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Arsenic Toxicity in Broiler Chicks and its Alleviation with Ascorbic Acid: A Toxico-patho-biochemical Study

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Abstract

To find out toxico-pathological effects of arsenic (As) and ameliorating effect of ascorbic acid (Vit C), broilers birds were administered 50 and 250 mg/kg arsenic and Vit C, respectively alone/in combination. As-treated birds exhibited severe signs of toxicity such as dullness, depression, increased thirst, open mouth breathing and watery diarrhea. All these signs were partially ameliorated with the treatment of Vit C. As-treated birds showed a significant decrease in serum total proteins while serum enzymes, urea and creatinine were significantly increased. Alkaline phosphatase and lactate dehydrogenase completely whereas proteins, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine were partial ameliorated in birds treated with As+Vit C as compared to As-treated and control birds. Pale and hemorrhagic liver and swollen kidneys were observed in As-treated birds. Histopathologically, liver exhibited congestion and cytoplasmic vacuolation while in kidneys, condensation of tubular epithelium nuclei, epithelial necrosis, increased urinary spaces, sloughing of tubules from basement membrane and cast deposition were observed in As-treated birds. Pathological lesions were partially ameliorated with the treatment of Vit C. It can be concluded that arsenic induces biochemical and histopathological alterations in broiler birds; however, these toxic effects can be partially attenuated by Vit C.

Keywords: Arsenic; Broiler chicks; Toxico-pathological effects; Serum enzymes; Vitamin C

Introduction

In Pakistan's GDP, livestock sector is contributing about 11% (Abubakar *et al*., 2011). Poultry industry among livestock sector, has its own importance. Other than animal proteins, it is providing employment opportunities (Mahmud *et al*., 2011). In Pakistan about 4518 million poultry birds yearly provide about 601 thousand tons meat. Layer and breeders at the end of egg production contribute more than 46,000 metric tons meat per annum (Javaid *et al*., 2012). Poultry is turning feed stuffs into meat (Hafez, 2011), but meat production is hindered by the presence of arsenic in drinking water (Sharaf *et al*., 2013) or various diseases (Mashkoor *et al*., 2013).

Heavy metal arsenic is categorized as a ubiquitous trace element and the 52nd most common element in the earth's crust (Hantson *et al*., 2003). Along with the beneficial effects, it is well known to be a potent toxic heavy metal since the ancient times. In broiler rearing, arsenic in the forms of roxarsone and arsenilic acid is used as additive in the feed which is used to control protozoan parasites and to enhance weight gain. If the recommended levels of arsenic in broiler feed are not observed strictly then it can accumulate in broilers flesh which might be detrimental to the consumers.

Arsenic may be present in the organic or inorganic form (Ahmed, 2003). Of the inorganic forms, arsenite (trivalent) and arsenate (pentavalent) are the two most prevalent and potential dangerous forms, while organic form of organoarsenicals is of importance (Dopp *et al*., 2004; Sharaf *et al*., 2013). Usual routes of As-assimilation could be through ingestion and inhalation, while some degree of skin absorption also occur (Monies, 1999). It is readily absorbed in the gastrointestinal tract (Aslam *et al*., 2011), although some of the inhaled arsenic may be absorbed in the intestines after clearance from the upper respiratory tract (Vahter and Norin, 1980).

Biotransformation of absorbed arsenic mostly takes place in liver and kidneys (Ford, 2002). Methylated metabolites formed during process of biotransformation are distributed throughout the body (Dopp *et al*., 2004). Urine is major gateway to excrete arsenic, as almost 60% of the dose is excreted in urine and only about 6% in feces (Goyer and Clarkson, 2002).

Arsenic-toxicity usually attributed to low methylating ability. Chimpanzee and the monkeys exhibit the highest toxic effects of arsenic than any other specie because they express poor methylating ability. Humans and guinea pigs are also more prone to toxicity because they seem to be poor methylators too. Disturbance in methylation ability in rats, chicken and rabbit leads to toxic effects (Vahter, 1994).

Ascorbic acid (Vit C) is a water-soluble antioxidant,

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which is necessary in the body to form collagen in bones, cartilage, muscle and blood vessels and aids in the absorption of iron (Bera *et al*., 2010). Vit C exerts its protective role by enhancing the speed of the bowel transit time to help the elimination of heavy metals through the intestines. It can terminate free radical reaction chains by being a stable electron donor in interactions with free radicals (Halliwell, 2002), being first converted into an ascrobate radical then monodehydroascorbate and dehydroascorbate. The oxidized forms of ascorbate are relatively stable and un-reactive, and do not cause cellular damage which can be reversed back to ascorbate by cellular enzymes (Banerjee *et al*., 2009). Ascorbate can also chelate and reduce transition metal ions and the reduced metal ions which in turn can reduce oxygen and H_2O_2 to superoxide and hydroxyl radicals, respectively (Carr and Frei, 2000). These observations of Vit C make it a suitable antidote for As-toxicity. This study deals with the ameliorating effect of Vit C on As-induced toxico-pathological manifestations in broiler chicks.

Materials and Methods

Before the execution, the experimental proposal was approved by the Graduate Studies and Research Board, University of Agriculture, Faisalabad. During the experiment, all the legislations regarding protection of animal welfare laid down by the Ethics Committee were followed.

Experimental Birds and Management

One-day old 72 Hubbard broiler chicks of mixed gender (as hatched) were procured from a local hatchery and kept in wire cages under standard management and housing conditions. The birds were provided with basal diet, i.e. chick starter crumbs having 21% total protein (Jalees *et al*., 2011) and clean water *ad libitum*. They were vaccinated with vaccines against Newcastle disease (Nobilis® ND Lasota, Intervet S.A. (Pty) Ltd) and infectious bursal disease (Nobilis Gumboro 228E, Intervet S.A. (Pty) Ltd) and formalized vaccine of hydropericardium syndrome (BioAngara Plus, Sana Lab) on the recommended days.

Experimental Procedure

After 10 days of acclimatization, all the birds were randomly divided into four equal groups. Treatments were started at 11^{th} day and continued till the age of day 42. The maximum tolerance level of arsenic for poultry was set as 100 mg/kg for organic and 50 mg/kg for inorganic arsenic (National Research Council, 1980). As the arsenic salt used in the present study was inorganic in nature, therefore, 50 mg/kg dose was selected. Standard dose of Vit C was collected from secondary source (Singh and Rana, 2007). Arsenic and Vit C are water soluble; therefore, distilled water was used as diluent. All the treatments were given orally via crop tubing on daily basis. Group A served as control. Group B was given arsenic (disodium hydrogen arsenate, E. Merck, AG, Germany) @ 50 mg/kg. Group C was provided with arsenic (50 mg/kg) along with Vit C (250 mg/kg) whereas group D received only Vit C (250 mg/kg). All the birds were monitored for clinical signs twice daily. Each bird was weighed weekly. Feed intake was measured on daily basis.

Biochemical Parameters

Randomly selected six birds from each group were killed humanly by neck dislocation on day 0, 16 and 32 of the experiment, and blood sample was collected for the procurement of serum. Total serum proteins were determined by using Biuret reagent (Njidda and Isidahomen, 2011; Ahmad *et al*., 2012) while albumin was determined by Bromocresol green dye binding method (Varley *et al*., 1980). Serum globulin concentration was calculated by subtracting albumin from total proteins (Benjamin, 1978). Serum concentration of ALT, AST, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), urea and creatinine were measured utilizing commercially available colorimetric kits (AMP Medizintechnic GmbH, Austria; Cat # BR0415, BR0615, BR0202, BR1412, BR04006 and BR2810, respectively). All analyses were carried out on spectrophotometer (U-2001, Hitachi, Japan).

Gross- and Histo-pathology

After killing, visceral organs (liver and kidneys) of each bird were weighed individually. Liver and kidneys were weighed and examined for gross lesions and tissues showing gross lesions were preserved in 10% buffered formalin. Specimens of 5 mm thickness from morbid organs were taken and processed for histopathological examination using the standard method of dehydration in ascending grades of ethanol, clearing in xylene and embedding in paraffin. Sections of 5µm thickness were cut and stained with Hematoxylin and Eosin (Bancroft and Gamble, 2007; Sikandar *et al*., 2012).

Statistical Analysis

The data collected throughout the experiment were subjected for statistical analysis by applying complete randomized design two factors factorial test. Multiple comparisons among the means were performed by least significant difference test using MSTAT-C computer statistical package (Plant and Soil Sciences, Michigan State University, East Lansing, Michigan, USA) for the significance of the result at $P \leq 0.05$.

Results

Physical and Biochemical Parameters

Arsenic treated birds were dull and depressed with ruffled feathers and pale combs. These were also having increased thirst, open mouth breathing, and watery diarrhea. All these signs were partially ameliorated with the treatment of Vit C. Feed intake in arsenic treated birds (Group B) decreased significantly ($P<0.05$) as compared with control (Group A) on experimental days 16 (79.1±1.09 vs 123.0±1.2 g/day) and 32 (180.0±2.01 vs 231.1±2.97 g/day). Similarly, control birds showed higher body weight as compared with As-treated birds on experimental day 16 (1261.1±11.8 vs 1174.3±1.3 g) and 32 (1824.1±12.6 vs 1567.1±1.8 g).

At experimental day 16, significantly ($P<0.05$) decreased serum total proteins and albumin values were recorded in As-treated birds as compared to control (Table 1). At day 32, significantly ($P<0.05$) decreased serum total proteins and albumin values were recorded in arsenic (group B) and As+Vit C (group C) treated birds as compared to control. The serum total proteins and albumin values in group D at experimental days 16 and 32 differ insignificantly than control. At experimental days 16 and 32 significantly ($P<0.05$) increased serum globulin values were recorded in As (group B), As+Vit C (group C) and Vit C (group D) treated birds as compared to control (Table 1).

Serum enzymes (ALT, AST and ALP) showed significant ($P<0.05$) increasing trend while LDH did not differ from that of control group (A) throughout the experiment in arsenic and/or Vit C treated groups as compared to control while LDH did not differ from that of control. (Table 2). Urea and creatinine on experimental day 16 and 32 showed significantly increased concentration in As, As+Vit C and Vit C treated birds compared to control. Absolute weight of liver and kidneys in As, As+Vit C and Vit C treated birds at experimental days 16 and 32 were significantly ($P<0.05$) decreased in as compared to control (Table 3).

Gross- and Histo-pathology

Grossly, kidneys were swollen and liver was pale and hemorrhagic in As-treated birds as compared to control birds (Fig. 1). Microscopically, congestion, nuclei condensation of tubular epithelium, epithelial necrosis, increased urinary spaces with atrophy of glomeruli, along with sloughing of tubules from basement membrane (Fig. 2) and cast deposition in the lumen of renal tubules were observed in As-treated birds (Fig. 3). These lesions were of less intensity in Vit C treated birds (Table 4). Microscopically severe congestion and cytoplasmic vacuolation (Fig. 4) and bile duct hyperplasia was observed in liver.

Table 1: Serum total proteins of arsenic and Vit C treated broiler birds along with control group

Parameters/Groups	Experimental Days		
	0	16	32
Total proteins (g/dL)			
A	4.01±0.01	4.11±0.03	3.91±0.02
B	4.03±0.01	3.74 ±0.02*	3.33±0.02*
C	4.00±0.01	4.01±0.09	3.79±0.02*
D	3.99±0.01	4.10±0.09	3.86±0.03
Albumin (g/dL)			
A	2.06±0.02	2.59±0.02	2.31±0.02
B	2.06±0.02	2.06±0.03*	1.93±0.02*
C	2.06±0.02	2.51±0.04	2.20±0.02*
D	2.06±0.02	2.52±0.01	2.26±0.02
Globulin (g/dL)			
A	1.96±0.02	1.52±0.05	1.48±0.09
B	1.94±0.02	1.68±0.04*	1.40±0.03*
C	1.94±0.02	1.55±0.03*	1.59±0.04*
D	1.95±0.02	1.58±0.11*	1.60±0.03*

Values (Mean±SE) bearing asterisk in a column differ significantly ($P<0.05$) than values in control group in each parameter. Birds in group A (Control) were provided feed and water without any treatment. Groups B and C received arsenic daily @ 50 mg/kg in drinking water. Group C received Vit C daily @ 250 mg/kg while group D was administered Vit C @ 250 mg/kg daily

Table 2: Biochemical parameters of broiler birds administered arsenic and Vit C along with control group

Parameters/Groups	Experimental Days		
	0	16	32
Alanine aminotransferase (U/L)			
A	2.67±0.39	2.74±0.22	3.23±0.19
B	2.64±0.39	3.84±0.14*	5.15±0.14*
C	2.60±0.39	3.06±0.34	4.07±0.32*
D	2.58±0.39	2.84± 0.32	3.20±0.16
Aspartate aminotransferase (U/L)			
A	3.93±0.34	4.36±0.39	5.24±0.55
B	3.95±0.34	5.93±0.43*	7.42±0.65*
C	3.90±0.34	4.80±0.34	6.40±0.32*
D	3.92±0.34	4.37±0.39	5.82±0.63*
Alkaline phosphatase (U/L)			
A	10.20±1.06	12.44±1.38	22.11±1.95
B	10.36±1.06	14.92±2.25	27.63±1.95*
C	10.40±1.06	12.44±1.85	20.89±0.55
D	10.52±1.06	12.43±1.38	20.71±1.36
Lactate dehydrogenase (U/L)			
A	377±8.00	357±3.10	365±5.93
B	375±8.00	378±4.18	385±5.60
C	375±8.00	365±9.29	374±5.84
D	373±8.00	361±3.58	364±2.93
Urea (g/dL)			
A	22.94±0.06	30.41±0.44	32.22±0.29
B	22.94±0.06	34.23±0.12*	49.05±0.03*
C	22.94±0.06	32.00±0.32*	33.33±0.11*
D	22.94±0.06	27.12±0.04*	30.78±0.03*
Creatinine (g/dL)			
A	0.47±0.01	0.52±0.01	0.62±0.01
B	0.50±0.01	1.11±0.03*	2.02±0.01*
C	0.52±0.01	0.83±0.01*	0.99±0.01*
D	0.49±0.01	0.79±0.01*	0.78±0.01*

Values (Mean±SE) bearing asterisk in a column differ significantly ($P<0.05$) than values in control group in each parameter. Birds in group A (Control) were provided feed and water without any treatment. Groups B and C received arsenic daily @ 50 mg/kg in drinking water. Group C received Vit C daily @ 250 mg/kg while group D was administered Vit C @ 250 mg/kg daily

Table 3: Absolute weight of liver and kidneys of arsenic and Vit C treated broiler birds along with control group

Parameters/Groups	Experimental Days		
	0	16	32
Liver			
A	11.53±0.01	36.28±0.01	42.24±0.01
B	11.79±0.01	32.77±0.01*	35.20±0.01*
C	11.85±0.01	33.72±0.01*	38.20±0.01*
D	11.01±0.01	34.60±0.04*	40.20±0.01*
Kidneys			
A	2.10±0.01	9.24±0.01	14.16±0.01
B	2.21±0.01	8.21±0.01*	12.25±0.01*
C	2.17±0.01	8.20±0.01*	12.75±0.01*
D	2.08±0.01	8.21±0.01*	13.05±0.01*

Values (Mean±SE) bearing asterisk in a column differ significantly ($P<0.05$) than values in control group in each parameter. Birds in group A (Control) were provided feed and water without any treatment. Groups B and C received arsenic daily @ 50 mg/kg in drinking water. Group C received Vit C daily @ 250 mg/kg while group D was administered Vit C @ 250 mg/kg daily

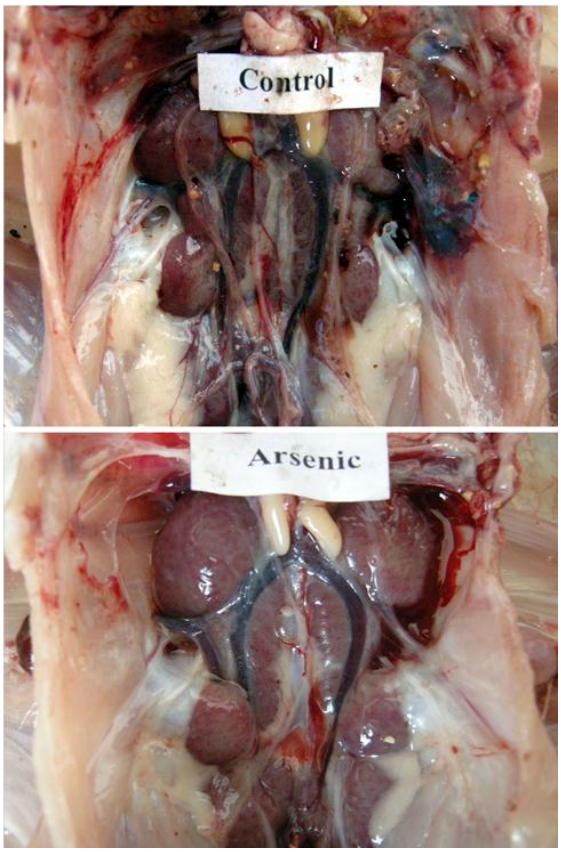

Fig. 1: Swollen kidneys in As-treated birds as compared to control birds

Discussion

As-toxicity signs like dullness, depression, ruffled feathers, open mouth breathing, and watery diarrhea observed in the present study might results in increased permeability of small blood vessels and inflammation and necrosis of the intestinal mucosa (Gordon, 2010). Impairment of intestinal functions leads to malabsorption of nutrients thus could result in dullness, depression (Benjamin, 1978; Stanely *et al*., 1994) and ruffled feathers (Vodela *et al*., 1997). With As-treatment, respiratory distress has been reported in goats (Patra *et al*., 2013).

In the present study, a decreasing trend in serum proteins was observed in As-treated birds, which can be attributed to decreased feed intake leading to catabolism of proteins to compensate the decreasing levels of blood glucose. It is well documented that arsenic leads to

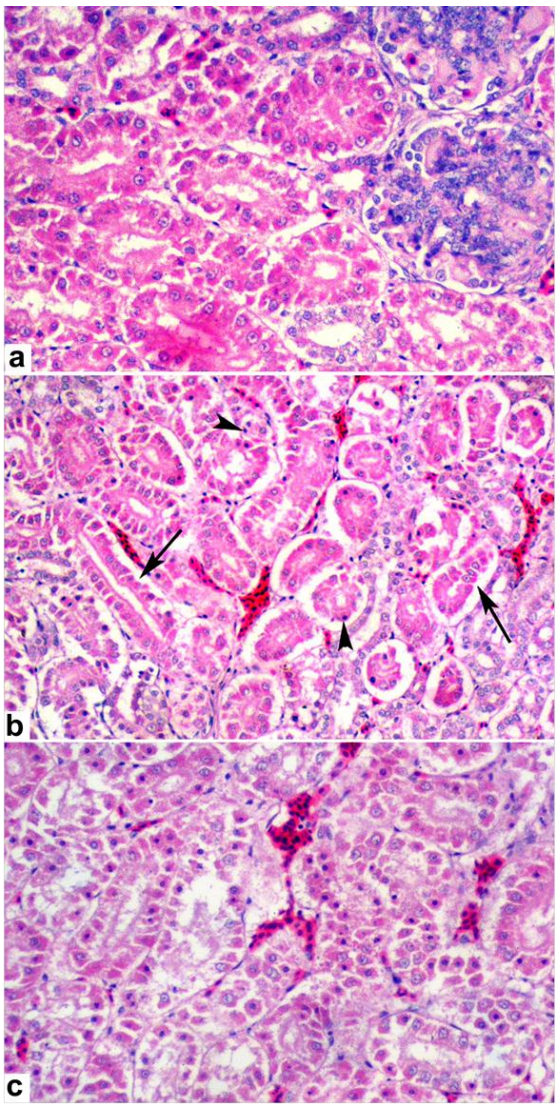

Fig. 2: Histopathology of kidneys. a) Control birds showing normal histological structure, b) As-treated broiler chicken showing congestion, condensed nuclei (arrow heads), epithelial necrosis, sloughing of tubular epithelium from basement membrane (arrows), and c) As+Vit C showing congestion and few condensation nuclei (H and E, X40 for all panels)

Table 4: Grading of lesions in liver and kidneys of broiler birds administered arsenic and Vit C along with control group

Organ/lesions	Groups			
	A (Control)	B (Arsenic)	C (As+Vit C)	D (Vit C)
Liver				
Vacuolation	-	++++	++	-
Congestion	-	+++	-	-
Bile Duct Hyperplasia	-	++++	+	-
Kidneys				
Condensed Nuclei in Tubular Epithelium	+	++++	+	+
Tubular Epithelium Necrosis	-	++++	-	-
Tubular Epithelium Sloughing	-	++++	++	-
Glomerular Atrophy/Tuft tuft Capillary shrinkage	-	+++	+	+
Increased Glomerular Space	-	++++	++	-
Cast Deposition in tubules	-	+++	+	-

Birds in group A (Control) were provided feed and water without any treatment. Groups B and C received arsenic daily @ 50 mg/kg in drinking water. Group C received Vit C daily @ 250 mg/kg while group D was administered Vit C @ 250 mg/kg daily

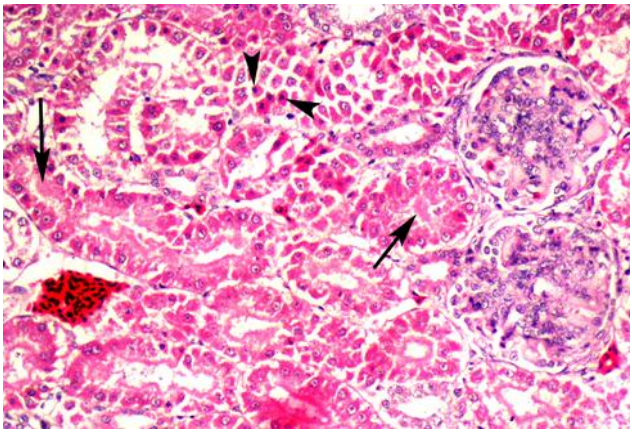

Fig. 3: Histopathology of kidney (As-treated bird) showing congestion, condensed nuclei (arrow heads), epithelial necrosis and cast deposition in renal tubules (arrows). (H and E, X100)

extensive damage to the capillaries causing increased permeability (Gordon, 2010) and exudation of serum into tissue spaces (Sarkar and Misra, 1991). As serum proteins synthesis occurs in liver, so a severely damage to hepatocytes could lead to poor protein synthesis (Benjamin, 1978). Accelerated proteolysis due to increase levels of hepatic enzymes (Table 2) could also lead to decrease in serum proteins (Abdel-Hameid, 2009). Decrease albumin is also used as an indicator of destruction in integrity of glomerular and mucus membranes (Abdel-Reheem, 2008). These results were corroborated with previous reports in cattle (Rana *et al*., 2008) and broilers (Padmaja *et al*., 2009).

In the present study, pathological lesions were observed in kidneys of As-treated birds (Table 4). These results could not be compared as such findings were not available in avian species, however, in goats (Biswas *et al*., 2000), fish (Roy and Bhattacharya, 2006) and mice (Ferzand *et al*., 2008; Li *et al*., 2010) various pathological lesions of As-toxicity have been reported. Increased urea level can be attributed to the kidneys failure to remove metabolic products (Bellomo *et al*., 2009). Increased absorption of urea from renal tubules could be due to failure of the selective reabsorption property of kidney tubules. Elevated level of creatinine indicated the signs of renal failure (Padmaja *et al*., 2009).

Grossly liver was hemorrhagic and pale, while severe congestion, cytoplasmic vacuolation and bile duct hyperplasia were observed microscopically. Moreover, a significant increase in serum enzymes (ALT, AST, ALP and LDH) was also recorded in As-treated birds. These results were in line with previous findings in Indian carp (Vutukuru *et al*., 2007), rats (Jadhav *et al*., 2007), cattle (Rana *et al*., 2008) and birds (Halder *et al*., 2008). Increased levels of ALT and AST are indicator of As-hepatotoxicity (Roy and Bhartacharya, 2006). It has been considered that increase in ALT and AST could be due to the cellular damage or increased plasma membrane permeability, so alteration of cell metabolism due to As-intoxication could increase the enzymic activity (Ramazzotto and Carlin, 1978).

Increased concentration of ALP and LDH were observed by Islam *et al.* (2009) in ducks as a result of As-treatment. ALP increase could be attributed to the irritation of liver, intestine, kidneys and to some extent linings of bile duct, particularly liver cell membrane which appears to act as stimulus to the increase synthesis of this enzyme (Teljon *et al*., 2006). Increased ALP could also be as a result of obstruction of intra-hepatic bile tract caused by cirrhosis of liver, causing regurgitation of enzyme back into the blood stream (Smith *et al*., 2008). Rise in LDH concentrations could be correlated to As-toxicity, as it results in decreased feed intake which paves the way to conversion of lactate to pyruvate to synthesize glucose to maintain energy levels (Abdel-Hameid, 2009).

In the current study, ameliorative effects of vitamin C were observed. These results were also reported previously in humans (Chattopadhyay *et al*., 2001), cattle (Singh and Rana, 2007) and mice (Banerjee *et al*., 2009; Bera *et al*., 2010). Antioxidants have long been attributed to be the reducers of the free radical-mediated oxidative stress. Vit C is a water-soluble antioxidant. Vitamin C speeds up the bowel transit time to help the elimination of heavy metals through the intestines. Free sulfhydryl groups (–SH) group

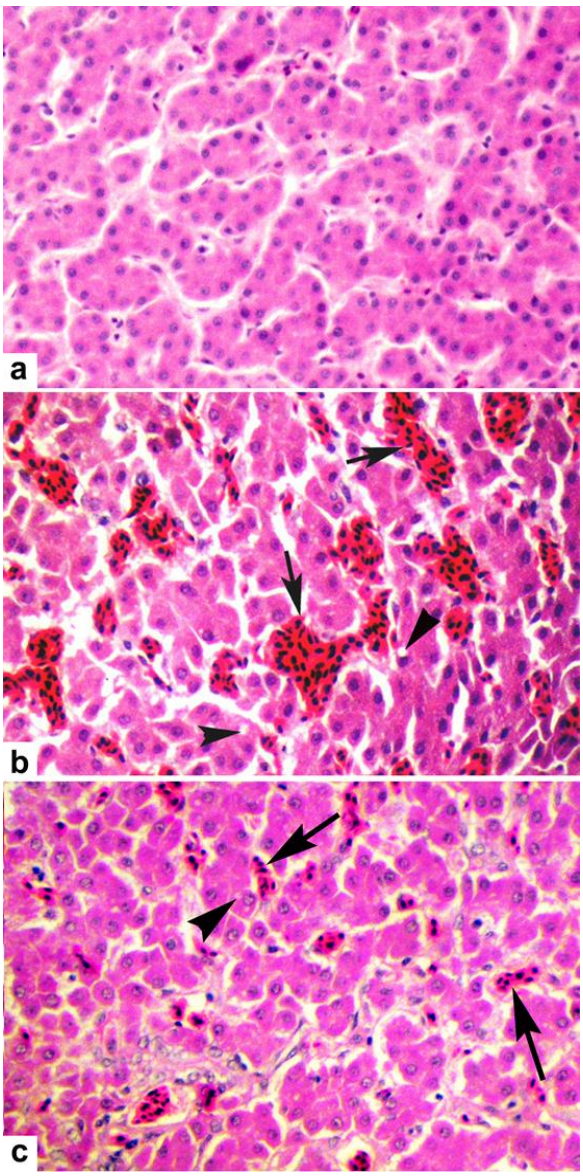

Fig. 4: Histopathology of liver. a) Control birds showing normal histological structure, b) As-treated broiler chicken showing severe congestion (arrows) and cytoplasmic vacuolation (arrow heads) and c) As+Vit C showing mild congestion and cytoplasmic vacuolation (H and E, X40 for all panels)

of Vitamin C causes its binding with heavy metals, resulting into reduction in the oxidative stress at tissue level and restoration of enzyme level (Rana *et al*., 2010).

Vit C can terminate free radical reaction chains as it is a stable electron donor in interactions with free radicals (Halliwell, 2002), being first converted into an ascrobate radical then monodehydroascorbate and dehydroascorbate. The oxidized forms of ascorbate are relatively stable and un-reactive, and do not cause cellular damage and can be reversed back to ascorbate by cellular enzymes (Banerjee *et al*., 2009). Ascorbate can also chelate and reduce transition metal ions and the reduced metal ions in turn can reduce oxygen and H_2O_2 to superoxide and hydroxyl radicals, respectively (Carr and Frei, 2000). Moreover, arsenic leads to depletion of glutathione (GSH) which cause damage to hepatic cells. Vit C reduces the depletion of GSH in As-toxicity which ultimately leads to prevention of cellular damage (Singh and Rana, 2007). These observations of vitamin C make it a suitable antidote for As-toxicity.

In conclusion, arsenic led to toxicity in broiler birds. Severe signs of depression were noted in birds treated with As. Biochemical indices, gross and histopathological lesions also indicated that arsenic was toxic. Notwithstanding, As-induced toxic effects can be partially nullified by treatment with Vit C.

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