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RESEARCH ARTICLE

Arsenic Induced Toxicity in Broiler Chicks and Its Amelioration with Ascorbic Acid: Clinical, Hematological and Pathological Study

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ABSTRACT

This study was conducted to observe the arsenic (As) toxicity lesions in birds and to know either Vit C ameliorates these toxic effects or not. One-day-old broilers chicks (n=72) procured from a local hatchery were randomly divided into four equal groups. First group was kept as control and second group was given As (50 mg/kg BW) via crop tubing. Third group received in addition to As, Vit C (250 mg/kg BW) whereas fourth group received only Vit C. Killing by neck dislocation of randomly selected six birds from each group was carried out on experimental days 0, 16 and 32 for collection of blood and tissues specimens. Arsenic treated birds showed clinical signs of toxicity throughout the experiment than all other groups. These clinical signs included decreased body weight and feed intake, dullness, open mouth breathing, increased thirst, ruffled feathers, pale comb, skin irritation and watery diarrhea which were not significant in any other group. As treated group showed a significant ($P<0.05$) decrease in hematological parameters. Severe gross and histopathological changes were observed in intestines, spleen and lungs of birds fed with As than all other groups. Decreased height of villi of middle portion of small intestines was also observed in As treated birds. Villi height in Vit C treated group increased as compared to control group. It was concluded that As induces severe toxic effects in broiler birds; however, these toxic effects can be partially ameliorated by Vit C.

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INTRODUCTION

Many heavy metals are used as a trace elements and feed additives in poultry feed. These metals are common in our environment some of these (iron, copper, manganese, zinc, etc) are essential for good health, however; other (As, mercury, lead, cadmium, etc) are poisonous and deleterious for health (Jadhav *et al.,* 2007; Ahmad *et al.,* 2009 & 2011; Khan *et al.,* 2012; Mashkoor *et al.,* 2013). Arsenic is most important and usually found in the environment in organic (combined with other elements such as oxygen, chlorine, and sulfur) and inorganic forms (combined with carbon and hydrogen). The organic arsenicals such as the derivatives of phenyl Arsenic acid are used as feed additives for half a century in poultry industry because it controls coccidiosis and improves production performance (Ghosh *et al*., 2012).

Along with these beneficial effects, As is recognized as one of the most toxic metalloids in poultry since the ancient times. The greatest commercial use of inorganic Arsenicals is in wood preservatives along with other major uses in pesticides (herbicides and termiticides). Most of the toxic effects arise from exposure to inorganic As and it affects nearly all body systems, especially keratinous material of poultry feathers (Nachman *et al*., 2012). Its toxicity usually attributed to low methylating ability. Arsenic toxicity varies with animals' species and also type of food they consume. Acute As toxicity by oral exposure can give symptoms with the rapid onset of headache, nausea, vomiting, diarrhea, dehydration, general vascular injury and muscular cramps **(**Rahman *et al.,* 2001). Severity and damage due to As toxicity in any organ depends upon dose and duration of exposure. Hematological parameters after As exposure showed marked decrease in hemoglobin, packed cell volume, erythrocytic count and total leukocytic counts, heterophils and lymphocytes **(**Halder *et al*., 2009). As induces free radical production, hence leads to lipid peroxidation and

damage cell membranes. Depending upon the transport in various tissues, As may react with sulfhydryl group enzymes and increased enzyme level occurs in liver and other related organs. The most affected organs are liver kidneys, lungs and small intestines (Horky *et al.,* 1998). Histopathological alteration due to As intoxication includes moderate to severe hyperemia, hemorrhages cellular swelling with granular cytoplasm, degeneration and coagulative necrosis in liver, intestines, kidneys and spleen (Biswas *et al*., 2000).

Antioxidants have long been known to reduce the free radical-mediated oxidative stress. Ascorbic acid (Vit C) is a water-soluble antioxidant, 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). Recently controlled trials with yeast, fish, mice, rats, chickens, clams, guinea pigs, and turkeys all came to the same conclusion that Vit C protects growing animals from heavy metals poisoning (Gajawat *et al*., 2005). 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 is also considered that it binds with heavy metals due to its free sulfhydryl group so reducing the oxidative stress in different tissues and restores enzyme level (Rana *et al*., 2010). Thus Vit C can be categorized to have cyto-protective role in all systems. Scanty information about arsenic toxicity in broilers is available; therefore, present study was designed and executed with the objectives to know the pathologic alterations induced by arsenic in broiler chicks and either vitamin C ameliorates these alterations or not.

MATERIALS AND METHODS

Experimental Birds and Management: This study was carried out on 72 one-day old broiler chicks of mixed gender (as hatched) procured from a local hatchery. After procurement, all the birds were kept in wire cages under standard and similar management and housing conditions. They were provided with basal diet, i.e. chick starter crumbs and clean water free from any chemical contaminants *ad libitum.* Birds were vaccinated against Newcastle Disease, Infectious Bursal Disease and Hydropericardium Syndrome on the recommended days using live vaccines.

Experimental Procedure: After 10 days of acclimatization, all the birds were randomly divided into four equal groups. The treatment started at the age of 11 days (it was day 1of the experiment) and continued till the age of 42 days (day 32, end of experiment). All the treatments were given orally via crop tubing on daily basis. Group A was kept as control and group B was given As (50 mg/ kg BW) (Howell and Hill, 1978). Group C in addition to As (50 mg/ kg BW) was provided with Vit C (250 mg/ kg BW) (Sahin and Kucuk, 2001) whereas group D received only Vit C (250 mg / kg BW). Both As and Vit C were mixed in distilled water.

Clinical observations: All the birds were monitored twice daily for clinical signs and behavioral abnormalities and subjectively categorized on the basis of severity {severe (++++), moderate (+++), mild (++) and very mild (+)}. Each bird was weighed weekly and feed intake was measured on daily basis.

Hematological studies: Randomly selected six birds from each group were killed by neck dislocation on experimental days 0, 16 and 32, and blood sample of each bird was collected with anticoagulant. Erythrocyte and total leukocyte counts were performed (Mushtaq *et al*., 2012). Hematocrit was determined using microhematocrit capillary tubes and hemoglobin was determined spectophotometrically by the cyanmethhemoglobin method using Drabkin's solution. Erythrocyte indices including mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated.

Gross and histopathology: After killing of individual bird, visceral organs (lungs, spleen and intestine) were weighed and along with these organs liver and kidneys were also examined grossly. Tissue specimens were fixed in 10% neutral buffered formalin (Shahzad *et al*., 2011). Specimens of 5mm 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). Furthermore, samples from middle portions of small intestine (from proventiculus upto ileo-cecal-colonic junction) were evaluated for quantification of histopathological lesions by micrometry, using stage and ocular micrometer (Samanya and Yamauchi, 2001).

Statistical analysis: The data thus collected from the above experiment were analyzed statistically by applying CRD two factors (treatment; time interval) factorial test. Different group means were compared by Least Significant Difference test using M-stat computer statistical package for the significance and non significance of the result. Significance level was $P<0.05$.

RESULTS

Arsenic treated birds showed significant ($P<0.05$) signs of toxicity than all other groups throughout the whole experiment. Clinical signs observed in As treated birds included dullness, open mouth breathing, increased thirst, ruffled feathers, pale comb, skin irritation and watery diarrhea. Birds treated with both As and Vit C showed non-significant signs of toxicity up to day 16 but after that no clinical signs up to day 32 as compared to control group. Feed intake and body weight was the highest in control group followed by Vit C treated group. Decreased feed intake and body weight was recorded in As treated birds as compared to control and Vit C treatment on experimental days 16 and 32, however, improvement in both these parameters was observed in As and Vit C treated birds (Table 1).

At day 0, all the groups showed a non-significant difference in all hematological parameters. At day 16, erythrocyte counts, hemoglobin concentration, hematocrit, mean carpuscular hemoglobin (MCH) and total leukocyte counts (TLC) were decreased significantly ($P<0.05$) in As

treated birds as compared to control and Vit C treatment, however, improvement in these parameters was observed in As and Vit C treated birds (Table 2). Mean carpuscular volume (MCV) and Mean carpuscular hemoglobin concentration (MCHC) decreased significantly in all treated groups; however, MCV differed non-significantly between each other. At day 32, As treated group showed significant ($P<0.05$) decrease in all hematological parameters, however, these parameters did not differ in between As+Vit C and Vit C groups except in TLC (Table 2).

Table 1: Feed intake and body weight of broilers administered arsenic and vitamin C

Parameters/	Age (Experimental Days)		
Groups	0	16	32
Feed Intake (g)			
Control	35.0±1.0	123.0±1.23a	231.1±2.97a
As	38.0±1.0	79.1±1.09d	180.0±2.01d
As+Vit C	33.1±1.0	99.9±1.22c	207.2±1.47c
Vit C	36.1±1.0	104.3±2.02b	215.9±2.00b
Body Weight (g)			
Control	255.0±10.0	1261.1±17.9a	1824.1±26.1a
As	249.8±9.0	1174.3±12.6d	1567.1±17.6d
As+Vit C	240.9±10.0	1184.2±12.2c	1705.4±19.5c
Vit C	252.2±11.0	1217.2±12.1b	1804.1±18.7b

Values (Mean±SE) bearing different alphabets in a column differ significantly ($P<0.05$) in each parameter.

Table 2: Hematological Parameters of broilers administered arsenic and vitamin C

Parameters/	Age (Experimental Days)		
Groups	0	16	32
Erythrocyte counts ($x10^6$)			
Control	2.01±0.01	3.00±0.02a	3.14±0.01a
As	2.05±0.01	2.38±0.01d	2.70±0.01c
As+Vit C	2.10±0.01	2.64±0.01c	2.94±0.01b
Vit C	2.03±0.01	2.83±0.01b	2.96±0.01b
Hemoglobin concentration (g/dl)			
Control	9.17±0.01	15.70±0.01a	16.90±0.07a
As	9.12±0.01	11.75±0.01d	13.50±0.07c
As+Vit C	9.13±0.01	13.39±0.01c	15.38±0.01b
Vit C	9.11±0.01	14.50±0.07b	15.50±0.11b
Hematocrit (%)			
Control	27.30±0.10	36.51±0.05a	39.08±0.15a
As	27.10±0.10	28.00±0.10d	32.00±0.19c
As+Vit C	27.00±0.10	31.20±0.04c	35.80±0.03b
Vit C	26.90±0.10	33.80±0.07b	35.80±0.09b
Mean Corpuscular Volume (fl)			
Control	131.1±0.86	121.6±0.54a	124.3±0.66a
As	132.1±0.86	117.3±0.30c	118.3±0.91c
As+Vit C	131.0±0.86	118.1±0.36c	121.7±0.69b
Vit C	131.6±0.86	119.3±0.33b	121.5±0.44b
Mean Corpuscular Hemoglobin (pg)			
Control	45.50±0.17	52.42±0.15a	53.88±0.11a
As	44.30±0.17	49.24±0.19d	49.88±0.27c
As+Vit C	44.60±0.17	50.70±0.11c	52.26±0.29b
Vit C	45.40±0.17	51.20±0.14b	52.32±0.24b
Mean Corpuscular Hemoglobin concentration (g/dl)			
Control	33.50±0.14	42.95±0.06a	43.20±0.16a
As	33.60±0.14	41.94±0.15b	42.14±0.14b
As+Vit C	34.00±0.14	42.86±0.07a	42.90±0.04a
Vit C	34.01±0.14	42.84±0.23a	43.00±0.25a
TLC ($x10^3$)			
Control	14.62±0.01	17.00±0.01a	17.50±0.06a
As	14.53±0.01	15.40±0.02d	15.98±0.01d
As+Vit C	14.59±0.01	16.40±0.01c	16.70±0.01c
Vit C	14.65±0.01	16.66±0.01b	17.10±0.01b

Values (Mean±SE) bearing different alphabets in a column differ significantly ($P<0.05$) in each parameter.

In case of relative weight (RW) of organs at day 0, a non-significant difference was present between all groups. A significantly ($P<0.05$) decreased in RW of organs of As treated birds was observed as compared to control, As+Vit C and Vit C treated groups at day 16 and 32 (Table 3) except spleen RW did not differ between As+Vit C and Vit C treated groups at day 32.

Grossly, intestines and spleen showed no signs of toxicity in any treated group but severe histopathological changes were observed in intestines and spleen of birds fed with As than all other groups. Microscopically, spleen showed cellular necrosis along with pyknotic nuclei in splenic cords. Sloughing of villus epithelium, infiltration of mononuclear cells and villus necrosis was observed in small intestine (Table 4). Grossly, lungs showed congestion and hemorrhages and a frothy material was also present in trachea and lungs of As fed birds. These lesions were not present in any other group. Decreased length of villi of middle portion of small intestines was also observed in As treated birds as compared to all other groups at days16 and 32. It was interesting to note that villi height in Vit C treated group even increased than control group (Table 5).

Grossly, liver and kidneys exhibited no lesions in any treated group. Histologically, congestion and vacuolation was observed in liver of As treated birds (Fig. 2). Kidneys of broiler chicks treated with arsenic showed congestion, detachment of tubular epithelium from the basement membrane, deposition of cast in the tubular lumen, atrophied glomerulous and increased urinary space (Fig. 3). These changes were partially ameliorated by the treatment of Vit C.

DISCUSSION

In the present study, the peculiar signs observed in As treated birds were decreased body weight, decreased feed intake, dullness, open mouth breathing, increased thirst, ruffled feathers, pale comb, skin irritation and watery diarrhea. These results are in line with previous reports like, in mammals (Rahman *et al.,* 2001), broilers (Vodela *et al.,* 1997) and in rats (Singh and Rana, 2007). Along with these, many morphological changes were also observed during the study period as lungs showed gross lesions like congestion, hemorrhages and frothy material accumulation in As treated birds. This could be the reason of respiratory distress as previously reported by Biswas *et al*. (2000) in goats.

Microscopically, spleen showed cellular necrosis along with pyknotic nuclei in spleenic cords which can be attributed to decreased values of erythrocyte counts, hemoglobin and hematocrit in As fed birds exhibited during the course of study. It could be corroborated with the reports of other workers in mammals (Rana *et al.,* 2008) and in birds (Halder *et al.,* 2009; Padmaja *et al.,* 2009). Reduction in blood parameters could also be due to hemolysis and hemorrhages (Simonato *et al.,* 2008) caused by As and bone marrow suppression. Another important factor to be considered in reduction of erythrocyte counts could be decrease in production of erythropoietin (EP). It is an important renal hormone as it increases the number of developing erythroid precursors and accelerates the release of reticulocytes from the marrow (Erslev, 1990). The decrease in EP could be correlated to kidney damage due to As toxicity as it is the major organ to regulate serum EP levels. In internal hemorrhages some erythrocytes are absorbed by lymphatic vessels. Remaining erythrocytes are

Table 3: Relative weight (%) of broiler birds in different groups

Parameters/ Groups	Age (Experimental Days)		
	0	16	32
Intestines			
Control	11.24±0.07	6.36±0.01a	5.28±0.01a
As	11.24±0.07	4.51±0.01d	5.09±0.01d
As+Vit C	11.24±0.07	6.07±0.01c	5.14±0.01c
Vit C	11.24±0.07	6.11±0.01b	5.15±0.01b
Spleen			
Control	0.07±0.01	0.09±0.00a	0.12±0.00a
As	0.07±0.01	0.06±0.01c	0.10±0.01c
As+Vit C	0.07±0.01	0.08±0.01b	0.11±0.01b
Vit C	0.07±0.01	0.09±0.01a	0.11±0.01b
Lungs			
Control	0.69±0.01	0.58±0.01a	0.53±0.01a
As	0.68±0.01	0.43±0.01d	0.45±0.01d
As+Vit C	0.69±0.01	0.51±0.01c	0.51±0.01b
Vit C	0.68±0.01	0.54±0.01b	0.50±0.01c

Values (Mean±SE) bearing different alphabets in a column differ significantly (P<0.05) in each parameter.

Table 4: Grading of lesions in intestines and spleen of broiler birds in different groups

Organ/lesion	Groups			
	A (Control)	B (As)	C (As+Vit. C)	D (Vit. C)
Spleen				
Cellular Necrosis	-	++++	+	-
Pyknotic Nuclei of Splenic Cords	-	++++	+	+
Small Intestine				
Sloughing of Villus Epithelium	+	++++	+	-
Infiltration of Mononuclear Cells	-	+++	+	+
Villus Necrosis	-	++++	++	-
Large Intestine				
Necrosis of Muscularis Mucosae	-	++++	+	-

Table 5: Variation in length of intestinal villi (µm) of broiler birds in different groups

Groups	Age (Experimental Days)		
	0	16	32
Control	709.2±12.9	868.9±13.5a	872.3±13.3b
As	713.2±13.0	726.7±10.5b	675.4±13.7d
As+Vit C	705.5±12.9	728.4±10.0b	771.9±11.3c
Vit C	707.3±13.0	892.8±17.0a	975.0±13.8a

Values (Mean±SE) bearing different alphabets in a column differ significantly (P<0.05).

lysed or phagocytized (Winski *et al.*, 1997). These findings were in negative relation to the report of Rezuke *et al.* (1991), showing a non-significant decrease in blood parameters.

A significant decrease in the erythrocyte indices was observed in As fed group than all other groups during the course of study. Anemia was classified as microcytic hypo-chromic. The decrease in erythrocyte indices could be due to acute hemorrhages or hemolysis (Simonato *et al.*, 2008), showing rapid decrease in all hematological parameters leading to anemia. The results differed with the results reported by Halder *et al.* (2009) in birds, who reported non-significant change in these parameters in birds fed with As. These results were also contrary to the previous findings of Pandey *et al.* (2007) who documented normocytic anemia due to non-significant increase in MCV values in As toxicity. The TLC, especially lymphocyte values of As fed group was also decreased throughout the study period which could be due to their decrease production from the germinal center of lymphoid organs due to their necrosis.

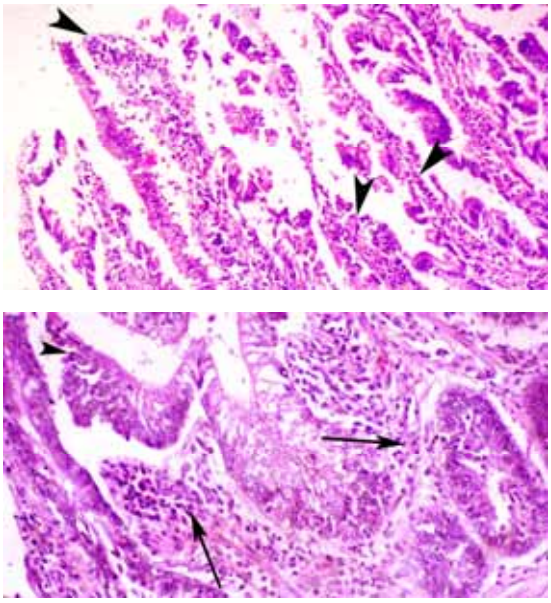

Fig.1: Photomicrograph of intestines of broiler chicks treated with arsenic showing sloughing of epithelium from the villi (arrow head) and infiltration of inflammatory cells between the crypts (arrow). H & E. upper) 200X and lower) 400X.

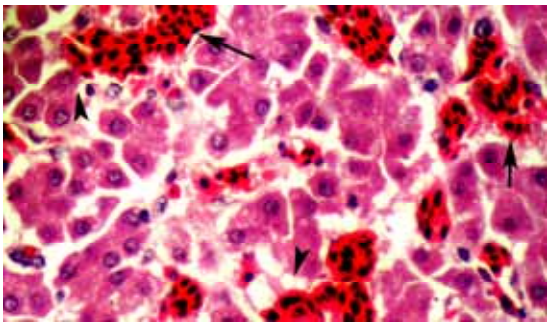

Fig. 2: Liver of broiler chicks treated with arsenic showing congestion (arrow) and hepatocyte vacuolation (arrow head). H & E. 400X.

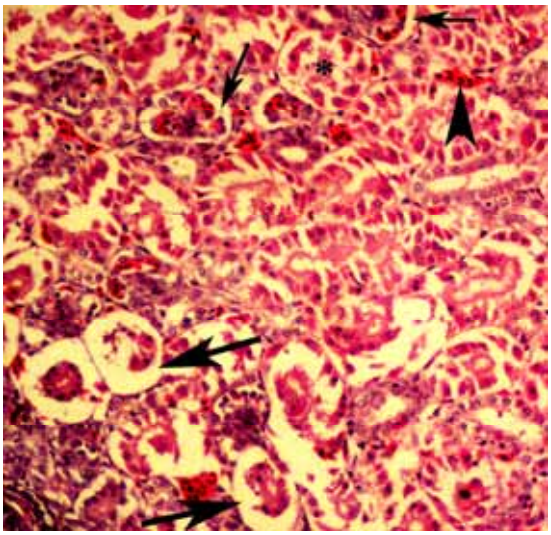

Fig. 3: Kidneys of broiler chicks treated with arsenic showing congestion (arrow head), detachment of tubular epithelium from the basement membrane (thick arrow), deposition of cast in the tubular lumen (*), atrophied glomerulous and increased urinary space (thin arrow) and numerous hyperchromatic nuclei. H & E. 200X.

In the present study, decreased length of villi of jejunum was observed in As treated birds as compared to other groups. This could be attributed to endothelial toxicity by damage of mucosal vascular supply but not by direct corrosive action of As which could lead to mucosal vesicle formation and sloughing causing mucosal erosions (Gorby, 1988).

Ameliorative effects of vitamin C were observed throughout the study period as the group treated with vitamin C along with As showed least toxic signs or no toxicity over all, in the present study. These results were also reported previously by Chattopadhyay *et al.* (2001) and Ramanathan *et al.* (2002) in humans, Singh and Rana (2007) in cattle, Banerjee *et al.* (2009) in mice and Bera *et al.* (2010) in rats. Antioxidants have long been attributed to be the reducers of the free radical-mediated oxidative stress. Vitamin C speeds up the bowel transit time to help the elimination of heavy metals through the intestines. Free SH group 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).

Conclusion: It was concluded that As exerts clinical signs birds including decreased body weight, decreased feed intake, dullness, open mouth breathing, increased thirst, ruffled feathers, pale comb, skin irritation and watery diarrhea. Arsenic treated group showed a significant ($P<0.05$) decrease in hematological parameters. Severe gross and histopathological changes were observed in intestines, spleen and lungs of birds fed with As than all other groups. Decreased height of villi of middle portion of small intestines was observed. Vit C partially ameliorated the toxic effects of arsenic in broiler birds.

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