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AMELIORATIVE EFFECT OF VITAMIN-C ON LEAD-INDUCED BIOCHEMICAL ALTERATIONS IN THE SERUM OF BROILERS

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ABSTRACT

The present study was aimed to elucidate the ameliorative effect of vitamin C on biochemical alterations in broilers treated with lead acetate. One hundred and eight (1 day old) broiler chicks were reared for 2 weeks and randomly divided into 6 equal groups. Group I birds served as control. Group II and III birds were given lead acetate @ 250 and 500 ppm, respectively, while group IV and V were supplemented with vitamin C @ 40 mg L^{-1} water in addition to lead acetate @ 250 and 500 ppm, respectively. Group VI birds were supplemented with vitamin C @ 40 mg L^{-1} water in their normal feed. Total serum protein analysis revealed a significant decrease in lead treated birds while as AST, ALT, blood urea nitrogen (BUN), creatnine and uric acid revealed a significant increase but the values were modulated in vitamin C supplemented birds.

Keywords: Broilers, **l**ead acetate, serum biochemistry, vitamin C

INTRODUCTION

The excessive amount of pollutants including heavy metals in animal feed and feed stuffs are mostly the consequences of anthropogenic activities (Aboul-Enein *et al*., 2010). Traces of lead are present in many rocks which find way into soil and water and hence enter food chain even in remote places where there is no use of this metal or its compounds (El-Beltagi and Mohamed, 2010). The toxicity of lead depends on its chemical form, route of administration and the amount administered to the animal (Baht and Moy, 1997). The absorbed lead is conjugated in liver and passed to kidneys where a small quantity is excreted with urine and the rest accumulates in various body organs and interferes with their function (Taib *et al*., 2004). Lead has a potential to induce oxidative stress and act as a catalyst in oxidative reactions of biological macromolecules. Therefore, toxicities associated with this metal might be due to oxidative tissue damage (Gurer and Ercal, 2000). Free radical scavengers (antioxidants) play an important role in prevention of per-oxidative tissue damage. Ascorbic acid is a well known antioxidant vitamin involved in several biochemical processes in biological systems. It breaks the chain of lipid peroxidation in cell membranes and scavenges free radicals such as reactive oxygen species (Carr and Frei, 1999). The present study was aimed to assess the effects of lead acetate on various blood biochemical parameters in broilers and assess the role of vitamin C as an ameliorative agent.

MATERIALS AND METHODS

One day old birds used in the present study were procured from Paras Hatchery, Jammu (India) and maintained under standard environmental conditions with *ad lib* feed and water in FVSc &AH, R.S. Pura Jammu from February to April 2011. The animals were treated humanely during the study period and the work was approved by the Institutional Animal Ethics Committee vide No.AU/FVSc/C-11/2470-72 on ethical standards in animal experimentation. After two weeks of rearing in brooder the chicks were randomly divided into six groups with 18 chicks in each group. All birds were fed normal feed. Chicks in group I served as control. Group II and III chicks were provided with lead acetate @ 250 and 500 ppm in feed, respectively. Group IV and V chicks were provided with lead acetate @ 250 and 500 ppm in feed, respectively, along with vitamin C @ 40 mg L^{-1} drinking water. Group VI chicks were provided with vitamin C @ 40 mg L^{-1} drinking water.

Clean tap water was provided to all the groups for drinking purpose. Blood samples (3-4 mL) were collected from wing vein from randomly selected six birds of each group at 2, 4 and 6 weeks post-exposure in dry clean and sterilized test tubes without the addition of anticoagulant and allowed to clot at room temperature. Serum was separated and preserved at -20˚C till analyzed for estimation of various parameters such as total serum protein by biuret method (Ubiali *et al.*, 2004), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine by DNPH colorimetric method (Henry, 1974) and serum urea nitrogen (SUN) and blood urea nitrogen (BUN) by kinetic enzymatic method (Sampson *et al.*, 1980) using standard Diagnostic kits of Erba Mannheim brand, Transasia Bio-Medicals, Solan H.P. (India) and Recon Diagnostics, Vadodara (India). Lead acetate (99.9%) and ascorbic acid (99.9%) from Hi-Media Mumbai (India) were used for experimentation. Standard statistical procedures were followed and data subjected to analysis of variance and significance tested using Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The mean concentration of total serum proteins (TSP) in birds fed with lead in dose and duration dependant manner were significantly lower ($P_{0.05}$) than control group birds. Lower TSP in lead toxicosis has been reported in rats, broilers and buffalo-calves (Brar *et al.*, 1997). Hypoproteinemia in birds may be due to renal tubular dysfunction (Kanakoudis *et al.*, 1988), liver damage (Youssef *et al.*, 1998), increased hepatic non-protein sulfhydryl concentration (Knowles and Donaldson, 1990). In the present study, the gross and histopathological examination of liver and kidney sections of affected birds revealed the damage to liver and renal tissues. Similar findings have been reported in poultry and cross-bred calves (Madej *et al.*, 1988). The decrease in feed consumption and greenish diarrhoea might be the other reason for decreased TSP in lead treated birds. Improvement in TSP in vitamin C supplemented birds might be due to decreased hepatotoxic and nephrotoxic effects (Patra and Swarup, 2004) which was evident from the reduced histopathological changes in sections of liver and kidneys in the present study.

The mean AST level in lead-fed groups was significantly higher throughout the experiment particularly in group III animals with lead @ 500 ppm kg^{-1} feed in comparison to control birds in dose and duration dependent manner. The finding are in confirmation to the reports of Madej *et al*. (1988) and Baxla *et al*. (2013) in case of cross bred calves and poultry, respectively. Comparatively low AST in lead-fed and vitamin C supplemented birds could be ascribed to the protective effect of vitamin C supplementation in lead toxicosis. Similarly the mean alanine aminotransferase (ALT) activity in lead-fed groups was significantly higher than control group. This is in agreement with earlier works in crossbred calves and sheep (Zong Ping *et al.*, 1997). Comparatively lesser activity

of ALT was observed in lead-fed and vitamin C supplemented groups than only lead-fed groups which may be ascribed to the protective effect of vitamin C as it has the ability of binding with lead thus rendering it less harmful. Patho-morphological changes observed in liver, kidneys and heart also support the increased levels of ALT and AST in the present study. AST is a mitochondrial enzyme but not liver-specific with increased serum concentration which revealed muscular degeneration (skeletal and cardiac) and hepatocellular damage.

The mean serum blood urea nitrogen (BUN) and serum creatinine concentrations showed significant increase in birds fed with lead acetate than in control group. Similar findings have been reported in poultry, rats, buffalo calves and goats (Haneef *et al*., 1998; Elgaml *et al.,* 2015). Increased BUN and serum creatinine levels, following the lead administration, in present study could be due to the accelerated rate of protein catabolism and/or kidney damage and altered kidney function due to the lead accumulation (Beyer *et al*., 1988). Histo-pathological observations of kidneys showed damage which supported our findings.

The mean serum uric acid concentrations showed significantly increase in birds fed with lead acetate than in control birds. Similar findings have been reported by Suleman *et al.* (2011) in broiler chicks and Azab *et al.* (2013) in albino mice. The increased uric acid in the present study reflects impaired kidney function which is evident from the histopathological alterations of kidney. Apart from this, there was also increase in protein catabolism.

Lead toxicity is a potential problem in poultry birds. It alters their biochemical and enzymatic status hence affects their health and productivity. Vitamin C ameliorates the negative effects of lead toxicity in dose dependent manner thus increases their productive potential.

Table 1: Effect of lead acetate and vitamin on various biochemical parameters in birds (n = 6)

Weeks post-exposure	Control	Lead acetate (ppm)		Lead acetate (ppm) + Vitamin C @ 40 mg L^{-1}		Vitamin C @ 40 mg L^{-1}
		250	500	250	500	
Total serum protein (TSP) concentration (g dL^{-1})						
2	2.61 ± 0.06^{a}	2.60 ± 0.05^{a}	2.30 ± 0.16^{b}	2.60 ± 0.05^{a}	2.60 ±0.05^{a}	2.61 ± 0.07^{a}
4	3.02 ± 0.09^{a}	2.31 ± 0.16^{b}	2.26 ± 0.31^{b}	2.86 ± 0.06^{a}	2.30 ±0.15^{b}	3.03 ± 0.08^{a}
6	3.37 ± 0.41^{a}	2.41 ± 0.16^{b}	2.29 ± 0.15^{b}	3.35 ± 0.28^{a}	2.35 ±0.19^{b}	3.39 ± 0.40^{a}
AST concentration (IU mL^{-1})						
2	203.33 ± 1.05^{b}	206.67 ± 2.47^{b}	230.83 ± 2.7^{a}	203.33 ± 1.52^{b}	208.83 ± 5.29^{b}	199.67 ± 2.26^{b}
4	200.00 ± 4.65^{b}	207.50 ± 4.03^{b}	255.00 ± 1.82^{a}	206.83 ± 3.16^{b}	253.00 ± 1.91^{a}	199.10 ± 3.20^{b}
6	198.83 ± 3.50^{b}	250.83 ± 2.00^{a}	260.00 ± 2.58^{a}	209.83 ± 5.47^{b}	254.00 ± 7.18^{a}	197.50 ± 2.14^{b}
ALT concentration (IU mL^{-1})						
2	4.53 ± 0.29^{a}	4.66 ± 0.18^{a}	4.72 ± 0.25^{a}	4.65 ± 0.22^{a}	4.71 ± 0.24^{a}	4.50 ± 0.28^{a}
4	4.45 ± 0.29^{a}	4.68 ± 0.18^{a}	4.79 ± 0.30^{a}	4.57 ± 0.28^{a}	4.74 ± 0.32^{a}	4.44 ± 0.29^{a}
6	4.18 ± 0.44^{a}	6.11 ± 0.57^{b}	6.23 ± 0.62^{b}	4.60 ± 0.23^{ab}	6.18 ± 0.74^{b}	4.14 ± 0.45^{a}
Blood urea nitrogen (BUN) (mg dL^{-1})						
2	0.62 ± 0.05^{a}	0.65 ± 0.06^{a}	0.69 ± 0.08^{a}	0.66 ± 0.05^{a}	0.67 ± 0.07^{a}	0.62 ± 0.04^{a}
4	0.68 ± 0.05^{a}	0.75 ± 0.14^{b}	0.78 ± 0.07^{b}	0.69 ± 0.14^{a}	0.77 ± 0.13^{b}	0.67 ± 0.07^{a}
6	0.71 ± 0.07^{a}	0.82 ± 0.15^{b}	0.85 ± 0.08^{b}	0.80 ± 0.09^{a}	0.83 ± 0.15^{b}	0.71 ± 0.09^{a}
Serum creatinine (mg dL)						
2	0.37 ± 0.05^{a}	0.39 ± 0.06^{a}	0.49 ± 0.08^{a}	0.38 ± 0.08^{a}	0.04 ± 0.07^{a}	0.35 ± 0.04^{a}
4	0.41 ± 0.05^{a}	0.75 ± 0.14^{b}	0.88 ± 0.07^{b}	0.62 ± 0.14^{a}	0.77 ± 0.14^{a}	0.39 ± 0.07^{a}
6	0.48 ± 0.07^{a}	0.89 ± 0.15^{b}	0.94 ± 0.08^{b}	0.78 ± 0.09^{a}	0.87 ± 0.15^{a}	0.46 ± 0.09^{a}
Serum uric acid (mg dL^{-1})						
2	3.05 ± 0.06^{a}	3.15 ± 0.06^{a}	3.28 ± 0.08^{a}	3.09 ± 0.07^{a}	3.12 ± 0.06^{a}	3.00 ± 0.04^{a}
4	3.07 ± 0.07^{a}	3.48 ± 0.07^{b}	3.69 ± 0.06^{b}	3.28 ± 0.09^{a}	3.35 ± 0.07^{a}	3.02 ± 0.06^{a}
6	3.08 ± 0.07^{a}	3.70 ± 0.09^{b}	3.89 ± 0.08^{b}	3.58 ± 0.07^{a}	3.69 ± 0.07^{b}	3.02 ± 0.06^{a}

Mean values within row with different superscripts differ significantly (P< 0.05)

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