Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Arsenic and Copper Sulfate in Combination Causes Testicular and Serum Biochemical Changes in White Leghorn Cockerels

Abdul Ghaffar[1]*, Riaz Hussain[2]*, Ghulam Abbas[3], Mirza Habib Ali[4], Hussain Ahmad[2], Javaria Nawaz[1], Iqra Rasheed Choudhary[1], Javaria Haneef[1] and Sundas Khan[1]

[1]Department of Life Sciences (Zoology) The Islamia University of Bahawalpur- 63100, Pakistan; [2]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur- 63100; [3]Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan; [4]Pakistan Science Foundation Islamabad, Pakistan
*Corresponding authors: dr.abdul.ghaffar@iub.edu.pk; driazhussain@yahoo.com

ARTICLE HISTORY (16-235)

Received: September 11, 2016
Revised: April 15, 2017
Accepted: April 24, 2017
Published online: July 17, 2017

Key words:
Arsenic
Birds
Copper sulfate
Histopathology
Serum biochemistry

ABSTRACT

The objective of present experimental study was to ascertain the reproductive and some serum biochemical effects following oral administration of arsenic and copper sulfate in adult male birds. For this purpose, adult cockerels (Lohmann Selected White Leghorn) were randomly allotted into seven equal groups. The male birds were fed with a standard control diet containing 20% crude proteins. Arsenic and copper sulfate significantly ($P \leq 0.05$) decreased the absolute weight of testes, liver, kidneys, spleen, lungs, heart and thymus in male birds. Histologically, the liver was congested and showed extensive fatty infiltration and biliary hyperplasia. Kidneys showed renal tubular necrosis, hypertrophy of glomeruli, and congestion. Different microscopic changes such as necrosis nuclei of spermatids, admixture of necrotic spermatids and disorganization of seminiferous tubules along with arrest of spermatogenesis were observed in testicular tissues. Furthermore, arsenic and copper sulfate significantly decreased plasma proteins, serum total proteins, serum globulins and albumins concentrations in birds. Conversely, the concentrations of serum urea, creatinine and alkaline phosphatase were increased in birds given arsenic and copper sulfate. The concentrations of lipid peroxidation product (malondialdehyde), levels of liver enzyme (aspartate aminotransferase) and cardiac biomarkers (CK-MB, triglyceride and cholesterol) increased significantly in birds treated with arsenic and copper sulfate. In summary, we showed that subacute exposure of birds to arsenic and copper sulfate induced oxidative stress and different tissue changes in birds.

To Cite This Article: Ghaffar A, Hussain R, Abbas G, Ali MH, Ahmed H, Nawaz J, Choudhary IR, Haneef J and Khan S, 2017. Arsenic and copper sulfate in combination causes testicular and serum biochemical changes in White Leghorn cockerels. Pak Vet J, 37(4): 375-380.

INTRODUCTION

Various compounds like fertilizers, pesticides, copper sulfate and arsenic are extensively in livestock, poultry industry and agri-food. These compounds are frequently used in some other industrial sectors to improve the nutritional security and sustainability. However, increasing applications of these compounds have led to their enhanced accumulation in natural water. Many agricultural and industrial processes are continuously releasing variety of chemicals and their wastes material into natural water resources which pose deleterious impacts on exposed biota (Witeska *et al*., 2014). The adverse impacts of these chemicals are manifold on various economically important species including avian population (Lima *et al*., 2010; Mashkoor *et al*., 2013).

The heavy metals are constantly contaminating natural environment though contamination level in natural ecosystem is usually below which cause mortality in exposed species. However, even low level of contamination can be sufficient to alter the usual and normal functioning of the vital organs. The application of these kinds of pollutants and heavy metals induce disturbance in different physiological and chemical mechanisms in many animals (Mandour *et al*., 2012; Sattar *et al.*, 2016). Monitoring of early adverse impacts of synthetic and natural pollutants is crucial and is only evident on the cellular levels than the prominent

behavioral or external changes (Witeska *et al*., 2014). The main source of different heavy metals and arsenic is usually food obtained from sea, mushrooms, rice and poultry (Nepusz *et al.*, 2009; Petroczi and Naughton, 2009). Particularly in southern areas of Pakistan the level of arsenic in drinking water is 3-30 fold higher than the permissible level (Baig *et al*., 2012).

Arsenic is well known heavy metal which influences the action of different enzymes responsible for heme synthesis and its degradation. Arsenic has been reported to persuade conditions leading to defective cell mediated immunity in numerous cell systems. Various mechanistic studies have indicated that arsenic induces mitochondrial alterations and generates reactive oxygen and nitrogen species during inorganic arsenic metabolic reactions at cellular levels which adversely alter the mitochondrial integrity (Cohen *et al*., 2006). Arsenic toxicological impacts depend on detoxifying mechanisms (Rahman *et al.,* 2012) and different interacting factors such as its bioavailability, exposure, environmental factors, and presence of other chemicals and resistance of the target organisms (Rahman *et al*., 2012). Reduced phagocytic activity, skin lesions, poor weight in poultry and rats due to alone arsenic toxicity have been reported through drinking water (Jun *et al*., 2008; Ghaffar *et al*., 2015). The biological actions also play crucial role in arsenic distribution (Rahman *et al.,* 2012; Sharaf *et al*., 2013).

Copper sulfate is an essential micro-element and frequently used in poultry sector in diets as a prophylactic, to control fungal infection, parasitic and bacterial diseases. Copper is important component of various metalo-enzymes. However, increased levels of copper induce toxicological impacts including poor health, less feed intake, poor weight gain and hemato-biochemicals in broiler birds (Rasool *et al*., 2013; Bhatti *et al*., 2015). Previously reports are available about the complications of copper and arsenic at higher levels as studied independently. There is no report about the combined impacts of these compounds in avian species. Therefore, the present experimental study was conducted to investigate adverse impacts of arsenic and copper sulfate together at low levels in different combinations in sexually mature birds.

MATERIALS AND METHODS

Birds and treatment: A total of 28 mature and sexually active male birds having similar age, weight and without any clinical ailment were procured from local poultry farm. The birds were randomly kept into seven groups having four birds each (A-G). The birds were remained in wire cages for 30 days under uniform environment having humidity (60-70%) and temperature (27-30°C). All the birds were given commercial feed having 20% proteins and fresh clean water daily. In poultry birds, the extreme tolerance level of inorganic arsenic is 50 mg/kg while organic arsenic is 100 mg/kg (National Research Council, 1980). Therefore, in this experimental study inorganic arsenic was selected and given with copper sulfate in different combinations at low doses to determine the possible combine toxic impacts. Different doses of arsenic and copper sulfate (Table 1) were administered via crop tubing.

Table 1: Treatment of arsenic and copper sulfate to the White Leghorn cockerels

Group	Arsenic (@mg/kg BW)	Copper sulfate (@mg/kg BW)
A (Control)	0	0
B	0	150
C	10	50
D	15	75
E	20	100
F	25	100
G	35	0

Pathology procedures: All the birds at the end of experiment were killed and examined for any gross changes. The absolute and relative weights of different tissues such as testes, liver, kidneys, spleen, thymus, heart and lungs were recorded. For histopathological changes about 2-3cm thickness tissues from all the visceral organs were separated and fixed in 10% neutral buffered formalin and processed following routine methods of dehydration and paraffin embedding procedures. Finally, about 4-5µm thick tissues were cut and stained with hematoxylin and eosin (Hussain *et al*., 2015).

Serum biochemical parameters: For serum, biochemical changes about 5 ml blood sample was collected without anticoagulant from wing vein of each bird on days 10, 20 and 30 of the experiment. The serum was separated on ice and stored at -20ºC. All the serum samples were analyzed for different biochemical changes spectrophotometrically using commercially available kits (Ghaffar *et al.,* 2016).

Statistical analysis: The data collected in present experimental study were subjected to statistical analysis. Means±SE values for various parameters were computed and their means were compared by Tukey's test with $P \leq 0.05$.

RESULTS

Gross and histopathological changes: No mortality was recorded in birds of all groups throughout the experiment. All the visceral organs including testes, liver, heart, kidneys, spleen, thymus and lungs were normal in color and consistency in birds of control group. However, in birds given higher levels of copper and arsenic alone showed gross changes in testes, liver, kidneys and thymus. The testes were smaller in size, liver was friable and pale to yellowish and kidneys were congested in birds given higher levels of arsenic and copper sulfate alone and at lower levels in combinations. The spleen and thymus were congested and smaller in size. However, mild to moderate similar gross lesions were also observed in birds given lower doses of copper sulfate and arsenic in combination. Histologically, the testes of various birds administered higher doses of copper sulfate (150 mg/kg) and arsenic (35 mg/kg) alone showed severe changes such as 2-3 cell layers having primary and secondary spermatocytes along with fewer round spermatids (Fig. 1). The lumen of seminiferous tubules had admixture of necrotic spermatids instead of mature sperms (Fig. 2). Similar testicular changes were also observed in birds of groups D-F. Histologically, liver tissues exhibited severe cytoplasmic vacuolation, hyperplasia of bile ducts and congestion in birds given higher doses of copper sulfate (150 mg/kg) and arsenic (35 mg/kg) alone (Fig. 3). Various nuclear changes

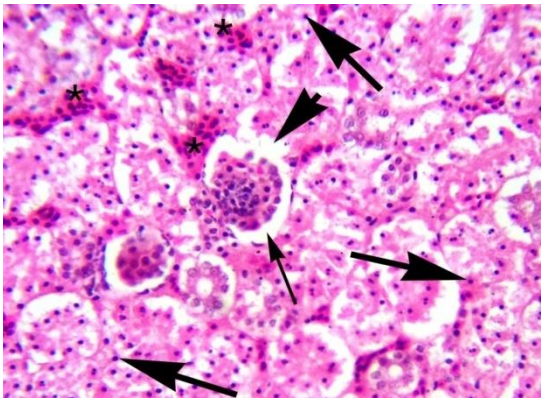

Fig. 1: Kidneys of male birds treated with arsenic and copper sulfate (Arsenic=20 mg/kg and copper sulfate=100 mg/kg) showing pyknosis (thick arrows), congestion (*), detachment of renal tubules from basement membrane (arrow heads) and increased urinary space (thin arrow). H&E 200X.

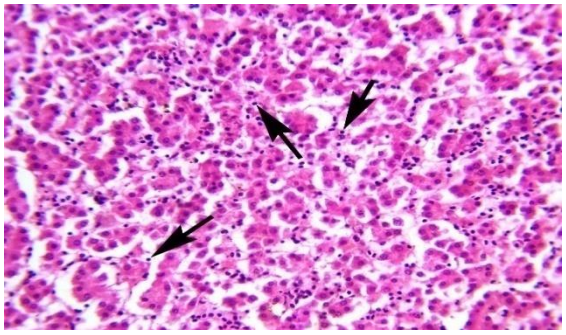

Fig. 2: Liver of male birds treated with arsenic and copper sulfate (Arsenic=20 mg/kg and copper sulfate=100 mg/kg) showing congestion pyknosis (arrow) of hepatocyte. H&E 200X.

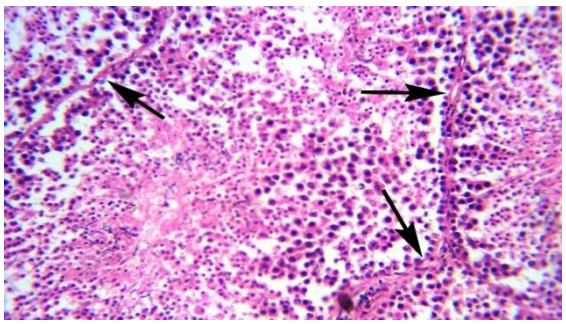

Fig. 3: Testes of male birds treated with arsenic and copper sulfate (Arsenic=15 mg/kg and copper sulfate=75 mg/kg) showing 1-2 number of spermatogenic cell layer with arrested of spermatogenic process at secondary spermatocyte level (arrows). H&E 200X.

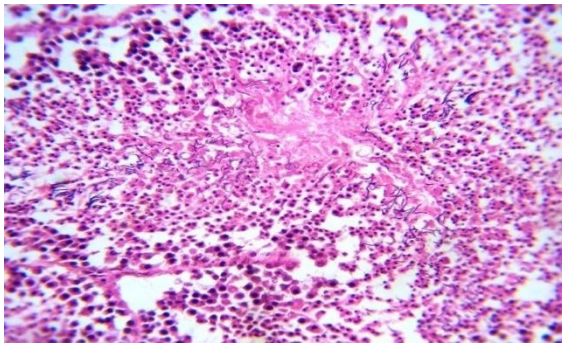

Fig. 4: Testes of male birds treated with arsenic and copper sulfate (Arsenic=20 mg/kg and copper sulfate=100 mg/kg) showing admixture of necrotic cells in lumen of tubules along with punctuation of chronic inflammatory cells. X 200 H&E.

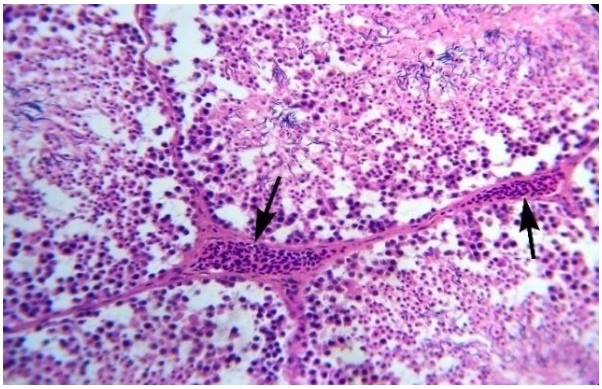

Fig. 5: Testes of male birds treated with arsenic and copper sulfate (Arsenic=25 mg/kg and copper sulfate=100 mg/kg) showing congestion (arrows) necrotic spermatids in lumen of tubules and punctuation of chronic inflammatory cells. X 200 H&E.

(condensation, fragmentation, granular appearance and disintegration of nucleus) were also observed. The intensity of these tissue changes in hepatocytes were similar in birds of groups (D-F) exposed to arsenic and copper sulfate in combination. Microscopically kidneys of birds in groups E and F showed severe necrosis of tubular epithelial cells (Fig. 4). Severe congestion, increased urinary space, hypertrophy of glomeruli along with loss of circular shape, detachment of renal tubules and necrosis of renal tubular epithelial (Fig. 5) was observed in birds of groups B and G exposed to higher levels of copper sulfate and arsenic alone. Microscopically, the different lungs tissues in birds exposed to copper sulfate (150 mg/kg) and arsenic (35 mg/kg) revealed congestion and edema. Minor to moderate congestion was also observed in birds kept in groups D-F. Congestion in spleen and thymus was observed at higher doses copper sulfate (150 mg/kg) and arsenic (35 mg/kg).

Absolute and relative organ weight: The absolute weight of testes, liver, kidneys, spleen, lungs and thymus was significantly decreased in birds given higher levels of copper sulfate and arsenic (Table 2). The relative weight of all the visceral organs was not significantly different in all treated birds (Table 2) as compared to the birds of control group.

Biochemical parameters: The plasma proteins, serum total proteins and albumins were significantly decreased in birds of groups D-G and B given different levels of arsenic and copper sulfate alone and in combinations. Serum globulin was significantly decreased in birds of groups E-G and B at days 10 and 20 while in D-G and B at day 30 of the experiment. Serum urea, creatinine and malondialdehyde concentrations were significantly increased in birds of groups E-G at day 10 and in groups D-F and B at days 20 and 30 of the experiment as compared to control group (Table 3). The values of serum aspartate aminotransferase, isoenzyme CK-MB, triglyceride and cholesterol were significantly higher at day 10 in groups E-G and at days 20 and 30 in birds of groups E-G and B as compared to control group. The values of serum alkaline phosphatase were significantly higher at day 10 in groups D-G and at days 20 and 30 in birds of groups D-G and B as compared to control group (Table 4).

Table 2: Absolute and relative weight (as a percent of body weight) of different visceral organs of birds given different levels of arsenic and copper sulfate

Organs	Groups						
	A	B	C	D	E	F	G
Absolute							
Testes	34.8±1.93	26.4±0.42*	30.3±0.36	29.0±0.46	27.7±0.18	26.6±0.21*	27.9±0.10*
Liver	55.0±1.97	41.9±0.45*	50.9±0.57	48.1±0.70	46.0±0.79	42.3±1.08*	43.7±0.98*
Kidneys	9.37±0.33	5.73±0.32*	8.16±0.02	8.10±0.01	7.86±0.01	6.47±0.03*	6.26±0.05*
Spleen	7.18±0.29	5.29±0.04*	6.16±0.02	5.89±0.02	5.72±0.02	5.28±0.02*	5.23±0.03*
Heart	7.24±0.04	7.13±0.01	7.17±0.02	7.18±0.02	7.08±0.01	6.99±0.04	7.70±0.76
Thymus	0.51±0.01	0.38±0.01*	0.49±0.00	0.46±0.00	0.42±0.01	0.39±0.02*	0.42±0.01*
Lungs	8.32±0.15	6.27±0.08*	7.69±0.04	7.90±0.02	8.12±0.06	6.31±0.05*	6.24±0.04*
Relative							
Testes	2.44±0.12	2.28±0.05	2.20±0.02	2.03±0.08	2.08±0.04	2.15±0.01	2.27±0.01
Liver	3.87±0.13	3.63±0.02	3.70±0.04	3.36±0.18	3.45±0.04	3.42±0.07	3.56±0.07
Kidneys	0.65±0.02	0.63±0.02	0.59±0.02	0.56±0.02	0.59±0.01	0.60±0.03	0.62±0.03
Spleen	0.47±0.02	0.42±0.00	0.44±0.00	0.41±0.01	0.42±0.00	0.43±0.01	0.45±0.02
Heart	0.50±0.00	0.51±0.00	0.52±0.00	0.50±0.02	0.53±0.01	0.56±0.00	0.62±0.06
Thymus	0.04±0.01	0.03±0.02	0.03±0.00	0.04±0.02	0.03±0.01	0.04±0.01	0.03±0.01
Lungs	0.58±0.01	0.57±0.01	0.56±0.00	0.55±0.02	0.61±0.01	0.60±0.02	0.61±0.02

Table 3: Hemato-biochemical parameters of birds given different levels of arsenic and copper sulfate

Parameter/Day	Groups						
	A	B	C	D	E	F	G
Total proteins (g/dL)							
10	3.24±0.00	3.15±0.01	3.09±0.01	3.05±0.01	2.37±0.03*	2.62±0.01*	2.62±0.01*
20	3.36±0.01	2.91±0.01*	2.94±0.01	2.85±0.01	2.69±0.01*	2.56±0.01*	2.58±0.00*
30	3.32±0.02	2.66±0.02*	2.83±0.01	2.74±0.02*	2.62±0.01*	2.49±0.00*	2.52±0.00*
Serum albumin (g/dL)							
10	1.70±0.03	1.59±0.01	1.67±0.00	1.61±0.01	1.44±0.01*	1.38±0.01*	1.41±0.01*
20	1.76±0.00	1.52±0.00*	1.61±0.00	1.58±0.01	1.38±0.00*	1.30±0.00*	1.38±0.00*
30	1.72±0.01	1.47±0.00*	1.58±0.00	1.51±0.00*	1.33±0.02*	1.25±0.00*	1.32±0.00*
Urea (mg/dL)							
10	6.86±0.04	7.09±0.03	6.97±0.02	7.22±0.01	7.52±0.02*	8.59±0.08*	8.05±0.09*
20	7.09±0.02	9.06±0.07*	7.52±0.06	8.47±0.09*	9.05±0.13*	10.16±0.26*	9.41±0.22*
30	7.09±0.00	10.3±0.09*	7.73±0.02	9.73±0.60*	10.4±0.03*	11.4±0.06*	10.9±0.02*
Creatinine (mg/dL)							
10	0.37±0.00	0.42±0.01	0.39±0.01	0.42±0.00	0.48±0.00*	0.57±0.00*	0.53±0.00*
20	0.33±0.00	0.45±0.00*	0.38±0.01	0.44±0.02*	0.49±0.01*	0.61±0.00*	0.58±0.00*
30	0.35±0.00	0.49±0.00*	0.39±0.02	0.48±0.01*	0.58±0.00*	0.64±0.01*	0.62±0.00*
Malondialdehyde concentration (μm/L)							
10	1.55±0.01	1.61±0.01	1.59±0.01	1.60±0.01	1.89±0.04*	2.10±0.01*	1.91±0.01*
20	1.54±0.01	1.74±0.03*	1.62±0.01	1.74±0.01*	1.93±0.04*	2.28±0.03*	2.11±0.01*
30	1.50±0.01	1.99±0.04*	1.66±0.01	1.82±0.01*	2.04±0.05*	2.38±0.04*	2.22±0.04*

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

DISCUSSION

Arsenic is widely distributed as a trace element throughout the world which causes numerous adverse impacts both on public health and animals. Prior to this experimental research it has been reported that increased concentration of copper sulfate and arsenic alone poses serious threats in avian species (Khan *et al.,* 2014). In present study, the birds given higher concentrations of copper sulfate and arsenic alone showed different gross lesions in various tissues. The testes were smaller in size, liver was friable and pale to yellowish and kidneys were congested. The spleen and thymus were congested and smaller in size. Mild to moderate gross abnormalities were also observed in birds given lower doses of copper sulfate and arsenic in combination. Similar pathological changes been reported in different animals such as mice, rats and rabbits (Kannan *et al.,* 2001; Gora *et al.,* 2014). These gross lesions in liver, testes, thymus, spleen and kidneys of birds might be due to increased oxidative stress amplified by copper sulfate. Previously degenerative changes in kidneys have also been observed (Sener *et al.,* 2015). The absolute weight of testes, liver, kidneys, spleen, lungs and thymus was significantly decreased while relative weight of all the visceral organs was non-significantly different. The lower weight of different tissues in present study could be due to increased oxidative stress leading to lower feed intake, poor absorption of nutrients and decrease metabolism of the exposed birds.

Microscopically, testes of birds given higher levels of copper sulfate (150 mg/kg) and arsenic (35 mg/kg) alone revealed 2-3 cell layers having primary and secondary spermatocytes along with fewer round spermatids. The lumen of seminiferous tubules had admixture of necrotic spermatids instead of mature sperms. Histologically, liver tissues showed cytoplasmic vacuolation, bile ducts hyperplasia and congestion at higher concentrations of copper sulfate (150 mg/kg) and arsenic (35 mg/kg). In hepatocytes various nuclear changes (condensation, fragmentation, granular appearance and disintegration of nucleus) were also observed. Microscopically, severe congestion, increased urinary space, necrosis of tubular epithelial cells, detachment of renal tubules and hypertrophy of glomeruli along with loss of circular shape in kidneys of birds. Microscopically, congestion in spleen and thymus was observed at higher doses. Congestion, focal mineralization, renal tubular epithelial cell degeneration

Table 4: Serum liver and cardiac biomarker of birds given different levels of arsenic and copper sulfate

Parameter/Day	Groups						
	A	B	C	D	E	F	G
Aspartate aminotransferase (IU/L)							
10	30.7±1.88	35.5±0.35	33.5±1.05	37.4±0.31	43.8±1.02*	50.2±0.99*	48.7±0.59*
20	29.8±0.76	43.2±1.30*	37.4±1.08	41.5±0.68	47.8±0.69*	55.3±1.12*	53.5±0.82*
30	32.0±0.59	50±0.88*	41.7±1.19	44.8±0.59*	52.2±1.02*	60.5±0.60*	57.6±0.84*
Alkaline phosphatase (IU/L)							
10	7.94±0.10	10.3±.28	8.91±0.15	9.52±0.20*	12.1±0.79*	15.4±0.36*	14.7±0.31*
20	8.23±0.14	12.8±0.25*	9.85±0.23	11.9±0.16*	13.8±o.54*	17.1±0.59*	16.9±0.27*
30	8.53±0.19	15.3±.39*	11.1±0.25	14.1±0.23*	14.9±0.59*	18.6±0.67*	18.2±0.34*
CK-MB (IU/L)							
10	7.51±0.19	8.05±0.06	7.98±0.02	9.40±0.12	11.7±0.13*	12.6±0.13*	11.3±0.24*
20	7.89±0.04	10.3±0.21*	8.61±0.16	10.5±0.24	12.8±0.13*	14.1±0.24*	12.7±0.07*
30	8.07±0.03	13.3±0.23*	10.0±0.12	11.4±0.07*	13.5±0.13*	15.0±0.16*	13.5±0.16*
Triglyceride (mg/dL)							
10	86.4±1.12	93.3±1.46	91.3±1.2	95.0±0.5	106.9±1.5*	114.6±1.5*	103.2±3.8*
20	87.1±0.55	97.7±0.69*	94.3±0.7	97.8±0.69	109.1±2.9*	119.2±1.7*	108.6±3.8*
30	87.0±0.83	109.1±2.8*	96.5±0.4	101.3±1.4*	115.2±0.9*	127.7±1.8*	117.8±2.6*
Cholesterol (mg/dL)							
10	92.2±0.78	104.0±1.8	106.9±1.2	109.7±1.5	124.1±2.8*	137.1±3.2*	126.7±2.8*
20	94.8±0.69	115.6±2.5*	107.3±1.3	111.0±2.9	129.4±2.7*	142.5±3.4*	133.3±2.4*
30	94.3±0.91	122.2±2.3*	109.9±1.9	118.8±1.6*	134.0±2.5*	146.9±3.7*	139.8±4.5*

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

and tubular casts in kidneys in rats due to arsenic exposure have been investigated (Gora *et al.*, 2014. It is reported that the degenerative changes in liver and kidneys in birds can be due to the oxidative stress enhanced by copper sulfate. Previously in kidneys apoptotic cells have been observed (Khan *et al*., 2013; Sener *et al*., 2015). It is reported that arsenic causes demethylation of IL-8 promoter by 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). Similar changes in kidneys have been observed in mice (Li *et al*., 2010). Furthermore, itis reported that arsenic exposure in liver tissues results the activation of prototypical AhR-regulated genes (Nqo1 mRNA, Cyp1b1 and Cyp1a1) which induces toxic effects by increasing nuclear localization (Elshenawy *et al*., 2015). The tissue changes in testes could be due to increased release of IL-8 and changes in IL-8 promoter followed by DNA methylation. The testicular changes in birds can also be related to generation of different intracellular DAMPS (N formal peptides, Neuropeptides HsP and), extracellular DAMPS (biglycan and hyloroune) and the release of IL-1a and IL-33 from necrotic cells (Ghaffar *et al*., 2015; Hussain *et al*., 2015). The microscopic abnormalities in this study could be due to generation of apoptotic inducing substances through ROS signaling pathway from mitochondria leading necrotic changes (Altikat *et al.*, 2014; Auon *et al.*, 2014; Hussain *et al*., 2014).

The decreased values of these blood biochemical parameters could be due to the inefficiency of birds to deliver sufficient amount of oxygen to hematopoietic tissues (Hussain *et al*., 2015; Ghaffar *et al.*, 2015). The abnormal values of these parameters at lower levels of arsenic and copper sulfate in present study are suggestive of interaction of these chemicals. Results showed that the serum urea, creatinine and malondialdehyde concentrations were significantly increased in exposed birds. The values of liver specific enzyme aspartate aminotransferase and the cardiac biomarkers such as isoenzyme CK-MB, triglyceride and cholesterol were significantly increased. Previously no information is available about the deleterious effects of copper sulfate and arsenic. However, similar reports are available in birds due to arsenic intoxication (Khan *et al*., 2013). The higher levels of urea, creatinine and lipid peroxidation product (MDA) in birds in our study have also been investigated due to arsenic toxicity in rats (Muthumani and Miltonprabu, 2015; Sener *et al*., 2015). Moreover, the higher values of kidneys function tests (creatinine and urea) and liver function test (ALT) could be due to increased process of oxido-nitrosative stress leading to up regulation of Caspase-3, TGF-β, KIM-1 and TNF-α mRNA expression in kidneys and liver. Similar results have been reported due to arsenic intoxication in rats (Adil *et al*., 2015). The increased levels of cardiac biomarkers (isoenzyme CK-MB, cholesterol and triglycerides) could be due toup-regulation of cardiac NADPH sub units (NOX2 and NOX4) and down-regulation of protein expressions Nrf2 and HO-1 (Muthumani and Miltonprabu, 2015).

Conclusions: From the results of our study it can be concluded that copper sulfate and arsenic at low levels in combinations and at higher doses alone poses deleterious effects in birds. Therefore, monitoring of these compounds is crucial to minimize the adverse impacts in public health.

Authors contribution: RH and AG designed the research. RH, IRC, JH and SK conducted the experiment. RH examined blood smears. RH and JN analyzed the data. RH, HA, GA, AG and MHA wrote the article. All the authors carefully read the manuscript.

REFERENCES

Adil M, Kandhare AD, Visnagri A, *et al*., 2015. Naringin ameliorates sodium arsenite-induced renal and hepatic toxicity in rats: decisive role of KIM-1, Caspase-3, TGF-β and TNF-α. Renal Fail 37:1396-407.

Altikat S, Uysal K, Kuru HI, *et al.,* 2014. The effect of arsenic on some antioxidant enzyme activities and lipid peroxidation in various tissues of mirror carp (*Cyprinus carpio carpio*). Environ Sci Poll Res Int 22:3212-8.

Auon M, Mahmood F, Khan A, *et al*., 2014. Testicular and genotoxic effects induced by subchronic oral administration of chlorpyrifos in Japanese quail (*Coturnix japonica*). Pak J Agric Sci 51:1005-10.

Baig JA, Kazi TG, Shah AQ, *et al.,* 2012. Arsenic speciation and other parameters of surface and ground water samples of Jamshoro, Pakistan. Int J Environ Anal Chem 92:28-42.

Bhatti SS, Javed MT, Kausar R, et al., 2015. Urea and copper sulphate in different combinations alter haematology and some serum proteins in broilers. Pak J Agri Sci 52:539-44.

Cohen SM, Arnold LL, Eldan M, *et al.*, 2006. Methylated arsenicals: the implications of metabolism and carcinogenicity studies in rodents to human risk assessment. Crit Rev Toxicol 36:99-133.

Elshenawy OH and El-Kadi AO, 2015. Modulation of aryl hydrocarbon receptor-regulated enzymes by trimethylarsine oxide in C57BL/6 mice: In vivo and in vitro studies. Toxicol Lett 238:17-31.

Ghaffar A, Hussain R, Aslam M, *et al.,* 2016. Arsenic and urea in combination alters the hematology, biochemistry and protoplasm in exposed Rahu fish (*Labeo rohita*) (Hamilton, 1822). Turkish J Fish Aquatic Sci 16:289-96.

Ghaffar A, Hussain R, Khan A, *et al.*, 2015. Butachlor induced clinicohematological and cellular changes in fresh water fish *Labeo rohita (Rohu)*. Pak Vet J 35:201-6.

Ghaffar A, Hussain R, Khan A, *et al.*, 2015. Clinico-hematological and mutagenic changes induced by arsenic and copper sulphate in adult poultry males. J Anim Plant Sci 25:1555-61.

Ghaffar A, Ashraf S, Hussain R, *et al.,* 2014. Clinico-hematological disparities induced by triazophos (organophosphate) in Japanese quail. Pak Vet J 34:257-9.

Gora RH, Kerketta P, Baxla SL, *et al.,* 2014. Ameliorative effect of *Tephrosia Purpurea* in arsenic-induced nephrotoxicity in rats. Toxicol Int 21:78-83.

Hussain R, Khan A, Mahmood F, *et al.*, 2014. Clinico-hematological and tissue changes induced by butachlor in male Japanese quail (Coturnix japonica). Pest Bioch Physiol 109:58-63.

Hussain R, Mahmood F, Khan A, *et al*., 2015. Genotoxic and pathological effects of malathion in male Japanese quail (*Coturnix japonica*). Pak J Agri Sci 52:1143-9.

Jun L, Jie W, Michael P, *et al.,* 2008. High dietary fat exacerbates arsenic induced liver fibrosis in mice. Exp Biol Med 233:377-84.

Kannan GM, Tripathi N, Dube SN, *et al.*, 2001. Toxic effects of arsenic (III) on some hematopoietic and central nervous system variables in rats and guinea pigs. J Toxicol Clin Toxicol 39:675-82.

Khan A, Hussain HI, Sattar A, *et al.,* 2014. Toxico-pathological aspects of arsenic in birds and mammals: a review. Int J Agric Biol 16:1213-24.

Khan A, Sharaf R, Khan MZ, *et al.,* 2013. Arsenic toxicity in broiler chicks and its alleviation with ascorbic acid: a toxico-patho-biochemical study. Int J Agric Biol 15:1105-11.

Li Z, Piao F, Liu S, *et al.*, 2010. Subchronic exposure to arsenic trioxide-induced oxidative DNA damage in kidney tissue of mice. Exp Toxicol Pathol 62:543-7.

Lima VJ, Mauricio RB and Josh MM, 2010. Arsenic toxicity in mammals and aquatic animals: A comparative biochemical approach. Ecotoxicol Environ Saf 74:211-8.

Mandour MA, Al-Shami SA and Hssein YA, 2012. Effect of feeding graded levels of urea on growing New Zealand white rabbit performance. Global Vet 9:761-8.

Mashkoor J, Khan A, Khan MZ, *et al.*, 2013. Arsenic induced clinico-hemato-pathological alterations in broilers and its attenuation by vitamin E and selenium. Pak J Agric Sci 50:131-8.

Muthumani M and Miltonprabu S, 2015. Ameliorative efficacy of tetrahydrocurcumin against arsenic induced oxidative damage, dyslipidemia and hepatic mitochondrial toxicity in rats. Chem Biol Interact 235:95-105.

Nepusz T, Petróczi A and Naughton DP, 2009. Food alert patterns for metal contamination analyses in seafoods: longitudinal and geographical perspectives. Environ Int 35:1030-3.

Petroczi A and Naughton DP, 2009. Mercury, cadmium and lead contamination in seafood: A comparative study to evaluate the usefulness of target hazard quotients. Food Chem Toxicol 47:298-302.

Rahman MA, Hasegawa H and Lim RP, 2012. Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain. Environ Res 116:118-35.

Rasool A, Javed MT, Akhtar M, *et al.,* 2013. Effects of urea and copper sulphate on some biochemical and meat parameters in broiler chicken. Pak Vet J 33:27-31.

Sattar A, Khan A, Hussain HI *et al.,* 2016. Immunosuppressive effects of arsenic in broiler chicks exposed to Newcastle disease virus. Immunotoxicology 13:861-9.

Sener U, Uygur R, Aktas C, *et al.*, 2015. Protective effects of thymoquinone against apoptosis and oxidative stress by arsenic in rat kidney. Renal Fail 29:1-7.

Sharaf R, Khan A, Khan MZ, *et al.*, 2013. Arsenic induced toxicity in broiler chicks and its amelioration with ascorbic acid: Clinical, hematological and pathological study. Pak Vet J 33:277-81.

Singh MK, Yadav SS, Yadav RS, *et al.*, 2015. Protective effect of *Emblica-officinalis* in arsenic induced biochemical alteration and inflammation in mice. Springerplus 4:438doi:10.1186/s40064-015-1227-9.

Witeska M, Sarnowski P, Ługowska K, *et al.*, 2014. The effects of cadmium and copper on embryonic and larval development of ide *Leuciscus idus* L. Fish Physiol Bioch 40:151-63.