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RESEARCH ARTICLE

Cumulative Effects of Sodium Arsenate and Diammonium Phosphate on Growth Performance, Hemato-Biochemistry and Protoplasm in Commercial Layer

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ABSTRACT

Recently the frequent use of different synthetic compounds like pesticides, herbicides, arsenic, insecticides and fertilizers had led to contamination of environment. Therefore, the current experimental study was conducted to ascertain the concurrent impacts of sodium arsenate and diammonium phosphate in commercial layer. For this purpose a total of 90, day old Lohmann Selected White leghorn chicks of similar weight and sex were procured and kept into six groups. Sodium arsenate and DAP were administered to experimental birds in groups (B-F) alone and in different combination. Group A was kept as control group. Various clinical and behavioral alterations such as watery droppings, dullness, gasping, depression, tremors, anemic comb and wattle were evident in birds. The body weight and relative weight of different organs was reduced significantly. Extensive fatty infiltration, pyknosis of hepatocyte and coagulative necrosis in liver, increased urinary space, necrosis of tubular epithelial cells and congestion in kidneys was observed. The total erythrocytes counts, hemoglobin concentration, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin concentration were significantly reduced. The plasma proteins, serum total proteins, albumin and globulins levels were significantly decreased while serum creatinine and different enzymes were significantly increased in birds. The incidence of erythrocyte with micronucleus, blebbed nucleus and binucleated erythrocyte was significantly increased at different experimental days. In conclusion the arsenic (25 mg/kg bw) and DAP (10%) alone and in combinations (15 mg/kg bw arsenic +6% DAP) were the most toxic to birds.

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INTRODUCTION

Among livestock sector, poultry industry in Pakistan has its own importance which plays significant role in generating animal proteins within the shortest possible time (Rasool *et al.*, 2013). Poultry meat being a high quality animal protein source plays significant role in maintaining the health and nutrition of the people (Shahzad *et al*., 2012). But the poultry meat production is hindered by the presence of arsenic in drinking water (Sharaf *et al*., 2013) or various diseases (Mashkoor *et al*., 2013). If the recommended levels of arsenic in broiler feed are not observed strictly then it can accumulate in poultry flesh which might be detrimental to the consumers.

With the expansion and development in industries, technology and frequent use of various synthetic chemicals like pesticides, herbicides, arsenic, insecticides and fertilizers had led to contamination of environment and many other ecosystems (Witeska *et al.*, 2014; Hussain *et al.*, 2015). Identification, management and monitoring of these pollutants is important to minimize their adverse impacts both on terrestrial and aquatic life. Although the contamination levels in natural ecosystems are usually well below than those which induce mortality in exposed animals. However, even low levels of contamination may be sufficient to impair the normal functioning of tissues. Arsenic is known as a highly toxic metalloid which is found in innumerable compounds in the earth's crust and

is a common environmental pollutant in aquatic as well as terrestrial ecosystems in diverse complex forms (Aruljothi *et al.,* 2013; Javed, 2015; Kousar and Javed, 2015). Natural sources of arsenic include weathering, volcanic and biological activities (Rahman and Hasegawa, 2012) while anthropogenic sources include mining activities, use of different pesticides and wood preservatives which result in contamination of soil and other particles (Baldissarelli *et al.,* 2012). Millions of people are exposed to arsenic in Asia, including China, Thailand, Vietnam, India and Bangladesh through drinking water, use of insecticides and herbicides and high level of arsenic in ground water. Currently in Pakistan, arsenic is one of the most important environmental contaminant (Mashkoor *et al.,* 2013) which induces its toxic effects through variety of mechanisms such as irregular cellular respiration, inhibition of different mitochondrial enzymes and disengagement of oxidative phosphorylation process (Magellan *et al.,* 2014). In particular, arsenic toxic impacts results due to the reason that it interacts with sulfahydryl groups of various enzymes, proteins and it substitutes phosphorus in several biochemical reactions (Khan *et al.*, 2013).

Diammonium phosphate (DAP) fertilizer causes injurious effects and changes the enzyme activity of tissues like kidney, liver, and muscles at capricious intervals and exposures. Consequently these alterations in enzyme activities of different body organs provide clue of the toxicity triggered by the fertilizer. Further investigation explored that toxicity caused by DAP resulted in an unexpected decrease in hematological parameters like erythrocyte count, hemoglobin and hematocrit while total leucocyte count (TLC) increased during DAP toxicity (Trivedi *et al*., 1990; Naqvi *et al.,* 1993). The toxicological and ecological effects produced by inorganic nitrogenous compounds can induce methemoglobinemia in humans and have a potential role in developing cancers of the digestive tract. Further indirect health threats are contributed from the potential relationship between inorganic nitrogen contamination and human infectious diseases. Several reports are available in Pakistan about the arsenic toxicity in poultry; however, there is no literature available about the clinico-hematological and mutagenic effects in concurrently As and DAP intoxicated birds. Therefore, this experimental research was conducted to determine the hemato-biochemical profile and cytogenetic damaging potential of arsenic and DAP in birds.

MATERIALS AND METHODS

Birds and management: A total of 90, day old Lohmann Selected White leghorn chicks of similar body weight and sex were procured from local hatchery (Sumandri, Pakistan). All the birds were kept in floor pens at a local commercial poultry farm under standard conditions, temperature (26-28°C) and humidity (60-65%). After the one week of acclimatization all the chicks were randomly allocated into six equal groups each having fifteen chickens. Birds in all groups were kept on starter diet having 21% crude protein and 2800 Kcal/Kg metabolizable energy. Clean water and feed were given to birds *ad libitum* throughout the experimental duration. Sodium arsenic mixed in water and DAP in feed alone and in different combinations were given to birds daily for 39 days (Table 1).

Table 1: Grouping and experimental treatments of layer birds

Groups	Treatments
Group 1	Control
Group 2	Arsenic (5mg/kg bw)+DAP (2%)
Group 3	Arsenic (10mg/kg bw)+DAP (4%)
Group 4	Arsenic (15mg/kg bw)+DAP (6%)
Group 5	Arsenic (25mg/kg bw)
Group 6	DAP (10%)

Blood analysis: Blood sample about 3-4 ml with anticoagulant (EDTA; 1 mg/ml) was collected from each bird during slaughtering separately at different sampling intervals. Serum samples were separated on ice and stored at -20°C. Blood samples were used for different parameters including erythrocyte count, hemoglobin concentration, hematocrit percent, total and differential leukocyte count (Hussain *et al.,* 2014). Serum biochemical parameters were estimated spectrophotometrically according to methods described (Ghaffar *et al*., 2015) using commercially available kits.

Micronuclei and nuclear changes: For nuclear, mutagenic and morphological changes in erythrocytes duplicate thin smears were made separately from each bird. A total of 2500 erythrocytes/smear/bird was observed under oil immersion lens (1000x) with the help of light microscope (Hussain *et al*., 2015) for the incidence of micronuclei, nuclear and morphological alterations.

Histological investigation: The birds were killed by cutting jugular vein on days 13, 26 and 39 of the experiment. Tissue samples of livers, kidney, brain and thymus were fixed in10% neutral buffered formalin. About 5 µm thick paraffin-embedded sections were stained with hematoxylin and eosin (H & E).

Statistical analysis: For statistical analysis all the data were subjected to Analysis of variance (ANOVA) techniques to estimate the variations among different hematological and serum constituents. Comparison of means in various groups was determined by Tukey's test. $P<0.05$ was accepted as statistically significant.

RESULTS

Various clinical and behavioral alterations such as watery droppings, dullness, gasping, depression, tremors, anemic comb and wattle were evident in pendent manner in birds. At necropsy the liver was fatty, friable and pale in color. At 26 days of experiment the liver appeared as greasy, easy to cut and the size was enlarged in birds of groups given higher levels of As and DAP alone or in combination as compared to those of control group. Similarly, at day 39 of the treatment congestion, areas of necrosis and hemorrhages were observed in the treated birds. However, the intensity of these lesions was varied from moderate to intense in a dose-dependent manner. Consistency of trachea and spleen was normal in all the treatment groups. However, the congestion was evident in spleen in birds of groups D-F. The size of spleen was decreased corresponding to the toxic impacts of As and DAP. The kidneys were congested, bulging out from their sockets and marked hemorrhages were observed. No gross changes were observed in the brain and bursa at necropsy. Congestion and hypotrophy of both thymic lobes was

observed in birds given higher doses alone and at lower levels in combinations. Extensive fatty infiltration, congestion, pyknosis of hepatocyte (Fig. 1) and coagulative necrosis in liver, increased urinary space, detachment of renal tubules, necrosis of tubular epithelial cells and congestion in kidneys (Fig. 2). The hematological parameters of birds showed that the total erythrocytes count, hemoglobin concentration, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin concentration was significantly reduced in birds of groups D-F throughout the experiment as compared to control group (Table 2). The values of immune response (monocyte and lymphocyte count), plasma proteins, serum total proteins, albumin and globulins were significantly increased in birds of groups D-F at all experimental days. Serum urea concentrations were significantly increased in birds of groups E-F. The values of serum creatinine at days 13 and 26 in birds of groups E-F, while at days 39 in groups D-F were significantly increased as compared to control group (Table 3). The activities of different enzymes such as alanine aminotransferase, lactate dehydrogenase, aspartate aminotransferase and alkaline phosphates were significantly increased in birds of groups D-F at all experimental days. The concentrations of lipid peroxidation product malondialdehyde, triglycerides, cholesterol and cardiac isoenzyme CK-MB were significantly increased in birds of groups D-F at all experimental day (Table 3). The percentile rate of incidence of erythrocyte with micronucleus (Fig. 3) at day 13 in groups E-F, at days 26 and 39 in groups D-F was significantly increased as compared to control group (Table 4). The frequency of erythrocytes with lobed nucleus and pear shaped erythrocytes increased significantly at days 13 in groups E-F and at days 26 and 39 in groups D-F. The percentile rate of erythrocyte with blebbed nucleus and binucleated erythrocyte was significantly increased throughout the experiment when compared to control group.

DISCUSSION

In the present study various clinical and behavioral alterations in birds including watery droppings, dullness, gasping, depression, tremors, anemic comb and wattle were observed. These physical alterations could be due to increased and impaired permeability of blood vessels and intestinal functions leadings to poor absorption of nutrients ultimately resulting to dullness and depression (Khan *et al*., 2013). Previously respiratory distress due to arsenic in birds, fish and goats has been reported (Khan *et al*., 2013; Ghaffar *et al*., 2015; Ghaffar *et al*., 2016).

Grossly the lungs were swollen and edematous. Congestion was evident in lungs and spleen. The kidneys were congested, bulging out their sockets and marked hemorrhages were observed. Grossly the liver was pale and hemorrhagic. Congestion and hypotrophy of both thymic lobes was observed only in birds given higher doses of As and DAP. The relative weight of liver, lungs trachea, kidneys, thymus, heart, spleen and brain reduced significantly in birds given higher doses of As and DAP alone and in combinations. The decreased relative weight of tissues might be due to oxidative stress associated with less feed intake, decrease metabolism and less absorption of nutrients. Similar results have been reported in other animals like in, birds, rats, mice and rabbits after supplementation of As (Khan *et al*., 2013). Toxicological effects of diammonium phosphate on liver, kidneys and muscles of fresh water fish (Naqvi *et al*., 1993) and rats have been reported (Gora *et al*., 2014).

The degenerative changes in kidneys and liver of exposed birds could be due to the increased oxidative stress augmented by DAP. Degenerative changes in kidneys and apoptotic cells have been observed (Sener *et al*., 2015). Scanty information is available about the histopathological changes in kidneys in birds due to arsenic intoxication (Khan *et al*., 2013). It is reported that As exposure in hepatocytes of exposed animals causes activation of prototypical AhR-regulated genes (Nqo1 mRNA, Cyp1a1 and Cyp1b1) and induces toxic impacts of As due to increased nuclear localization (Elshenawy *et al*., 2015). The tissue changes in kidneys in present study can be related to increased generation of IL-8 and changes in IL-8 promoter as a result of histone acetylation and DNA methylation. Furthermore, it has been reported that arsenic causes demethylation of IL-8 promoter by

Table 2: Hematological parameters of birds given different levels of arsenic and diammonium phosphate

Parameters/ Days	Groups A	B	C	D	E	F
Erythrocyte counts (10^{12}/L)						
13	2.87±0.04	2.71±0.01	2.7±0.02	2.70±0.01*	2.58±0.01*	2.46±0.00*
26	2.85±0.02	2.68±0.01	2.63±0.01	2.62±0.01*	2.51±0.01*	2.41±0.00*
39	2.69±0.02	2.60±0.04	2.55±0.01	2.37±0.08*	2.37±0.03*	2.32±0.03*
Hemoglobin concentration (g/dl)						
13	13.02±0.2	11.6±0.2	10.75±0.3	10.35±0.3*	9.15±0.02*	9.11±0.21*
26	13.07±0.2	10.8±0.2	11.9±0.32	9.92±0.13*	8.67±0.14*	8.29±0.14*
39	13.25±0.1	10.7±0.2	11.37±0.1	9.32±0.62*	8.65±0.19*	8.25±0.11*
Hematocrit (%)						
13	46.5±0.8	45.47±0.5	43.77±0.4	38.7±0.6*	37.4±1.3*	34.1±2.6*
26	45.2±0.7	41.72±0.6	39.35±0.3	38.4±0.2*	34.4±1.2*	33.2±2.1*
39	42.5±0.5	39.05±0.2	37.47±0.5	35.1±1.1*	29.9±2.6*	31.7±2.1*
Mean corpuscular volume (fl)						
13	137.8±0.5	135.2±0.6	132.8±0.2	128.7±0.8*	127.1±0.7*	123.5±0.1*
26	135.4±0.4	131.5±0.8	131.3±0.1	127.3±0.4*	128.1±0.8*	122.6±0.1*
39	134.5±0.4	133.1±0.4	131.2±0.1	125.1±0.1*	124.6±0.1*	122.4±1.4*
Mean corpuscular hemoglobin concentration (g/dl)						
13	38.7±0.4	36.9±0.2	35.3±0.6	31.1±0.4*	30.8±0.6*	30.3±0.8*
26	36.7±0.4	34.2±0.7	31.8±0.5	28.8±0.4*	28.3±0.7*	29.7±0.7*
39	38.2±0.5	36.2±1	34.3±1.5	29.6±0.4*	29.5±0.5*	29.7±0.5*

Values (Mean±SE) in each row with asterisk differ significantly (P<0.05) than control group.

Table 3: Serum biochemical parameters of birds given different levels of arsenic and diammonium phosphate

Parameters/Days	Groups					
	A	B	C	D	E	F
Plasma proteins (g/dL)						
13	3.56±0.02	3.54±0.02	3.46±0.03	2.84±0.03*	2.81±0.03*	2.62±0.01*
26	3.49±0.01	3.48±0.01	3.41±0.01	2.81±0.0*	2.66±0.02*	2.57±0.02*
39	3.50±0.01	3.34±0.02	3.39±0.01	2.73±0.0*	2.68±0.01*	2.49±0.03*
Total protein(gm/dL)						
13	3.92±0.01	3.86±0.01	3.83±0.01	3.38±0.01*	3.11±0.02*	2.96±0.01*
26	3.92±0.01	3.83±0.01	3.79±0.01	3.32±0.01*	3.16±0.02*	2.86±0.02*
39	3.90±0.01	3.78±0.01	3.73±0.01	3.29±0.0*	3.15±0.01*	2.65±0.0*
Albumin (g/dL)						
13	1.75±0.01	1.70±0.01	1.67±0.02	1.51±0.02*	1.45±0.01*	1.39±0.01*
26	1.68±0.01	1.68±0.01	1.66±0.01	1.53±0.01*	1.41±0.01*	1.36±0.02*
39	1.76±0.01	1.65±0.01	1.64±0.02	1.50±0.01*	1.41±0.01*	1.33±0.02*
Globulins (g/dL)						
13	2.35±0.01	2.32±0.01	2.27±0.01	2.05±0.01*	2.01±0.02*	1.92±0.01*
26	2.34±0.01	2.26±0.02	2.22±0.01	2.02±0.01*	1.98±0.01*	1.89±0.01*
39	2.34±0.01	2.24±0.01	2.20±0.01	2.01±0.01*	1.92±0.01*	1.85±0.02*
Urea (mg/dL)						
13	9.12±0.01	9.28±0.00	9.32±0.01	9.69±0.01*	10.8±0.01*	9.70±0.04*
26	9.02±0.01	9.31±0.01	9.37±0.01	9.75±0.01*	10.9±0.02*	9.70±0.4*
39	9.09±0.02	9.41±0.01	9.47±0.01	9.79±0.02*	12.1±0.05*	11.9±0.25*
Creatinine (mg/dL)						
13	1.11±0.02	1.17±0.01	1.19±0.0	1.38±0.03*	1.44±0.01*	1.35±0.01*
26	1.06±0.01	1.18±0.01	1.21±0.0	1.39±0.03*	1.54±0.01*	1.37±0.01*
39	1.05±0.02	1.20±0.01	1.23±0.0	1.45±0.02*	1.57±0.01*	1.39±0.01*
Alanine aminotransferase (IU/L)						
13	5.48±0.01	5.92±0.00	5.96±0.00	6.10±0.02*	6.47±0.05*	6.58±0.02*
26	5.89±0.01	6.03±0.02	6.17±0.03	6.84±0.05*	7.52±0.08*	7.93 ±0.08*
39	5.90±0.01	6.18±0.02	6.32±0.01	6.95±0.03*	7.91±0.03*	8.26±0.05*
Lactate dehydrogenase (IU/L)						
13	117±1.04	121.2±2.8	124.1±1.3	130.7±1.4*	139±1.2*	144.6±0.6*
26	118.5±2.1	124.7±1.9	127.3±1.4	132.6±1.2*	146±3.8*	157.5±2.2*
39	118±2.63	125.8±1.3	129.2±2.1	134.2±1.3*	156±2.2*	163.9±1.8*
Aspartate aminotransferase (IU/L)						
13	93.1±3.98	97.9±3.68	100.2±2.6	102.3±2.9*	117.3±1.9*	119.8±3.9*
26	94.5±4.63	98.9±2.48	101.3±1.7	103.1±1.1*	121.3±2.1*	126.3±1.9*
39	92.1±3.77	99.4±2.63	105.4±1.3	107.8±1.6*	121.4±3.7*	138.6±2.6*
Alkaline phosphates (IU/L)						
13	15.1±0.2	15.7±0.2	16.3±0.1	19.7±1.7*	19.0±0.4*	20.6±0.2*
26	15.4±0.3	17.2±0.1	17.7±0.4	19.3±1.3*	20.3±1.3*	21.2±0.2*
39	15.6±0.4	17.4±0.8	17.7±0.08	19.1±0.9*	21.8±1.1*	24.8±0.3*
Malondialdehyde concentration						
13	2.15±0.03	2.19±-0.0	2.27±0.01	2.74±0.2*	3.19±.01*	3.22±0.1*
26	2.16±0.01	2.26±0.0	2.32±0.01	2.77±0.1*	3.28±0.1*	3.33±0.02*
39	2.16±0.01	2.30±0.0	2.35±0.01	2.89±0.1*	3.32±0.1*	3.43±0.01*
Triglycerides (mg/dL)						
13	74.9±0.8	76.8±0.5	77.8±0.6	86.5±0.6*	87.4±0.8*	89.4±0.9*
26	75.3±0.4	78.4±0.5	78.5±0.6	87.8±0.8*	92.2±1.4*	98.4±0.4*
39	75.7±0.1	79.6±0.4	79.5±0.5	86.2±0.3*	92.2±0.4*	99.1±1.5*
Cholesterol (mg/dL)						
13	108.3±0.6	110.8±0.6	111.8±0.7	123.9±0.7*	123.3±.1*	131.2±0.5*
26	108.7±0.3	113.1±0.3	115.3±0.3	129.5±0.2*	134.7±.3*	136.6±1.4*
39	108.7±0.2	114.2±0.3	116.1±0.3	137.1±0.1*	141.2±0.8*	142.1±1.1*
CK-MB (IU/L)						
13	9.32±0.02	9.44±0.01	9.45±0.01	10.57±0.02*	11.81±0.2*	11.97±0.01*
26	9.29±0.01	9.54±0.01	9.62±0.02	11.74±0.01*	12.32±0.6*	13.92±0.02*
39	9.24±0.01	9.65±0.02	9.85±0.5	11.81±0.1*	13.52±0.5*	13.92±0.2*

Values (Mean±SE) in each row with asterisk differ significantly (P<0.05) than control group.

modulation of CpG leading to upstream of transcription of its bases which increases cell cycle dysregulation and cell migratory capabilities leading to renal toxicity (Singh *et al*., 2015).The necrotic changes in our study might be due to release of apoptotic inducing factors from mitochondria through reactive oxygen species (ROS) signaling pathway and ultimately leading to cell death (Altikat *et al.* 2014). In addition, congestion in lymphoid organs, hemorrhages, tubular epithelial cell degeneration, focal mineralization and tubular casts in kidneys in rats due to As alone (Jomova *et al*., 2011; Gora *et al*., 2014) and birds have been reported (Khan *et al*., 2013).

The values of immune response (monocyte and lymphocyte count), plasma proteins, serum total proteins, albumin and globulins were significantly decreased in birds. The decreased values of erythrocyte counts, hematocrit concentration and hemoglobin (Hb) in present study could be due to exhaustion of both hemopoietic and metabolic activities of exposed birds. Similar results have been reported previously in rats (Hussain *et al*., 2014; Hussain *et al*., 2015). Arsenic exposure causes lower values of erythrocytes, plasma total protein and total antioxidant activity by reactive oxygen species (ROS) production (Hussain *et al.*, 2015). Scanty information is also available about the adverse impacts of DAP in fish at higher concentrations in terms of decreased erythrocyte counts, hemoglobin, hematocrit while increased total leucocyte count (Trivedi *et al*. 1990, Naqvi *et al*. 1993).

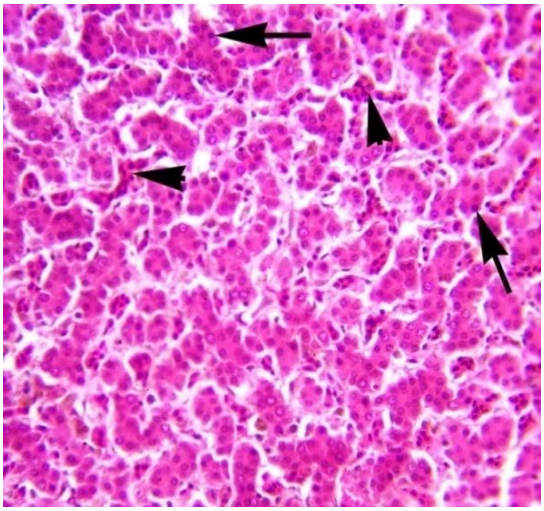

Fig. 1: Liver of birds treated with arsenic and diammonium phosphate showing congestion (arrow heads), pyknosis of hepatocyte (arrows) and coagulative necrosis in liver. H & E. 200X.

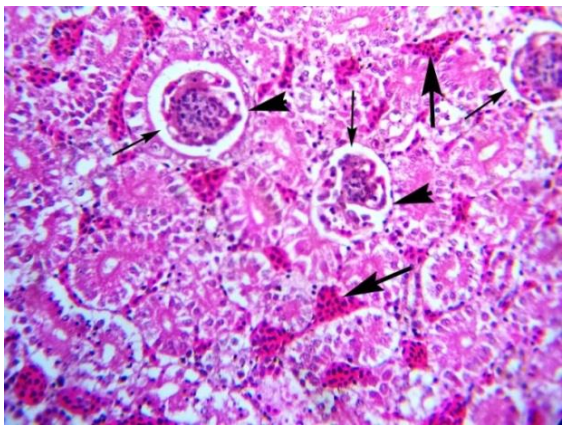

Fig. 2: Kidneys of birds treated with arsenic and diammonium phosphate showing congestion (arrows), detachment of renal tubular epithelium from basement membrane (arrow heads) and increased urinary space (thin arrows). H & E. 200X.

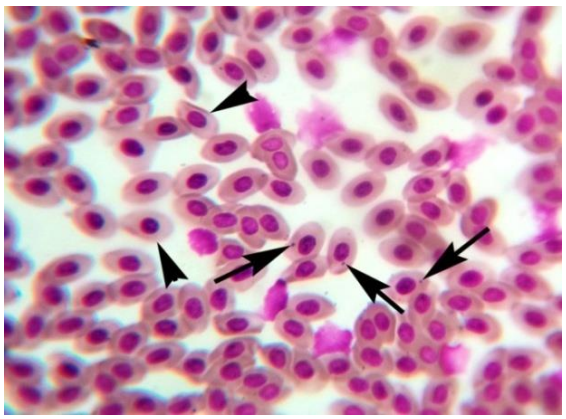

Fig. 3: Blood smear of arsenic and diammonium phosphate treated birds showing different nuclear and morphological changes in erythrocytes. Arrows=micronucleus; Arrow heads=pear shaped erythrocyte. Wright-Giemsa stain: 1000x.

Higher values of MCV and MCH in birds in our study could also be due to increased number of immature erythrocyte suggesting macrocytic anemia (Ghaffar *et al.*, 2014), while the reduced values of MCHC could be due to poor synthesis of hemoglobin and swelling of erythrocyte. The reduced values of hematological parameters in this study could be due to the inability of birds to carry suitable amount of oxygen to blood forming tissue (Hussain *et al.*, 2014).

The serum triglycerides, cholesterol, cardiac isoenzyme CK-MB and the concentrations of lipid peroxidation product malondialdehyde were significantly increased in birds. Similar findings have also been reported in birds alone due to arsenic intoxication (Khan *et al.*, 2013). In published literature, no report is available about the adverse impacts of both As and DAP together in birds in different combinations. The increased values of serum urea and creatinine in this study have also been reported due to increased exposure to As in rats (Sener *et al*., 2015). The increased values of biomarkers of kidneys (urea and creatinine) and liver (ALT and AST) in birds in present study might be due to higher levels of oxido-nitrosative stress and up regulation of renal Caspase-3, KIM-1, TGF-β, and TNF-α mRNA expression in kidneys and hepatic tissues in rats (Adil *et al.*, 2015). Previously significantly increased values of serum cardiac biomarkers (triglycerides, creatine kinase-MB and cholesterol), liver function tests (AST, ALT and ALP), renal function tests (urea and creatinine) and lipid peroxidation product (MDA) have been reported in rats (Muthumani and Prabu, 2015). In present study the increased cardiac markers in birds can be related to down-regulation of protein expressions Nrf2 and HO-1 while up-regulation of myocardial NADPH sub units (NOX2 and NOX4) and Keap-1.

The percentile rate of erythrocyte with blebbed nucleus and binucleated erythrocyte was significantly increased throughout the experiment in treated birds. So far as our knowledge is concerned, no information is available in published literature about these kinds of nuclear and morphological changes in erythrocyte of birds exposed to As and DAP. The presence of micronucleus in erythrocytes, lobed nuclei and blebbed nucleui in our study could be due to higher production of caspase activated DNase leading to cleavage of cytoskeleton (vimentin, fodrin and gelsolin) and nuclear proteins resulting to mitochondrial damage (Ghaffar *et al*., 2014; Hussain *et al.*, 2014). Erythrocytes anomalies have also been reported in fish exposed to heavy metal (As) and toxicants (Witeska *et al.*, 2014). The various morphological changes in erythrocytes in present study in birds like pear shape erythrocytes might be due to morphological changes in their plasma membrane associated with nitration of DNA proteins and oxidation of mRNA when the organisms encounter to different chemicals which induce oxidative stress potential ultimately causing DNA assault leading to molecular damages (Hussain *et al.*, 2014).

Based on the results of our experiment it is determined that arsenic (25 mg/kg bw) and DAP (10%) alone and in combinations (15 mg/kg bw+6% DAP) were the most toxic than all other combinations to birds.

Conclusions: The As even at low levels in the presence of nitrogenous chemicals in ecosystem poses clinico-hematological, histopathological, biochemical and genotoxic impacts in birds.

Table 4: Various nuclear and morphological abnormalities in erythrocytes of birds administered different levels of arsenic and diammonium phosphate

Parameters/Days	Groups					
	A	B	C	D	E	F
Erythrocyte with micronucleus (%)						
13	0.11+0.01	0.12+0.01	0.13+0.02	0.14+0.01	1.22+0.04*	1.56+0.04*
26	0.14+0.01	0.15+0.01	0.15+0.01	0.26+0.01*	1.42+0.02*	1.83+0.03*
39	0.15+0.01	0.16+0.01	0.16+0.01	0.48+0.02*	1.68+0.01*	2.09+0.04*
Erythrocytes with Lobed Nucleus (%)						
13	0.20+0.01	0.23+0.01	0.22+0.01	0.24+0.02	0.62+0.02*	0.94+0.08*
26	0.23+0.01	0.24+0.01	0.25+0.01	0.25+0.03	0.76+0.02*	1.06+0.04*
39	0.24+0.01	0.25+0.01	0.23+0.01	0.33+0.02*	0.82+0.07*	1.11+0.04*
Erythrocyte with Blebbed Nucleus (%)						
13	0.35+0.01	0.38+0.02	0.35+0.01	0.46+0.02*	0.86+0.01*	1.05+0.01*
26	0.33+0.01	0.34+0.01	0.36+0.01	0.61+0.01*	1.12+0.01*	1.29+0.02*
39	0.35+0.01	0.35+0.01	0.39+0.02	0.69+0.01*	1.24+0.02*	1.40+0.34*
Binucleated Erythrocyte (%)						
13	0.07+0.01	0.07+0.01	0.09+0.01	0.12+0.01*	0.23+0.02*	0.43+0.02*
26	0.06+0.00	0.07+0.00	0.10+0.00	0.19+0.01*	0.31+0.02*	0.50+0.03*
39	0.08+0.00	0.08+0.00	0.10+0.00	0.23+0.01*	0.34+0.02*	0.60+0.05*
Pear Shaped Erythrocytes (%)						
13	0.24+0.01	0.24+0.01	0.26+0.01	0.30+0.01	0.79+0.01*	0.97+0.01*
26	0.23+0.01	0.26+0.00	0.29+0.01	0.35+0.00*	1.06+0.01*	1.25+0.03*
39	0.28+0.02	0.30+0.01	0.31+0.01	0.48+0.01*	1.20+0.01*	1.36+0.02*

Values (Mean±SE) in each row with asterisk differ significantly (P<0.05) than control group.

Authors contribution: RH and AG planed and supervised the research. RH, MS, TK and RM conducted the experiment. RH made and examined blood smears. RH analyzed the data. RH, AG, HA, GA and MHA wrote the manuscript. All the authors approved the manuscript.

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