.43	130.0±0.22	135.0±0.66*	136.7±0.41*	131.9±0.69*
20	127±0.65	132.0±0.62*	129.0±0.67	131.7±0.42	135.3±0.29*	138.2±0.42*	135.3±0.39*
30	123.9±0.23	134.3±0.68*	129.0±0.44	132.4±0.45	136.3±0.24*	138.8±0.25*	136.9±0.19*
Mean corpuscular hemoglobin concentration (g/dl)							
10	36.9±0.47	32.3±0.32	33.9±0.63	32.2±0.36	32.6±0.24*	29±0.24*	29.4±0.09*
20	36.1±0.43	28.3±0.34*	32.2±0.53	32.0±0.15	28±0.21*	26.6±0.14*	27.4±0.39*
30	37.2±0.46	27.2±0.41*	32.7±0.20	30.1±1.25	27.4±0.17*	25.7±0.29*	26.6±0.21*
Total leukocyte count (10^3/µl)							
10	12.5±0.43	14.3±0.17	12.6±0.07	13.6±0.11	14.2±0.12	16.7±0.19*	16.0±0.15*
20	12.4±0.35	16.8±0.22 *	13.7±0.20	14.7±0.16	15.3±0.19	17.8±0.24*	17.1±0.29*
30	12.6±0.14	17.7±0.25*	14.5±0.12	15.7±0.20	17.6±0.27*	19.2±0.38*	18.1±0.20*
Lymphocyte (%)							
10	44.6±0.36	43.9±0.59	41.7±0.58	41.1±0.49	39.2±0.22	39.4±0.21	39.8±0.23
20	43.3±0.30	41.02±0.20	40.2±0.16	39.5±0.47	38.4±0.21	38.3±0.17	39.1±0.32
30	42.4±0.31	38.5±0.21	38.6±0.19	37.9±0.32	37.5±0.21	36.7±0.15	37.4±0.21

Values (mean±SE) in a row bearing asterisk are significantly (P<0.05) different from control group.

Table 3. Various nuclear/protoplasmic alterations observed in erythrocytes of birds given different doses of Arsenic and copper sulphate.

Parameters/day	Groups						
	A	B	C	D	E	F	G
Erythrocyte with micronuclei (%)							
10	0.17±0.02	0.15±0.01	0.15±0.02	0.21±0.01	0.24±0.02	0.65±0.03*	0.59±0.01*
20	0.15±0.01	0.19±0.01	0.22±0.01	0.22±0.02	0.23±0.02	1.09±0.08*	0.92±0.02*
30	0.18±0.01	0.22±0.02	0.23±0.01	0.23±0.01	0.24±0.01	2.43±0.02*	1.50±0.05*
Notched Nuclei (%)							
10	0.15±0.01	0.15±0.01	0.16±0.01	0.17±0.01	0.17±0.02	0.55±0.03*	0.47±0.01*
20	0.15±0.02	0.17±0.02	0.18±0.01	0.18±0.02	0.18±0.01	0.60±0.01*	0.54±0.02*
30	0.15±0.02	0.18±0.01	0.19±0.02	0.19±0.02	0.20±0.02	0.90±0.02*	0.67±0.01*
Cells with nuclear Remnants (%)							
10	0.24±0.01	0.25±0.02	0.25±0.01	0.27±0.02	0.28±0.02	0.60±0.01*	0.52±0.02*
20	0.25±0.02	0.27±0.01	0.28±0.01	0.30±0.01	0.32±0.01	0.73±0.02*	0.61±0.01*
30	0.25±0.01	0.28±0.02	0.31±0.02	0.32±0.01	0.33±0.01	0.85±0.03*	0.73±0.01*
Blebbed Nuclei (%)							
10	0.20±0.01	0.23±0.01	0.24±0.01	0.25±0.02	0.25±0.02	0.32±0.01*	0.29±0.02*
20	0.22±0.02	0.24±0.02	0.25±0.01	0.26±0.02	0.26±0.01	0.39±0.01*	0.33±0.02*
30	0.23±0.02	0.25±0.01	0.25±0.02	0.26±0.01	0.26±0.01	0.49±0.01*	0.39±0.01*
Condensed nuclei (%)							
10	0.35±0.01	0.36±0.02	0.35±0.01	0.36±0.02	0.43±0.01*	0.47±0.01*	0.45±0.02*
20	0.35±0.02	0.37±0.01	0.37±0.02	0.38±0.01	0.45±0.02*	0.56±0.01*	0.50±0.01*
30	0.35±0.02	0.37±0.01	0.38±0.02	0.39±0.02	0.47±0.01*	0.64±0.01*	0.56±0.02*
Binucleated erythrocytes (%)							
10	0.12±0.02	0.14±0.01	0.15±0.02	0.14±0.01	0.16±0.01	0.68±0.04*	0.59±0.02*
20	0.14±0.02	0.15±0.01	0.16±0.01	0.17±0.02	0.18±0.01	0.85±0.02*	0.81±0.03*
30	0.15±0.01	0.16±0.02	0.19±0.02	0.2±0.02	0.21±0.02	1.15±0.07*	0.88±0.02*

Values (mean±SE) in a row bearing asterisk are significantly (P<0.05) different from control group.

Table 4. Various morphological alterations observed in erythrocytes of birds given different doses of Arsenic and copper sulphate.

Parameters/Days	Groups						
	A	B	C	D	E	F	G
Leptocyte (%)							
10	0.33±0.01	0.34±0.02	0.35±0.01	0.36±0.02	0.41±0.01*	0.60±0.03*	0.55±0.01*
20	0.34±0.01	0.37±0.02	0.38±0.02	0.39±0.01	0.43±0.02*	1.19±0.09*	0.74±0.03*
30	0.34±0.01	0.38±0.01	0.39±0.02	0.40±0.01	0.45±0.01*	1.5±0.03*	1.28±0.02*
Microcyte (%)							
10	0.18±0.01	0.19±0.02	0.22±0.01	0.22±0.02	0.22±0.01	0.40±0.02*	0.38±0.02*
20	0.18±0.02	0.20±0.02	0.22±0.01	0.23±0.01	0.24±0.02	0.46±0.03*	0.45±0.01*
30	0.18±0.02	0.22±0.01	0.23±0.01	0.24±0.01	0.24±0.02	0.54±0.03*	0.49±0.01*
Lobed nucleated erythrocyte (%)							
10	0.29±0.01	0.32±0.02	0.34±0.02	0.35±0.02	0.47±0.01	0.85±0.03*	0.73±0.03*
20	0.31±0.01	0.34±0.01	0.35±0.02	0.37±0.01	0.38±0.02	1.22±0.10*	0.84±0.03*
30	0.34±0.02	0.36±0.01	0.38±0.01	0.39±0.02	0.40±0.01	1.44±0.03*	1.20±0.03*
Vacuolation in cytoplasm of erythrocytes (%)							
10	0.39±0.01	0.39±0.02	0.42±0.01	0.43±0.01	0.44±0.01	0.85±0.03*	0.76±0.04*
20	0.40±0.02	0.41±0.02	0.44±0.02	0.45±0.01	0.47±0.02	1.15±0.04*	0.99±0.02*
30	0.41±0.01	0.44±0.01	0.48±0.01	0.49±0.02	0.51±0.02	1.75±0.08*	1.46±0.06*
Pear shaped erythrocytes (%)							
10	0.32±0.01	0.34±0.01	0.37±0.01	0.38±0.02	0.48±0.01*	0.82±0.02*	0.64±0.02*
20	0.35±0.02	0.38±0.02	0.41±0.02	0.41±0.02	0.52±0.02*	1.30±0.07*	1.18±0.02*
30	0.37±0.01	0.40±0.01	0.42±0.01	0.44±0.01	0.54±0.01*	1.64±0.07*	1.49±0.04*

Values (mean±SE) in a row bearing asterisk are significantly (P<0.05) different from control group.

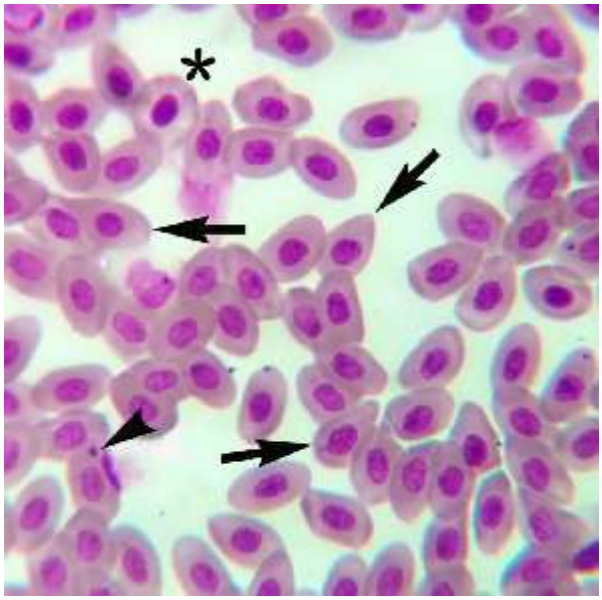

Fig. 1. Blood smear of arsenic and copper sulphate treated birds showing various morphological and nuclear alterations in erythrocytes. Arrow = pear shaped erythrocytes; Arrow head = micronucleus; Asterisk = Macrocyte. Giemsa stain; 1000X

DISCUSSION

Arsenic and copper sulphate have been shown to produce some deleterious effects alone at higher levels and in combination even at low concentrations in birds and these effects appear to be due to stress produced by interaction of these chemicals (Zhang *et al*., 2014). In the present study, various clinical and behavioral signs such as depression, dullness, ocular discharge, salivation, dysponea, watery droppings and emaciation were observed in birds given higher doses of copper sulphate and arsenic alone. These clinical signs were also evidentwhen both arsenic and copper sulphate were given in combination at low levels, suggesting their interacting toxic effects. Previously, different clinical signs in broiler birds due to copper sulphate and arsenic alone at higher levels have also been reported (Khan *et al*., 2013).A significant lower feed consumption and reduced body weight of birds was reported in birds when they were exposed to copper and arsenic at higher concentration (Shahzad *et al*., 2012). But the difference in our study is that when copper sulphate and arsenic were used in combination at low levels, they induced different clinical and behavioral changes.

In the present study, total erythrocyte counts, pack cell volume, hemoglobin concentrations and mean corpuscular hemoglobin concentration were significantly reduced, while the values of leukocyte counts and mean corpuscular volume were significantly higher in copper and arsenic intoxicated birds. The blood cells and other various hematological parameters are known to be the best biomarkers to evaluate toxic potential of different toxicants alone and in combination, even at low levels. Moreover, these parameters also reflect the patho-physiological condition of various animals exposed to any toxicants (Hussain *et al*., 2012; Khan *et al*., 2013). The lower values of erythrocyte counts, hemoglobin concentration and pack cell volume in the present experimentcan be related tofailure of birds to carryadequateamount of oxygen to blood forming tissuessuggestive ofreducedphysical activity (Hussain *et al*., 2014). Significantly lower values of erythrocyte counts and hemoglobinconcentrations might be due to exhaustion of both hemopoietic and metabolic activities of birds exposed to copper sulphate and arsenic (Mashkoor *et al*., 2013; Khan *et al*., 2014). Previous studies have shown that hematological indices such as erythrocyte counts, hemoglobin concentration, mean corpuscular volume and mean corpuscular hemoglobin concentration are vital tools to diagnose anemia in birds (Sharaf *et al*., 2013; Ghaffar *et al*., 2014). Changes in hematological parameters (increasedMCV and lower values of MCHC) seems tobe due to toxic effects of copper sulphate and arsenic resulting in higher number of immature erythrocytesleading to macrocytic anemia which is also supported by decreased values of hemoglobin, erythrocytes and pack cell volume in this study. The significantly lower values of MCHC in the present study may be related to decreased synthesis of hemoglobin and swelling of erythrocytes. Previously, similar results have also been reported in rats, cat fish and birds (Halder *et al*., 2009; Padmaja *et al*., 2009). MCHC was significantly decreased in the present study with arsenic intoxication, which is in agreement with results of Halder *et al.* (2009) in rats. Higher values of leukocyte count in treated birds in the present study could be due to increased sensitivity of the immune system against to the stress condition due to copper and arsenic toxicity.

In the present study, nuclear anomalies in the erythrocytes of birds were the formation of micronuclei, notched nuclei, nuclear remnants, blebbed nuclei, condensed nuclei and binucleated erythrocytes.Formerly no report is available in published literature about the nuclear changes in erythrocytes of avian species exposed to arsenic and copper sulphate. These nuclear abnormalities in erythrocytes of birds could be due to increasedproduction and release of caspase activated DNase, leading to cleavage of different nuclear and cytoskeleton proteins, mitochondrial damage, nitration of DNA proteins, failure of tubulin polymerization and oxidation of mRNA (Campos-Pereira *et al*., 2011; Hussain *et al*., 2012).It is well established that many environmental toxicants/pollutants having the capacity of oxidativestress can also attack DNA,resulting in mutation

and clastogenic damages (Jha, 2008; Khan *et al*., 2014; Hussain *et al*., 2014). Moreover, it has been reported that inorganic arsenic causes DNA damage through different mechanisms such as by denaturation of cellular enzymes, cellular damage through increased oxidative stress, reactive oxygen species and altered gene expression (Khan *et al*., 2014). In the present study, various morphological anomalies such as leptocyte, microcyte, cytoplasmic vacuolation of erythrocytes and erythrocytes with pear shapein birds exposed to arsenic alone at higher levels and copper sulphate might be due to over production of lipid peroxidation products. Moreover, it is hypothesized that morphological alterations in erythrocytes result when they are exposed to different clastogenic/mutagenic agents which induce morphological abnormities in plasma membrane. The nuclear and morphologicalchanges in erythrocytes make them more susceptible to burst in micro vascular system at the time of crossing.Increased frequency of morphological changes in erythrocyte could also be related to increased process of erythropoiesis to eliminate the damaged cells and to balance the impaired function (Witeska *et al*., 2011). Previously, various erythrocyte anomalies such as irregular nucleus, poikilocytosis, erythrocyte swelling and deterioration of cell membrane were seen in fish exposed to heavy metal intoxication (Banerjee *et al.,* 2008; Strunjak-Perovic *et al*., 2009). Based on results of the present study, it can be suggested that copper sulphate alone at 200 mg/kg poses deleterious clinico-hematological effects without mutagenic potential, while the arsenic alone induces both clinico-hematological and mutagenic effects when given alone at higher levels or even at low levels in the presence of copper sulphate.

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