

Original Research Article

Ameliorating Effect of Ascorbic Acid on Clinicopathological Changes of Induced Sub-Acute Arsenic Toxicity in Broiler Birds

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ABSTRACT

The present research work was conducted in broiler chickens by daily oral administration of 1/10th of ALD₅₀ of As₂O₃ i.e. 16.25 mg/kg b. wt. starting from day-old to 6 weeks of age to study the clinicopathological changes in sub-acute arsenic toxicity and also to record the ameliorating effect of ascorbic acid in arsenic intoxicated broiler birds. Clinical signs of toxicity developed in the arsenic treated birds on 3 weeks onwards, consisting of partial loss of appetite and body weight gain, gastrointestinal signs, mild depression, pale combs, ruffled feather and weakness without mortality in both the arsenic induced and arsenic + ascorbic acid treated birds. Maximum growth depression 22.46% and 7.9% was observed on 6 weeks post treatment in the arsenic induced and arsenic + ascorbic acid treated birds respectively. Haematological changes comprised a significant (P<0.01 or P<0.05) decrease in hemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) in arsenic induced broiler birds only or with ascorbic acid in contrast to control birds after 3 and 6 weeks of treatment. Birds of the ascorbic acid ameliorated group showed significant improvement when compared to the values of the arsenic treated birds but the values were lower than the values of the birds of the control group. A significant (P<0.01) decrease in total leucocyte count (TLC) with the significant (P<0.01 or P<0.05) decrease in lymphocyte count was also observed in arsenic treated birds indicating the suppressive effects of arsenic on haemopoietic system. Arsenic + ascorbic acid treated birds showed significant (P<0.01) improvement in the TLC values though the values were lower than the control. Both the arsenic treated and arsenic + ascorbic acid treated birds had a significant (P<0.01 or p<0.05) decrease in total serum protein, albumin, with simultaneous reduction in A: G ratio and a significant (P<0.01 or P<0.05) increase in serum glucose, creatinine and serum transaminase activities (AST and ALT) and alkaline phosphatase (ALP) activity on 21 and 42 days post- treatment. But, ascorbic acid treatment showed its protective effect against arsenic induced toxicity in broiler birds as there was a marked improvement in the values of the biochemical indices.

Keywords

Arsenic,
Ascorbic acid,
Broiler birds,
Clinico-
pathology,
Toxicity

Introduction

Arsenic (As) is a widely occurring toxic metalloid in the natural ecosystems. 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). Arsenic pollution in the environment has gained importance owing to its widespread toxic effect on aquatic and

terrestrial animal. The contamination of ground water with arsenic is one of the serious problems encountered in different states of India. Soil contamination with arsenic occurs through the contaminated ground water being used for irrigation and thereby arsenic contamination in plants and vegetable may cause serious threat to livestock animal and poultry birds. The accumulation of arsenic in ground water (0.20-3.70 mg/litre) has posed a serious threat to millions of population in India and many other countries like Bangladesh, China, Thailand and Taiwan (Chakraborty and Das, 1997). 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). Vit. C exerts its protective role by enhancing the speed of the bowel transit time to help the elimination of heavy metals through the intestine. During last few decades, constant use of agro-chemicals such as heavy metals and pesticides in various agricultural operations, animal husbandry practices and public health program has polluted the environment and alarmingly contaminated food grains, animal products like meat and meat products, eggs, milk and milk products etc., vegetables, soil, water and air imposing a serious threat to the healthy existence of flora and fauna in the ecosystem. Arsenic also inhibits various vital enzyme systems including iso-enzymes of cytochrome P-450 (Albores *et al.*, 1992) and thus produces adverse effect on growth and productivity of livestock and poultry causing a substantial economical loss to animal husbandry sector. Considering the significance of the problem, the present study was undertaken to evaluate the clinico-pathological effects of sub-acute toxicity of arsenic trioxide in broiler chickens and the ameliorating effect of ascorbic acid in arsenic intoxicated broiler birds.

Materials and Methods

Sixty (60) day-old broiler chicks weighing 40-50 gm were obtained from a commercial hatchery for the present study. They were divided into 3 equal groups (T₁, T₂ and C) of 20 each to determine clinicopathological effects of induced sub-acute arsenic toxicity. Birds were housed in electrically heated thermostatically controlled metal cages throughout the experimental period. All the birds were fed with a commercial chick starter ration containing 22% protein and 3000 Kcal ME/Kg up to 3 weeks, followed by a finisher ration having 19% protein and 2900 Kcal ME/Kg, formulated to meet nutrient requirement as recommended by NRC (1999). Feed and water were provided *ad libitum* throughout the study.

About 1/10th of acute LD₅₀ i.e., 16.25 mg/kg body weight of arsenic trioxide (Loba Chemie Pvt. Ltd, Colaba, Mumbai, India) was dissolved in 0.2 ml of deionized water and fed orally with the help of a specially prepared syringe to each birds of treatment groups (T₁ and T₂) consisting of 20 birds daily starting from day-old to 6 weeks or until death. Birds of T₂ group were also provided with ascorbic acid 250 mg/kg b.wt. (Khan *et al.*, 2013) from day-old to 6 weeks or until death. Twenty birds of other group (C) received only the deionized water, serving as control.

All the experimental birds (T₁, T₂ and C) were observed daily for the development of clinical signs of toxicity including mortality (if any). Feed consumption was evaluated on a group basis every other day by weighing and weekly average was determined, starting from 1 to 6 weeks of age. The initial and weekly body weights were analyzed for the same periods to measure the effects of arsenic toxicity and its alleviation with ascorbic acid (Vit. C) on the growth of birds.

Blood samples (10 ml) were collected at 3 and 6 weeks of age from the wing vein of birds of all the groups (T₁, T₂ and C) using sterilized disposable syringe for hematological and biochemical studies. Blood (4 ml) was immediately transferred to heparinised tube (heparin @ 1 mg/ml) and mixed by gentle horizontal rotation. Blood smears were prepared in duplicate on grease free glass slides from the fresh blood collected. Remaining 6 ml of blood transferred to a sterile wide mouth glass test tube without any anticoagulant was used for serum separation for biochemical analysis. Blood samples with anticoagulant were used for estimation of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte and leucocyte count (TEC and TLC) and differential leucocyte count (DLC). The separated serum was utilized for estimation of total serum proteins and albumin by Biuret method, serum glucose, serum creatinine, aspartate transaminase and alanine transaminase (AST and ALT) by using SPAN diagnostic kits. All the estimated data were analysed by analysis of variance and the significant result were shown by difference in superscripts with respective means (Snedecor and Cochran, 1967).

Results and Discussion

Clinical signs

There was no apparent clinical manifestation in the arsenic induced chickens upto 2 weeks of age. From the beginning of the third week, mild signs of toxicity consisting of partial loss of appetite and body weight, mild diarrhoea and mild depression were observed. These were followed by loss of appetite, weakness, progressive loss of body weight, profuse watery diarrhoea, pale combs, ruffled feather and staggering gait in both the arsenic induced (T₁) and As +

ascorbic acid treated birds (T₂). But the intensity of all the clinical manifestations were less in birds in the ascorbic acid ameliorated group (T₂). The birds were dull and depressed. Some birds also showed open mouth breathing. All these signs were partially ameliorated in the birds (T₂ group) with the treatment of ascorbic acid. None of the control birds had any clinical signs of toxicity and mortality.

Feed consumption and body weight

The results relating to the feed consumption of all birds of all the groups (C, T₁ and T₂) at weekly intervals are presented in Table 1. From the table, it is clear that arsenic treatment caused reduction in feed intake in arsenic induced birds, though ascorbic acid supplementation had caused mark ameliorative effect in the consumption of feed. The feed consumption rate was significantly ($P<0.01$) lower in arsenic induced broiler birds on 4, 5 and 6 weeks post treatment, when compared with the mean values of control birds. Whereas, feed consumption rate was significantly lower ($P<0.01$) in As + ascorbic acid treated birds on 5 and 6 weeks post treatment than the control birds (Table 1). The results relating to the body weight of the control, arsenic induced and arsenic + ascorbic acid induced broiler chickens at weekly intervals are presented in Table 2. Body weights of arsenic induced broiler chickens were significantly ($P<0.05$, 0.01) lowered from 3 weeks onwards when compared with the control chickens (Table 2). The growth depression effects persisted throughout the experimental periods. There was approximately 17.04% body weight loss in sub-acute arsenic induced birds at 6 weeks than the control birds of the same age. The growth depression effects were partially ameliorated in the birds with the treatment of ascorbic acid. There was approximately

7.90% body weight loss in ascorbic acid + arsenic induced birds (T₂) at 6 weeks than the control birds of that age.

Haematological changes

Results of hematological findings are summarized in Table 3 which showed a significant (P<0.05 or P<0.01) decrease in Hb, PCV and TEC in arsenic induced broiler birds only or with ascorbic acid in contrast to control birds after 3 and 6 weeks of treatment. Birds of the T₂ group showed significant improvement when compared to the values of the birds of group T₁ but the values were lower than the values of the birds of the control group (C). A significant (P<0.01) decrease in TLC was also observed both after 3 and 6 weeks in arsenic induced birds when compared with the control. Birds of T₂ group showed significant improvement in the TLC values, though the values were lower than the control. Differential leucocyte count (DLC) showed a significant (P<0.01 or P<0.05) decrease in lymphocyte count in the arsenic treated birds but did not reveal any alteration in heterophil and basophil count, though there was an increasing trend in the monocyte and eosinophil count in the arsenic induced birds than those of control on 21 and 42 days post-treatment. The birds treated with ascorbic acid showed partially ameliorative effect on DLC values when compared to the values of the (T₁) group.

Biochemical changes

Results on bio-chemical changes in blood are presented in Table 4. The level of total serum protein and albumin declined significantly (P<0.01, 0.05) in the birds of both the treatment groups (T₁ and T₂) at 3 and 6 weeks post treatment. But the level of total serum protein and albumin in T₂ group was higher than the T₁ group and below the level of control group indicating

ameliorating action of ascorbic acid on total serum protein. Similarly, the serum globulin and A: G ratio decreased significantly (P<0.01, 0.05) in both the treatment and ameliorative group at 6 weeks post treatment. There was a significant (P<0.01, 0.05) increase in serum glucose in arsenic induced and As+ ascorbic acid treated birds at 3 and 6 weeks post treatment. The concentration of glucose (mg/dl) was 198.36^b±3.14 and 203.16^b±3.22 in arsenic treated birds in comparison to 185.78^a±3.28 and 186.89^a±2.78 in control group at 3 and 6 weeks post treatment respectively. The arsenic+ ascorbic acid treated birds (T₂) had glucose level (mg/dl) 188.23^b±2.96 and 123.89^b±3.18, respectively at 3 and 6 weeks post treatment. Serum creatinine (mg/dl) level increased significantly (P<0.01, 0.05) in arsenic treated birds in comparison to control birds both at 3 and 6 weeks post treatment indicating nephrotoxicity. The level of serum creatinine was also increased in the birds treated simultaneously with arsenic and ascorbic acid than the control birds but the mean values were lower than only arsenic treated birds indicating protective effects of ascorbic acid. The serum transaminase activities (AST, ALT) and alkaline phosphatase (AP) in arsenic treated birds (T₁) and As + ascorbic acid induced birds (T₂) was increased than those of control birds (C) on 21 and 42 days post-treatment. Ascorbic acid treatment showed its protective effect against arsenic induced toxicity in broiler birds as the values of different enzymes in the birds of T₂ group showed higher values than the control and lower values than the arsenic treated birds (Table 4).

Clinical signs

Khan *et al.*, (2013) also reported similar clinical signs in both arsenic induced and arsenic + ascorbic acid treated birds. The

observed clinical signs in the arsenic treated birds in present study corroborated with the findings of Niyogi (2009) and Mashkooor *et al.*, (2013). Arsenic has an affinity for the tissues rich in oxidative enzymes such as intestine, kidney and liver. The onset of gastrointestinal signs from 3 weeks onwards might be due to a local corrosive action of arsenic on gut and gastrointestinal tract. Arsenic 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 (Stanely *et al.*, 1994) and ruffled feathers (Vodela *et al.*, 1997). Respiratory distress had also been reported in arsenic treated goats (Patra *et al.*, 2013).

Effect on body weight and Feed consumption

Kerr *et al.*, (1963) observed reduction in feed intake in chickens induced orally with organic arsenical compound. On the other hand, an enhanced feed intake of adult white leghorn layers kept on the diet containing arsenic feed additive roxarsone @ 11, 22, 44 and 88 mg/kg body weight was reported by Chiou *et al.*, (1997). Such differences might be due to variation of arsenical compound and dosage of the compound used in the study.

The results clearly indicated that trivalent arsenic (As₂O₃) had growth depressing effect which confirmed the reports of Kerr *et al.*, (1963) in chickens, Shapiro *et al.*, (1992) in broiler chickens, Islam *et al.*, (2005) in Evans rats and Niyogi, (2009) in broiler birds. Khan *et al.*, (2013) reported partial ameliorating effect of Vitamin C or ascorbic acid on the body weight loss of arsenic

treated birds. Similar reduction in body weight gain and feed intake have also been recorded after oral administration of different levels of As in broiler chicks (Vodela *et al.*, 1997), dullness and depression, rough body coat with erected hairs, profound muscular weakness, and in coordination in goats at the dose rate of 75, 100, 125, 150 mg/kg sodium arsenate goat (Halder *et al.*, 2007).

In the present study, maximum growth depression effects of arsenic were observed on 5 and 6 weeks post treatment. Sub-acute arsenic poisoning caused severe diarrhoea causing fluid loss, loss of appetite and a gradual loss of general condition. Reduced appetite, frequent diarrhoea along with the damage of liver, kidney and gastro-intestinal tract due to irritant effect of arsenic might be played an important role in body weight loss in chronic toxicity.

Haematological changes

These results of a significant (P<0.05 or P<0.01) decrease in Hb, PCV and TEC in arsenic induced broiler birds also corroborated with the findings of Islam *et al.*, (2005) in Evans rats, Niyogi (2009) in arsenic induced broiler birds, Tanju and Madhuri (2013) and Mashkooor *et al.*, (2013) in arsenic poisoning in broiler chicks.

Khan *et al.*, (2013) also recorded the similar findings observed in the present study in the ascorbic acid ameliorated birds. The resultant leucopenia in this study simulated with the findings of Niyogi (2009) in arsenic induced broiler birds. The alteration in haematological parameters might have occurred due to direct suppressive effects of arsenic on haemopoietic system (including spleen and bone marrow) indicating the possibility of depression of bone marrow activity and spleen.

Table.1 Feed consumption (g) in control (C), arsenic treated (T₁) and arsenic + ascorbic acid induced (T₂) broiler chickens (Mean ± SE)

Age in Week(s)	Average feed consumption / birds/ day/ week (n=20)		
	Control	T ₁	T ₂
1	16.82 ^a ± 1.48	15.86 ^a ± 2.12	16.18 ^a ± 1.74
2	29.07 ^a ±1.96	26.38 ^a ±2.09	27.96 ^a ±1.21
3	56.14 ^a ±2.08	49.43 ^a ±1.96	53.64 ^a ±1.45
4	98.37 ^a ±3.72	86.49 ^b ±2.84	91.56 ^{ab} ±2.83
5	126.49 ^a ±5.26	107.74 ^b ±4.92	110.39 ^b ±5.14
6	168.92 ^a ±4.84	136.08 ^b ±4.45	154.28 ^c ±4.43

Means in a row having different superscripts differ significantly (p<0.01)

Table.2 Body weight (g) in control (C), arsenic treated (T₁) and arsenic + ascorbic acid induced (T₂) broiler chickens (Mean ± SE)

Age in Week(s)	Average body weight (g) of broiler chickens (n=20)			Percent of reduction in growth in birds of T ₁ Group	Percent of reduction in growth in birds of T ₂ Group
	Control (C)	T ₁	T ₂		
1	116.6 ^a ± 3.14	111.4 ^a ± 3.22	113.8 ^a ± 3.24	4.44	2.44
2	236.8 ^a ±3.56	224.8 ^a ±4.42	229.6 ^a ±4.18	5.06	3.04
3	512.6 ^a ±4.56	476.2 ^b ±6.12	492.4 ^{ab} ±5.25	7.10	3.90
4	872.2 ^a ±5.84	779.5 ^b ±8.14	828.4 ^c ±7.13	10.6	5.02
5	1264.7 ^a ±6.58	1086.6 ^b ±8.92	1176.2 ^c ±7.98	14.08	6.99
6	1722.4 ^a ±12.08	1428.8 ^b ±7.98	1586.2 ^c ±9.94	17.04	7.90

Means in a row having different superscripts differ significantly (p<0.01)

Table.3 Haematological changes in control, subacute arsenic treated and arsenic + ascorbic acid induced broiler birds at 3 weeks and 6 weeks post treatment. (Mean values of 20 estimations ± S.E.)

	3 weeks			6 weeks		
	Control (C)	T ₁	T ₂	Control (C)	T ₁	T ₂
Haemoglobin (g%)	10.60 ^a ±0.11	8.80 ^b ±0.14	9.90 ^b ±0.20	10.80 ^a ±0.16	7.90 ^b ±0.14	10.10 ^b ±0.20
Packed cell volume.	32.30 ^a ±0.38	27.40 ^b ±0.28	30.60 ^b ±0.24	32.40 ^a ±0.24	25.10 ^b ±0.28	31.80 ^b ±0.25
Total erythrocyte count (x 10 ⁶ /cu.mm)	3.58 ^a ±0.17	2.94 ^b ±0.14	3.42 ^b ±0.12	3.60 ^a ±0.19	2.61 ^b ±0.16	3.46 ^b ±0.18
Total leucocyte count (x 10 ³ /cu.mm)	26.48 ^a ±0.26	24.88 ^b ±0.34	25.92 ^b ±0.31	26.36 ^a ±0.42	23.39 ^b ±0.35	25.98 ^b ±0.45
Heterophils	32.00 ^a ±0.60	34.78 ^b ±0.64	32.78 ^b ±0.52	32.5 ^a ±0.49	34.56 ^b ±0.64	32.67 ^b ±0.61
Lymphocytes	61.00 ^a ±0.85	55.56 ^b ±0.58	59.25 ^b ±0.82	59.5 ^a ±0.86	50.50 ^b ±1.16	58.45 ^b ±1.27
Monocytes	4.5 ^a ±0.40	6.95 ^{ab} ±0.46	5.25 ^{ab} ±0.42	5.35 ^a ±0.66	9.0 ^b ±0.50	6.5 ^b ±0.55
Eosinophils	1.78±0.38	2.16±0.46	1.87±0.38	1.47±0.41	4.43±0.72	1.67±0.33
Basophils	0.72±0.24	0.68±0.22	0.85±0.31	1.18±0.27	1.51±0.36	0.71±0.38

Means in a row having different superscripts differ significantly (P<0.05 or 0.01)

Table.4 Biochemical alterations in control, arsenic induced and arsenic + ascorbic acid treated broiler birds after 3 weeks and 6 weeks post treatment

	Mean± S.E. (Average of 20 estimations)					
	After 3 weeks post treatment			After 6 weeks post treatment		
	Control (C)	T ₁	T ₂	Control (C)	T ₁	T ₂
Serum glucose (mg/dl)	185.78 ^a ±3.28	198.36 ^b ±3.14	188.23 ^b ±2.96	186.89 ^a ±2.78	203.16 ^b ±3.22	123.89 ^b ±3.18
Total serum protein (g/dl)	4.64 ^a ±0.06	3.96 ^b ±0.08	4.28 ^b ±0.06	4.56 ^a ±0.04	3.71 ^b ±0.07	3.88 ^b ±0.04
Serum Albumin (g/dl)	2.54 ^a ±0.03	2.12 ^b ±0.02	2.31 ^b ±0.02	2.48 ^a ±0.04	1.96 ^b ±0.06	2.08 ^b ±0.03
Serum Globulin	2.10 ^a ±0.02	1.84 ^b ±0.04	1.97 ^{ab} ±0.04	2.08 ^a ±0.03	1.75 ^b ±0.05	1.80 ^b ±0.02
A:G	1.20 ^a ±0.01	1.15 ^b ±0.02	1.17 ^{ab} ±0.02	1.19 ^a ±0.04	1.12 ^b ±0.03	1.15 ^b ±0.05
Serum creatinine (mg/dl)	1.07 ^a ±0.04	1.92 ^b ±0.08	1.19 ^b ±0.09	1.1 ^a ±0.03	2.18 ^b ±0.07	1.21 ^b ±0.06
Aspartate aminotransferase (IU/L)	164.38 ^a ±3.18	179.82 ^b ±3.76	176.08 ^b ±2.28	166.10 ^a ±3.24	204.28 ^b ±4.39	194.18 ^b ±3.68
Alanine aminotransferase (IU/L)	24.08 ^a ±1.26	33.89 ^b ±1.31	26.94 ^b ±1.18	23.93 ^a ±1.26	40.08 ^b ±1.19	33.18 ^b ±1.48
Alkaline phosphatase (K.A. Units)	28.06 ^a ±1.24	36.42 ^b ±1.27	31.02 ^b ±1.08	28.92 ^a ±1.34	46.54 ^b ±1.78	35.68 ^b ±1.46

Means in a row having different superscripts differ significantly (P<0.01, P< 0.05)

The reduction might be due to its effect on haem and porphyrin synthesis. Arsenic causes inhibition of blood aminolevulinic acid dehydratase (ALAD) activity resulting in disturbed haem synthesis pathway (Gupta and Flora, 2006). Binding ability of arsenic to Hb led to inhibition of haem synthesis and other parameter were also disturbed because these are dependent on each other. In addition arsenic also acts as a capillary poisons and increases the fragility of red blood cells (Biswas *et al.*, 2000 and Radostits, 2003).

Biochemical changes

Hypoproteinemia due to chronic arsenic toxicity was also reported in cockerels (Sajan, 2006), in rats (Islam *et al.*, 2005), in goats (Biswas *et al.*, 2000) and in broiler chicks (Khan *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 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 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). The hypoproteinemia observed in the present study might have resulted on account of hepatic dysfunction or liver

damage, increased catabolism and excess renal elimination of protein.

Arsenic induced hyperglycemia observed in the present study in the broiler chickens corroborated with the findings of Biswas *et al.*, (2000) in goats and Sajan (2006) in cockerels. Hyperglycemia in the arsenic treated birds was possibly resulted from enhanced enzymic activity of neoglucogenesis due to activation of sympathetic component and release of adrenaline from adrenal medulla and secretion of glucocorticoids from adrenal cortex under stress condition (Kaneko, 1980) produced as a toxic effect of arsenic in the broiler chickens.

The increased level of creatinine found in the present study was an indicator of its nephrotoxic effect in the treated birds. Sajan (2006) also reported the elevated level of serum creatinine in arsenic induced cockerels. The arsenic is known to cause degenerative changes in the renal capillary, glomeruli and renal tubules (Kleinfeld, 1980). Elevated level of creatinine indicated the signs of renal failure (Padmaja *et al.*, 2009).

In this study, the increase in transaminase activities and alkaline phosphatase activity in arsenic treated birds confirmed the reports of Chiou *et al.*, (1997) in white leghorn chicken, Jadhav *et al.*, (2007) and Ramanathan *et al.*, (2003) in rats, Biswas *et al.*, (2000) in goats, Sajan (2006) in cockerels, Rana *et al.*, (2007) in cattle, Halder *et al.*, (2009) in birds, Niyogi (2009) in broiler birds and Tanju and Madhuri (2013) in broiler chicken. Khan *et al.*, (2013) observed the similar findings of serum enzymatic activities in broiler chicks treated with arsenic alone or with ascorbic acid. The increase in the activity of transaminases is known to be the indicator

of degenerative changes in organs or tissues like liver and myocardium (Kaneko, 1980). Increased levels of transaminases and AP activities are known to occur in a wide range of diseases of liver like cholestasis, biliary obstruction and hepatic necrosis (Tennant, 1997). Increased levels of ALT and AST are indicator of As-hepatotoxicity (Roy and Bhattacharya, 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). The elevation of serum enzymic activity in the present study is attributed to arsenic induced hepatic damage/ or necrosis as confirmed from histopathological observations.

In the current study, ameliorative effects of ascorbic acid 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. Ascorbic acid is a water-soluble antioxidant. Ascorbic acid speeds up the bowel transit time to help the elimination of heavy metals through the intestines. Free sulfhydryl groups (-SH) group of ascorbic acid 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).

In conclusion, arsenic led to toxicity in broiler birds. Severe signs of depression including less feed intake and poor body weight gain were noted in birds treated with arsenic. Haematological and biochemical indices, gross and histopathological lesions also indicated that arsenic was toxic to the broiler birds. Notwithstanding, As-induced

toxic effects can be partially nullified by treatment with ascorbic acid.

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