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Effect of Dietary Lead Exposure on Hematological Parameters and Their Alleviation by Antioxidants in Broilers

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Abstract

A total number of 126 day old broiler chicks were randomly assigned into 6 treatments groups A, B, C, D, E and F. The birds of group A were kept as control, while group B received lead acetate @ 200 mg kg^{-1} alone, whereas, birds of group C, D, E and F received Lead acetate @ 200 mg kg^{-1}+Ascorbic acid @ 200 mg kg^{-1}, lead acetate @ 200 mg kg^{-1} along with α-tocopherol @ 100 mg kg^{-1}+Selenium @ 0.1 mg kg^{-1}, Lead acetate @ 200 mg kg^{-1}+DL-methionine @ 100 mg kg^{-1} and Lead acetate @ 200 mg kg^{-1}+methanolic extract of *Cissus quadrangularis* (CQE) @ 400 mg kg^{-1} respectively. In group-B TEC, hemoglobin and MCV declined significantly ($p<0.05$) as compared to control on day 21. At day 42 significant ($p<0.05$) decrease in Hb, PCV and MCV was recorded in lead treated group. Treatment with Vit-E and Se (group-D) was found effective in restoration of TEC and Hb. Evaluation of leucocyte parameters revealed no significant ($p<0.05$) difference in total and differential leucocytic count on day 21 and 42. However, significant ($p<0.05$) increase in lymphocyte count was recorded in lead exposed group as compared to other groups. Vit-E @ 100 mg kg^{-1} and Se @ 0.1 mg kg^{-1} having highest efficacy against lead intoxication followed by Vitamin-C @ 200 mg kg^{-1}, DL-methionine @ 100 mg kg^{-1} and methanolic extract of CQE @ 400 mg kg^{-1} to alleviate lead toxicosis upto the level of 200 mg kg^{-1} in broiler diet.

Keywords: Lead, hematological parameters, antioxidants, broilers, *Cissus quadrangularis*

1. Introduction

Lead a potent heavy environmental pollutant is naturally occurring elements that has been mobilized and redistributed to a large extent in the environment by industrialization and urbanization process. Berg et al. (1980) stated that lead contamination of environment due its prolonged exposure and slow rate of elimination (Demichele, 1984; Ercal et al., 2001). Lead is a common source of poisoning in domestic animals all over the world (Khan et al., 2008). A good source of lead contamination of poultry feed is bone and blood meals, majority of which comes from cattle. However, in cattle, highest lead accumulation has been reported to occur in bones (Heaney, 2000). A main effect of lead poisoning is anemia, which consequences from inhibition of the heme synthesizing enzymes with concurrent elevation of protoporphyrin (Lee, 1981). This statement was further buttressed by Osweiler (1996) who reported that lead slows down haemosynthesis through inhibition of enzymes. Ascorbic acid (AA) is a well-known antioxidant vitamin involved in several biochemical processes in biological systems. This vitamin breaks the chain of lipid peroxidation in cell membranes and scavenges free radicals such as reactive oxygen species (Carr & Frei, 1999; Kucuk et al., 2003). Lead has reached to such a harmful level that it can affects the growth, productivity and health of poultry as well as animals due to cumulative poisoning. But poultry has not been studied to the desired extent in comparison with other farm animals. With a view to study the effect of lead on hematological parameters of broilers, present investigation was under taken. We also aimed to determine whether the effects of lead, attributed on hematological parameters, could be reversed by adding antioxidants.

2. Materials and Methods

The present study was undertaken in the Department of Veterinary Medicine, College of Veterinary Science and A.H., Anjora, Durg (Chhattisgarh), India in the year of 2010. In this study 126 day old broiler chicks (Ven-Cobb strain, IB Group, Rajnandgaon, Chhattisgarh) were randomly divided into six treatment groups (n=21) as A, B, C, D, E and F and all treatments were given to chicks on the basis of kg^{-1} basal diet daily for the period of 42 days. The birds of group-A were kept as healthy

control received only basal diet, birds of group- B received lead acetate alone @ 200 mg kg^{-1} where as birds of group-C received lead acetate @ 200 mg kg^{-1} along with ascorbic acid @ 200 mg kg^{-1}, group-D got vit-E @ 100 mg kg^{-1} and Se @ 0.1 mg kg^{-1}, group-E received DL-methionine @ 100 mg kg^{-1} and methanolic extract *Cissus quadrangularis* (CQE) @ 400 mg kg^{-1} was given to group-F respectively for 42 days. About 5–6 ml of blood sample were collected aseptically from metatarsal vein of birds by using 22 gauge needle in the sterilized, neat and clean plastic vials containing heparin anticoagulant @ 0.1 ml 10 ml^{-1} of blood (Heparin as a 1% solution 1,000 USP units ml^{-1}) on 21st and 42nd day of experiment in the morning hours from 6 broilers that were randomly chosen from each group. Haematology was performed to evaluate the effect of lead on blood parameters. The following blood parameters were evaluated at 21 and 42 days by using Automated Haematology Blood Cell Counter of Melet Schloesing Laboratories of Sussi France (MS9). Total Erythrocyte count (TEC in Million μl^{-1}), Haemoglobin (Hb in g % or g dl^{-1}), Packed cell volume (PCV in %), Mean corpuscular value (MCV in fl), Mean corpuscular haemoglobin (MCH in pg), Mean corpuscular haemoglobin concentration (MCHC in g dl^{-1}), Total Leukocyte count (TLC in Thousand μl^{-1}), Differential Leukocyte count (DLC in %).The data were analyzed statistically using analysis of variance with one way classification followed by Dancan's Multiple Range Test (DMRT) to find out the significance of difference between mean values of different groups as per the procedure outlined by Snedecor and Cochran (1994).

3. Results and Discussion

3.1. Haematological changes

The results of haematological profile are summarized in the Table 1 for day 21 and Table 2 for day 42 and comparison between day 21 and 42 are presented in Table 3.

3.1.1. Total erythrocytic count (TEC)

The obtained values in group A (control), B, C, D, E and F were 1.28±0.04, 0.93±0.01, 1.42±0.04, 1.16±0.07, 1.14±0.06 and 1.23±0.67 10^6 μl^{-1} respectively on 21 days, while on day 42 TEC values in group A (control), B, C, D, E and F was 2.30±0.11, 1.82±0.11, 2.06±0.30, 2.45±0.13, 2.44±0.26 and 2.44±0.27 10^6 μl^{-1} respectively. A significant higher ($p<0.05$) value of RBC count was recorded in control and other treatment group as compared to lead treated groups on day 21. However comparison among group C, D, E and F revealed, significant ($p<0.05$) difference in the RBC count of group C from the others treatment groups but group D, E and F was not significantly ($p>0.05$) comparable to each other. Though on day 42 TEC values were significantly ($p<0.05$) increased on day 21 onwards in all groups but at the end of the study lead treated group showed no significant ($p>0.05$) changes from the control (group A) and other treatment groups. RBC count of different treatment group was comparable to each other on day 42.

Table 1: Effect of lead and different treatments on haematological profile in broiler chickens on day 21 (n=6)

Haematological parameters	Various treatment groups showing mean±SE values					
	A	B	C	D	E	F
TEC ($10^6\mu l^{-1}$)	1.28 ± 0.04^{bc}	0.93 ± 0.01^{a}	1.42 ± 0.04^{c}	1.16 ± 0.07^{b}	1.14 ± 0.06^{b}	1.23 ± 0.67^{b}
Hb (gdl^{-1})	9.77 ± 0.36^{b}	7.43 ± 0.13^{a}	10.44 ± 0.23^{bc}	11.70 ± 1.29^{c}	9.60 ± 0.09^{b}	9.33 ± 0.30^{b}
PCV (%)	14.47 ± 0.41^{bc}	9.83 ± 0.15^{c}	15.43 ± 0.29^{c}	13.27 ± 0.79^{b}	13.22 ± 0.62^{b}	13.90 ± 0.71^{bc}
MCV (fl)	113.09 ± 0.61^{bc}	105.35 ± 1.02^{a}	109.25 ± 2.11^{ab}	114.28 ± 1.64^{bc}	116.33 ± 3.44^{c}	113.13 ± 1.25^{bc}
MCH (pg)	76.41 ± 2.11^{a}	79.53 ± 0.79^{a}	74.91 ± 1.80^{a}	101.64 ± 11.11^{b}	85.26 ± 4.34^{a}	76.51 ± 2.30^{a}
MCHC (g dl^{-1})	67.54 ± 1.60^{a}	75.53 ± 1.04^{a}	68.74 ± 2.42^{a}	89.46 ± 10.73^{b}	73.26 ± 3.10^{a}	67.66 ± 2.10^{a}
TLC (10^3 dl^{-1})	139.88 ± 5.49^{bc}	146.91 ± 4.00^{c}	127.91 ± 1.98^{ab}	122.22 ± 10.66^{a}	138.41 ± 0.61^{bc}	140.04 ± 2.50^{bc}
Lymphocyte(%)	33.10 ± 1.84^{a}	52.47 ± 1.28^{c}	31.27 ± 0.17^{a}	41.87 ± 3.62^{b}	35.77 ± 0.80^{a}	35.27 ± 02.28^{a}
Monocyte (%)	6.60 ± 0.27^{ab}	7.06 ± 0.11^{bc}	6.37 ± 0.09^{a}	7.30 ± 0.15^{c}	7.00 ± 0.10^{bc}	6.67 ± 0.21^{ab}
Heterophil (%)	59.16 ± 2.24^{bc}	54.63 ± 0.97^{ab}	60.90 ± 0.43^{c}	49.70 ± 3.22^{a}	55.23 ± 0.65^{abc}	56.56 ± 2.32^{bc}
Eosinophil (%)	1.00 ± 0.36^{a}	1.67 ± 0.33^{a}	1.17 ± 0.31^{a}	1.00 ± 0.51^{a}	1.33 ± 0.42^{a}	1.17 ± 0.31^{a}
Basophil (%)	0.50 ± 0.22^{a}	0.67 ± 0.21^{a}	0.33 ± 0.21^{a}	0.50 ± 0.22^{a}	0.67 ± 0.21^{a}	0.56 ± 0.21^{a}

a-c Mean values bearing different superscripts within a row differ significantly ($p<0.05$)

3.1.2. Haemoglobin (Hb)

Mean value of hemoglobin was significantly higher ($p<0.05$) in control (9.77±0.36 g dl^{-1}) and other treatment group C (10.44±0.23 g dl^{-1}), D (11.70±1.29 g dl^{-1}), E (9.60±0.09 g dl^{-1}) and F (9.33±0.30 g dl^{-1}) as compared to lead treated group B (7.43±0.13 g dl^{-1}) on day 21. Comparison among the different treatment groups showed highest Hb level in group D followed by group C, A, E and F. On day 42 lead treated group (8.90±0.22 g dl^{-1}) showed significant ($P<0.05$) reduction in Hb as compared to group A (13.30±0.64 g dl^{-1}), D (14.66±0.90 g dl^{-1}) and E (15.60±2.09 g dl^{-1}) and non-significant ($p>0.05$) reduction from group C (12.67±1.71 g dl^{-1}) and F (12.71±0.98 g dl^{-1}).

Table 2: Effect of lead and different treatments on haematological profile in broiler chickens on day 42 (n=6)

Haematological parameters	Various treatment groups showing mean±SE values					
	A	B	C	D	E	F
TEC (10^6 µl^{-1})	2.30±0.11^a	1.82±0.11^a	2.06±0.30^a	2.45±0.13^a	2.44±0.26^a	2.44±0.27^a
Hb (g dl^{-1})	13.30±0.64^b	8.90±0.22^a	12.67±1.71^{ab}	14.66±0.90^b	15.60±2.09^b	12.71±0.98^{ab}
PCV (%)	24.86±1.38^b	16.13±0.52^a	22.28±3.09^{ab}	26.42±1.39^b	26.30±2.99^b	26.09±2.81^b
MCV (fl)	107.87±1.60^b	89.91±3.73^a	108.87±2.30^b	107.56±0.86^b	107.35±1.42^b	107.13±0.47^b
MCH (pg)	57.83±0.61^{ab}	49.75±2.49^a	62.06±2.41^b	59.58±0.78^{ab}	63.03±1.79^b	55.36±7.00^{ab}
MCHC (g dl^{-1})	53.65±0.42^a	55.29±1.20^a	56.96±1.56^a	55.41±0.82^a	58.69±1.37^a	51.63±6.41^a
TLC (10^3 dl^{-1})	98.27±1.37^{ab}	83.97±2.36^a	106.59±9.94^{ab}	119.46±0.80^b	102.25±10.54^{ab}	94.56±10.70^a
Lymphocyte(%)	22.83±1.61^a	20.65±0.66^a	27.63±3.58^a	28.63±0.48^a	23.00±2.76^a	22.46±3.10^a
Monocyte (%)	5.13±0.32^a	6.13±0.21^a	5.70±0.64^a	5.16±0.31^a	5.17±0.53^a	4.75±0.70^a
Heterophil (%)	70.54±1.42^a	71.86±1.06^a	65.32±3.78^a	64.78±0.64^a	70.00±2.71^a	71.12±4.14^a
Eosinophil (%)	1.00±0.36^a	1.67±0.33^a	0.83±0.31^a	0.67±0.21^a	1.17±0.31^a	1.17±0.40^a
Basophil (%)	0.50±0.22^a	0.67±0.21^a	0.50±0.22^a	0.33±0.21^a	0.67±0.33^a	0.50±0.22^a

a-c Mean values bearing different superscripts within a row differ significantly ($p<0.05$)

3.1.3. Packed cell volume (PCV)

Mean value of packed cell volume (PCV) was no significantly ($p>0.05$) higher in group A (14.47±0.41%), group C (15.43±0.29%) and group F (13.90±0.71%), but significantly ($p<0.05$) higher in group D (13.27±0.79%) and group E (13.22±0.62%) as compared to lead treated group B (9.83±0.15%) on day 21. Comparison among the different treatment groups showed highest PCV level in group C followed by group A, F, D and E. Although on 42 days PCV values were significantly ($p<0.05$) increased from the day 21 in all groups. But on day 42 lead treated group (16.13±0.52%) showed significant ($p<0.05$) reduction of PCV from the group A (24.86±1.38%), D (26.42±1.39%), E (26.30±2.99%) and F (26.09±2.81%) but no-significant ($p>0.05$) reduction from group C (22.28±3.09%).

3.1.4. Mean corpuscular volume (MCV)

The mean values of MCV are significantly ($p<0.05$) higher values were obtained in control group A (113.09±0.61 fl), D (114.28±1.64 fl), E (116.33±3.44 fl) and F (113.13±1.25 fl) but non-significantly ($p>0.05$) higher in group C (109.25±2.11 fl) as compared to lead treated group B (105.35±1.02 fl) on day 21. Comparison among the different treatment groups on day 21 showed highest MCV level in group-E followed by group D>F>A>C. Although on day 42 MCV values were significantly ($p<0.05$) increased from the day 21 in all groups except lead treated group which showed significantly ($p>0.05$) lower MCV values from day 21 till end of the study. On day 42 lead treated group (89.91±3.73 fl) showed significant ($p>0.05$) reduction of PCV from the group A (107.87±1.60 fl), C (108.87±2.30 fl), D (107.56±0.86 fl), E (107.35±1.42 fl) and F (107.13±0.47 fl).

3.1.5. Mean corpuscular haemoglobin (MCH)

The mean MCH values were non-significant ($p>0.05$) comparable in group A (76.41±2.11 pg), B (79.53±0.79 pg), C (74.91±1.80 pg), E (85.26±4.34 pg) and group F (76.51±2.30 pg) but significantly ($p<0.05$) higher in group D (101.64±11.11 pg) as compared to lead treated group on day 21. Comparison among the different treatment groups on day 21 showed highest MCH level in group-D while others showed non-significant ($p>0.05$) changes. Although on 42 days MCH values were significantly ($p<0.05$) decreased from the day 21 in all groups. But on day 42 lead treated group B (49.75±2.49 pg) showed non-significant ($p>0.05$) reduction of MCH from the group A (57.83±0.61 pg), D (59.58±0.78 pg), F (55.36±7.00 pg), but significant ($p<0.05$) reduction of MCH from group C (62.06±2.41 pg) and E (63.03±1.79 pg).

3.1.6. Mean corpuscular haemoglobin concentration (MCHC)

The mean MCHC values were non-significantly ($p>0.05$) decreased in lead treated group B (75.53±1.04 g dl^{-1}) from control group A (67.54±1.60 g dl^{-1}), C (68.74±2.42 g dl^{-1}), E (73.26±3.10 g dl^{-1}) and F (67.66±2.10 g dl^{-1}) but significant ($p<0.05$) reduction was observed in group D (89.46±10.73 g dl^{-1}) on day 21. Comparative changes in different groups from day 21 to 42 showed significant ($p<0.05$) alteration. On day 42 lead treated group B (55.29±1.20 g dl^{-1}) showed non-significant ($p>0.05$) reduction of MCHC from the group C (56.96±1.56 g dl^{-1}), D (55.41±0.82 g dl^{-1}), E (58.69±1.37 g dl^{-1}) and group F (51.63±6.41 g dl^{-1}) except group A (53.65±0.42 g dl^{-1}). But no significant ($p>0.05$) comparative changes in MCHC were found in group-A, C, D, E and F.

3.1.7. Total leucocyte count (TLC)

The obtained values of TLC in group A (control), B, C, D, E and F were 139.88±5.49, 146.91±4.00, 127.91±1.98, 122.22±10.66, 138.41±0.61 and 140.04±2.50 10^3 µl^{-1} respectively on day 21 while on day 42 TLC value in group A (control), B, C, D, E and F were 98.27±1.37, 83.97±2.36, 106.59±9.94, 119.46±0.80,

Table 3: Comparative effect of lead and different treatments on haematological profile in broiler chicken between day 21 and 42

Haematological parameters	Day	Various treatment groups showing mean±SE values					
		A	B	C	D	E	F
TEC (10^6 µl^{-1})	21	1.28±0.04^a	0.93±0.008^a	1.42±0.04^a	1.16±0.07^a	1.14±0.06^a	1.23±0.06^a
	42	2.30±0.11^b	1.82±0.11^b	2.06±0.30^a	2.45±0.13^b	2.44±0.26^b	2.44±0.27^b
Hb (g dl^{-1})	21	9.77±0.36^a	7.43±0.13^a	10.44±0.23^a	11.70±1.28^a	9.60±0.09^a	9.33±0.29^a
	42	13.30±0.64^b	8.90±0.22^b	12.67±1.72^a	14.66±0.90^b	15.60±2.09^b	12.71±0.98^b
PCV (%)	21	14.47±0.41^a	9.83±0.15^a	15.43±0.29^a	13.27±0.79^a	13.22±0.62^a	13.90±0.71^a
	42	24.86±1.37^b	16.13±0.52^b	22.28±3.10^a	26.42±1.39^b	26.30±2.99^b	26.09±2.81^b
MCV (fl)	21	113.09±0.61^a	105.35±1.02^a	109.25±2.11^a	114.28±1.65^a	116.33±3.45^a	113.13±1.25^a
	42	107.87±1.60^b	89.91±3.73^b	108.87±2.30^a	107.57±0.86^b	107.35±1.42^a	107.13±0.47^b
MCH (pg)	21	76.41±2.11^a	79.53±0.79^a	74.91±1.80^a	101.64±11.11^a	85.26±4.44^a	76.51±2.29^a
	42	57.83±0.61^b	49.75±2.49^b	62.06±2.41^b	59.58±0.78^b	63.03±1.79^b	55.36±7.00^b
MCHC(g dl^{-1})	21	67.54±1.60^a	75.53±1.04^a	68.74±2.42^a	89.46±10.73^a	73.26±3.09^a	67.66±2.10^a
	42	53.65±0.42^b	55.29±1.21^b	56.96±1.56^b	55.41±0.83^b	58.69±1.37^b	51.63±6.41^b
TLC (10^3 dl^{-1})	21	139.88±5.49^a	146.91±4.00^a	127.91±1.98^a	122.22±10.66^a	138.41±0.61^a	140.04±2.51^a
	42	98.27±1.37^b	83.97±2.36^b	106.59±9.94^a	119.46±0.80^a	102.25±10.54^b	94.56±10.70^b
Lymphocyte (%)	21	83.10±1.84^a	52.47±1.28^a	31.27±0.17^a	41.87±3.62^a	35.77±0.80^a	35.27±2.28^a
	42	22.83±1.61^b	20.65±0.66^b	27.63±3.58^a	28.63±0.48^b	23.00±2.76^b	22.46±3.98^b
Monocyte (%)	21	6.60±0.28^a	7.06±0.11^a	6.37±0.09^a	7.30±0.15^a	7.00±0.09^a	6.67±0.21^a
	42	5.13±0.32^b	6.13±0.21^b	5.70±0.64^a	5.16±0.31^b	5.17±0.53^b	4.75±0.69^b
Heterophil (%)	21	59.16±2.24^a	54.63±0.97^a	60.90±0.43^a	49.70±3.22^a	55.23±0.65^a	56.56±2.32^a
	42	70.54±1.42^b	71.86±1.06^b	65.32±3.78^a	64.78±0.64^b	70.00±2.72^b	71.12±4.15^b
Eosinophil (%)	21	1.00±0.37^a	1.67±0.33^a	1.17±0.31^a	1.00±0.52^a	1.33±0.42^a	1.17±0.31^a
	42	1.00±0.37^a	1.67±0.33^a	0.83±0.31^a	0.67±0.21^a	1.17±0.31^a	1.17±0.40^a
Basophil (%)	21	0.50±0.22^a	0.67±0.21^a	0.33±0.21^a	0.50±0.22^a	0.67±0.21^a	0.67±0.21^a
	42	0.50±0.22^a	67±0.21^a	0.50±0.22^a	0.33±0.21^a	0.67±0.33^a	0.50±0.22^a

a-b Mean values bearing different superscripts within a column differ significantly (*p<0.05*)

102.25±10.54 and 94.56±10.70 10^3 µl^{-1} respectively. There was significant (*p<0.05*) increased in values of TLC in lead treated group B as compared to group C, D and non-significant (*p>0.05*) increase in group A, E and F on day 21. However comparison among treatment groups on day 21 showed significant (*p<0.05*) decrease in the WBC count of group D from the treatment group E and F but group C and D and group E and F was non-significantly (*p>0.05*) comparable to each other. Observations of day 42 revealed that TLC values were significantly (*p<0.05*) decreased in the group A, B, E and F and non-significantly (*p>0.05*) decreased in the group C and D as compare to day 21. At the end of the study lead treated group showed non-significant reduction (*p>0.05*) from the group A (control), C, E and F but significant decrease (*p<0.05*) from group D.

3.1.8. Differential leucocyte count (DLC)

The mean lymphocyte count was significantly (*p<0.05*) higher in lead treated group B (52.47±1.28%) as compared to group A (33.10±1.84%), C (31.27±0.17%), D (41.87±3.62%), E (35.77±0.80%) and F (35.27±02.28%) on day 21 respectively. However, on day 42 a significant (*p<0.05*) reduction in lymphocytes number was seen in all groups except group C which showed non-significant (*p>0.05*) reduction from day 21. At the end of experiment lead exposed (group-B) birds (20.65±0.66%) showed non-significant (*p>0.05*) decrease in lymphocyte count as compared to group A (22.83±1.61%), C (27.63±3.58%), D (28.63±0.48%), E (23.00±2.76%) and F (22.46±3.10%). Non-significant (*p>0.05*) change was also observed in different treatment groups and was comparable to each other. Non-significant (*p>0.05*) changes was also observed in lead treated group from the normal and other treatment groups in respect to monocytes, heterophils, eosinophils and basophils on day 21 and 42 except group C which showed significant (*p<0.05*) increased in heterophils from group B and

D while significant ($p<0.05$) decreased monocyte from group B, D and E. A significant ($p<0.05$) decrease in monocytes and heterophils count was observed in lead treated (group B) birds on day 42 as compared to its corresponding value on day 21. However, in other groups the values remained non-significantly ($p>0.05$) lower except group C.

Rahman et al. (2008) reported significant ($p<0.05$) reduction in haemoglobin, pack cell volume, total erythrocyte count, MCV, MCH, MCHC in experimentally lead exposed broilers. These findings are in agreement with the findings of present study indicating decrease in all the haematological value in lead exposed group as there was significant ($p<0.05$) reduction in TEC, Hb and PCV on day 21, and Hb, PCV and MCV on day 42, and non significant ($p>0.05$) decrease in MCV, MCH and MCHC on day 21 and TEC, MCH, and MCHC on day 42. Though at the end of the study these haematological parameters either increased significantly or non-signficantly in all the other groups except lead treated group B which showed no improvement in all the haematological values. This clearly indicated that lead had either suppressive or destructive effect on the haematopoitic activities in the body. The mechanism by which lead alters the erythrocytic parameters has been suggested by various workers. It was proposed that anaemia was the functional effect of lead poisoning due to interference with haem synthesis and decrease life span of RBCs (Waldron, 1966). Madej (1988) also suggested inhibition of haembiosynthesis, interference with porphyrine metabolism due to lead induced alterations in mitochondria and ribosome. Subsequent studies (Abou et al., 2000) in ducks also revealed marked reduction in erythrocyte count, haemoglobin (Hb), packed cell volume (PCV%) and mean corpuscular volume (MCV%) which are in support of our finding. In their study Khurana et al. (1999) observed reduction in haematological parameters like total erythrocyte count, haemoglobin, packed cell volume which were decreased significantly ($p<0.05$) in the lead exposed chicks as compared to control group chicks. Lead induced anemia was a constant finding in most of affected animals could be due to destruction of erythrocytes (due to increased fragility), depressant action on bone marrow, delaying erythrocyte maturation, and inhibition of haemsynthesis (Garg, 2000). In cases of chronic lead exposure, a microcytic, hypochromic, regenerative anemia may be present. These previous reports are in support with the present findings. Present study revealed the highest therapeutic efficacy of DL-methionine in reducing the lead induced toxic effect on TEC and Vit-E & Se on haemoglobin followed by CQE and Vit-C. These might be due to the protective effect of antioxidants used in the present study. Earlier studies showed that Vit-E protects erythrocyte against haemolysis and it has been suggested that membrane damage resulting from the LPO is the cause of Vit-E deficiency (Chow, 1991). It appears that scavenging of free radicals by Vit-E used in the present study might have protected erythrocytes in lead exposed birds. Previous studies indicated that dietary lead increases the liver glutathione concentration (Seshadri and Khanna, 1992) and enhances the excretion of glutathione conjugates (Leeming and Donaldson, 1984). This could be correlated with low blood lead concentration, obtained in the present study, which might be due to enhanced excretion of lead in lead exposed birds, thereby protecting the erythrocytes from haemolysis. Fair et al., 2002 found that Quail (*Coturnix coturnix japonica*) chicks orally received either one lead shot (0.05 g), four lead shots (0.2 g), or no lead at the age of 8 days. WBC numbers increased 7 days after antigen injection on days 27 and 49 of age which is in agreement with present finding on day 21 but not with day 42 finding. In the present study the total white blood cell count was decreased non significantly ($p>0.05$) in lead treated group B on day 42 as compared to control group. Studies by various workers have also shown leucopenia with decrease in differential leucocyte count (Avadhesh et al., 1998; Kumar et al,. 1998). On the contrary Abou et al. (2000) recorded significant ($p<0.05$) increase in leucocyte count in ducks following lead administration for 12 weeks. The difference in the finding of present study and that of Abou et al. (2000) might be due to differences in experimental birds, dose and period of exposure. The possible mechanism by which lead induces leucopenia in birds might be due to decreased production or direct cytotoxic action of lead and increased lysis attributed to accumulation of lead (Brar et al., 1997). In the present study lymhopenia and leucopenia in lead exposed birds was observed which might be due to suppressive effect of lead on lymphoid tissues of bursal follicles. Youssef et al. (1995) have also described dose dependent lymphocytic depletion of lymphoid population in bursal follicle and spleen in lead poisoning. All the treatment groups showed least alterations in leucocytic parameters except group B indicating ameliorative potential of different therapeutic agent used in the present study.

4. Conclusion

It is concluded that Vit-E @ 100 mg kg^{-1} and Se @ 0.1 mg kg^{-1} having highest efficacy against lead intoxication followed by Vitamin-C @ 200 mg kg^{-1}, DL-methionine @ 100 mg kg^{-1} and methanolic extract of *Cissus quadrangularis* (CQE) @ 400 mg kg^{-1} to alleviate lead toxicosis upto the level of 200 mg kg^{-1} in broiler diet.

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