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EFFECTS OF GARLIC (*Allium sativum*) FEED SUPPLEMENT ON HEMATO-BIOCHEMICAL PROPERTIES IN BROILER CHICKENS WITH SUB-CLINICAL TOXICITY OF LEAD

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ABSTRACT

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The present study was conducted to estimate the garlic feed supplement on hemato-biochemical parameters in lead exposed broiler chickens. 350 commercial broiler chickens were grouped into five, as T₀, T₁, T₂, T₃ and T₄ consisting of 70 birds each, where T₀ served as control. T₁ was provided with lead acetate @ 100mg/kg body weight, T₂ @ 100mg/kg lead acetate + 1% garlic supplement, T₃ was fed @ 100mg/kg lead acetate + 2% garlic supplement and T₄ @ 100mg/kg lead acetate + 4% garlic supplement. It was observed that Garlic supplementation in feed has got some ability to maintain normal values of erythrocytes, leukocytes, ALT, AST & Cholesterol parameters in spite of treatment with lead acetate. It was also found highly significant (P<0.001) in 2% garlic supplemented group (T₃) compared to that of 1% (T₂) and 4% (T₄) garlic supplemented groups. The mean values of erythrocyte, Hb. and PCV significantly reduced from 2.217±0.020 to 2.062±0.047, 7.697±0.247 to 6.172±0.198 and 25.183±0.8122 to 23.532±1.001, respectively in group T₁. The ameliorating effects of garlic in heavy metal lead revealed significantly (P<0.01) increased erythrocyte, hemoglobin and PCV values from 2.16±0.034 to 2.46±0.077, 8.362±0.262 to 10.44±0.26 and 23.64±0.90 to 30.68±0.75 in group T₃, respectively. The mean values of ALT, AST and Cholesterol with lead Acetate @ 100mg/kg treatment were significantly (P<0.01) increased from 30.11±1.135 to 36.274±1.192, 51.162±1.422 to 103.641±2.023 and 152.34±17.47 to 364.28±12.33, respectively in T₁ group on 42nd Day of treatment. The calculated significant mean values (mg/dl) of ALT, AST and Cholesterol were 24.51±0.63, 57.95±1.68 and 203.62±12.76, respectively in T₃ group. The protection action of garlic against lead toxicity could be attributed to the antioxidant action of its sulfhydryl groups.

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INTRODUCTION

Lead toxicity remains a global problem. Lead is one of the ubiquitous environmental pollutants, particularly widespread in industrial areas. Animals are exposed to lead from numerous sources as well as from the general environment. The main sources of contamination of food by lead are soil, industrial pollution, agricultural technology and food processing. Lead is toxic in most of its chemical forms, whether it is inhaled or ingested in water or feed. However, due to its slow rate of elimination, harmful levels of lead can accumulate in food chain after prolonged exposure to low quantities. Lead produces acute and chronic poisoning and induces a broad range of physiological, hematological, biochemical and behavioral dysfunctions in animals. Lead is also known to reduce erythrocyte membrane stability (Humphreys, 1991). Lead poisoning continues to be a common occupational disease affecting several organ systems. Many studies have demonstrated that lead affects the function of a variety of cell types, including those of the nervous system, the microvascular endothelium, the kidney and the immune system (Fischbein et al., 1993). *In vivo* studies have shown that lead is an immunotoxicant that can depress humoral immunity increase host susceptibility to bacterial and viral infections. Lead also increases the susceptibility of cells to oxidative attack by altering the membrane integrity. The assumption of oxidative stress as a mechanism in lead toxicity suggests that antioxidants may play a role in the treatment of lead poisoning (Wabel et al., 2007).

Exposure to lead significantly decreased red blood cell counts, hemoglobin levels and hematocrit values (Terayama, 1993). Anemia accompanying lead poisoning has the inhibitory effects of lead on heme biosynthesis. Exposure to lead in drinking water significantly decreased red blood cell count, hemoglobin concentration and hematocrit value (Bersenyi et al., 2003). The decreased RBC count depends on dose and duration of lead acetate. A shortening of erythrocyte survival time was also observed in rats exposed to lead (Terayama et al., 1993). The precise mechanism underlying lead toxicity on RBC is still to be defined. However, lead could affect the erythrocyte membrane and decrease their mobility (Terayama et al., 1993). Lead may inhibit the ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Two authors pointed out different types of anemia developed in animals exposed to lead (Bersenyi et al., 2003 and Terayama et al., 1993). Treatment of animals with garlic resulted in some improvement in the RBC count, hemoglobin concentration and hematocrit value. However, the prophylactic effect of garlic on blood parameters was more pronounced than that with olive oil. It was pointed out that garlic oil contains natural sulfur compounds which act as anti-lead active substances (Attia et al., 1993). The protection action of garlic against lead toxicity could be attributed to the antioxidant action of its sulfhydryl groups (Ashour, 2002). Efforts have been focused on using chelating agents including meso-2,3-dimercaptosuccinic acid (DMSA) and calcium disodium ethylenediaminetetraacetic acid (CaNa₂-EDTA) to protect both human and laboratory animals from lead toxicity (Yokoyama, et al., 1998). However, not much data are available on natural products therapy like garlic (Ashour, 2002 and Yassin, 2005). Therefore, the aim of this study was to evaluate the efficacy of natural product garlic to combat lead toxicity in Broiler chickens.

MATERIALS AND METHODS

The experiment was conducted in the Department of Pharmacology and Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Preparation of Poultry House for Experimental Birds

The experimental animal house particularly floor, walls, wire net, ceiling etc. were properly brushed with broom and then washed by forced water using a hosepipe. After washing with clean water, the room was disinfected by bleaching powder. Then the house was left for 7 days and then

the shed was again disinfected with Virkons (Antes International Limited, England). The house was also fumigated by formalin and potassium permanganate for a period of 24 hours for disinfection.

Rearing of Experimental Birds

A total of 350 day old commercial broiler chickens (Hubbard Classic) of both sexes were collected from a local breeder farm. The chicks were housed in floor pens containing litter composed of rice husk and saw dust and received a corn-based starter diet. The chicks were reared under fluorescent lighting. Chicks had access to feed and water *ad libitum*. All chicks were weighed individually at day 1, 7, 14, 21, 28, 35 and 42. The light was continuous during the experiment. The temperature was gradually decreased by 5° F at every week from 90° F to 75° F and continued throughout the experimental course. Chickens were reared under standard management conditions throughout the experimental course.

Ranikhet and Infectious Bursal disease vaccine were administered accordingly. The overall management of rearing was well organized in order to prevent cross contamination effectively throughout the experimental course.

Use of Lead Acetate and Garlic (*Allium sativum*) in Different Treatment Groups

The chicks were randomly divided to five (05) separate pens named Group T₀, Group T₁, Group T₂, Group T₃ and Group T₄, consisting of 70 birds in each group. Group T₀ was kept as control group. Group T₁ was given only lead acetate @ 100mg/kg. Group T₂ was treated with lead acetate @ 100 mg/kg + 1% garlic supplement. Group T₃ was treated with lead acetate @ 100 mg/kg + 2% garlic supplement and Group T₄ was treated with lead acetate @ 100 mg/kg + 4% garlic supplement. The experimental course was operated for 42 uninterrupted days. Three experimental diets were formulated to have 1%, 2% and 4% garlic powder for Group T₂, Group T₃ and Group T₄, respectively. Control group diet was free from both dietary garlic and lead acetate. Broiler feed was formulated from the locally commercially available ingredients. Garlic was prepared without skin and dried in a Freeze Drier for 72 hours, and then ground to make powder. Ten (10) birds were sacrificed from each group on every week at Day1, Day7, Day14, day21, day28, day 35 and Day42. Analytical grade lead acetate that used in this study was obtained from Merck Company (Germany). Garlic was purchased from local market. The doses of lead acetate and garlic were based on previous studies (Hanafy, *et al.*, 1994; Ashour *et al.*, 2000; Vengris, 1974 and Yassin, *et al.*, 2005). The garlic powder was not deodorized.

Collection of Blood and Sampling Procedures

Collected blood samples were used for hematological analysis at 1st, 7th, 14th, 21st, 28th, 35th and 42nd day. At each sampling date, ten chickens were randomly sacrificed from each group. Approximately 2-3ml of blood samples was collected from wing vein into a screw cap test tube containing ethylene-diaminetetra-acetic acid (EDTA) by sterile disposable syringe immediately before the sacrifice of the chickens for hematological study. The whole blood samples having EDTA were stored at 4°C and processed within 2 hours. The hematological analyses were performed according to Jain (1986).

a. Total Erythrocyte Counts

Total erythrocytes count were performed by a manual method using hemocytometer. Erythrocyte counts were made by using a Coulter Counter. The total RBC numbers were expressed in million/cumm. The counting and calculation was performed according to the method as described by Lamberg and Rothstein (1977).

b. Estimation of Hemoglobin

The hemoglobin concentration was measured using the cyanomethomoglobin technique. Sahli-Hellige method as described by Lamberg and Rothstein (1977).

c. Estimation of PCV Values

Packed cell volume (PCV) was determined by the micro hematocrit method using capillary tubes and the percentage of packed erythrocytes was determined (Lamberg and Rothstein, 1977). The result was expressed in percentage (%).

Estimation of Biochemical parameters

Blood samples were collected for biochemical analysis at 1st, 7th, 14th, 21st, 28th, 35th and 42nd day. At each sampling date, ten chickens were randomly sacrificed from each group. Having no anticoagulant, approximately 2-5 ml of blood was collected in a screw cap test tube for biochemical analysis with the aim for determination of biochemical parameters in lead toxicity induced broilers chickens. All kits required for the biochemical analysis were obtained from RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, UK. The considered biochemical parameters operated in the present study was ALT, AST and Cholesterol. Then result was recorded from Reflectron® (Imahnheim, Boehringer, Germany) display.

Statistical Analysis

The statistical analyses of variance were analyzed by Duncan's Multiple Range Test (DMRT) using the General Linear Models (GLM) procedure of SAS software. Duncan's multiple range tests were also used to locate the calculated means that are significantly different. Results were displayed as means \pm standard error (SE).

RESULTS AND DISCUSSION

Effect of Garlic on Total Erythrocyte Counts (TEC)

Analysis of variance of data on hematological examinations revealed significant difference between Group T₂, T₃ and T₄, groups. The results of Total erythrocyte count (TEC) are presented in Table 01. The mean values of TEC corresponding to the different treatments was statistically significant ($P < 0.01$). The following treatment with lead acetate @ 100mg/kg for 42 days long experimental course, the mean value of TEC significantly ($P < 0.01$) reduced from 2.21 ± 0.021 to 2.06 ± 0.042 in T₁ group. Following 42 days of garlic supplementation at different therapeutic doses in lead toxicity induced chickens, the mean value of TEC was increased significantly ($P < 0.01$). Following 42 days of garlic supplementation the TEC values were increased from 2.26 ± 0.092 to 2.28 ± 0.024 , 2.16 ± 0.034 to 2.46 ± 0.077 and 2.21 ± 0.042 to 2.35 ± 0.036 in Lead Acetate @ 100mg/Kg + 1% Garlic supplement (T₂), Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T₃) and Lead Acetate @ 100mg/Kg + 4% Garlic supplement (T₄), respectively.

Effect of Garlic on Hemoglobin in Lead Toxicity Induced Broiler Chickens

The content of hemoglobin in only lead acetate @ 100mg/kg treated groups of chickens was also significantly lower ($P < 0.01$) in comparison with the control group. Following the treatment with garlic supplement in lead toxicity induced chickens, the present study indicates significant ($P < 0.01$) increase of hemoglobin concentration in experimental groups on 42 days in comparison with the Lead Acetate @ 100mg/Kg (T₁ group). The mean value of hemoglobin content in Lead Acetate @ 100mg/Kg (T₁) group was recorded as 7.697 ± 0.241 on Day 07, however the decreased mean value 6.17 ± 0.19 was recorded on 42nd days post- treatment. Following the treatment with dietary garlic, the present study revealed a significant ($P < 0.01$) increased value of hemoglobin content from 7.418 ± 0.166 to 8.49 ± 0.22 , 8.362 ± 0.262 to 10.44 ± 0.26 and 8.044 ± 0.381 to 8.88 ± 0.25 in Lead Acetate @ 100mg/Kg + 1% Garlic supplement (T₂), Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T₃) and Lead Acetate @ 100mg/Kg + 4% Garlic supplement (T₄) group, respectively after 42 days observation. The ameliorating effect was more obvious with Lead Acetate @ 100mg/Kg + 2% Garlic supplement treatment group (T₃) which returned towards the normal values compared to the control group. Lead acetate @ 100mg/kg + 1% garlic feed

supplement group (T₂) and Lead acetate @ 100mg/kg + 4% garlic feed supplement group (T₄) also denotes the improved content compared to the Lead acetate @ 100mg/kg group (T₁).

Table 1. Effect of Garlic on Total Erythrocyte Counts (million/cu.mm) in Lead Toxicity Induced Broiler Chickens

Treatment	Mean ± SE					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T ₀	2.22±0.028 ^a	2.26±0.042 ^a	2.21±0.036 ^a	2.32±0.03 ^a	2.36±0.027 ^a	2.42±0.022 ^a
T ₁	2.21±0.021 ^a	2.19±0.072 ^a	2.17±0.028 ^b	2.13±0.02 ^b	2.11±0.041 ^c	2.06±0.042 ^c
T ₂	2.26±0.092 ^b	2.21±0.022 ^b	2.23±0.027 ^b	2.24±0.03 ^c	2.24±0.050 ^c	2.28±0.024 ^a
T ₃	2.16±0.034 ^a	2.18±0.043 ^a	2.25±0.032 ^b	2.28±0.03 ^b	2.32±0.057 ^b	2.46±0.077 ^d
T ₄	2.21±0.042 ^a	2.16±0.013 ^b	2.19±0.115 ^b	2.22±0.05 ^c	2.28±0.054 ^c	2.35±0.036 ^d
P values	0.154 NS	0.0427 **	0.0034 *	0.001 **	0.0042 **	0.0051 **

T₀, T₁, T₂, T₃ and T₄ indicates Control, Lead Acetate @ 100mg/Kg, Lead Acetate @ 100mg/Kg + 1 % Garlic supplement, Lead Acetate @ 100mg/Kg + 2 % Garlic supplement and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement groups, respectively. Values in the figures indicate mean ±Standard error (SE). Means belonging the same superscripts did not differ significantly. Each value is the mean of 10 birds. Data were calculated at 99% level of significance (P< 0.01). * = Significant, ** = Highly Significant, NS = Non-significant).

Effect of Garlic on Packed Cell Volume in Lead Toxicity Induced Broiler Chickens

The mean PCV values of broilers is influenced by different therapeutic doses of dietary garlic in lead toxicity induced broiler chickens and presented in Table 03. Analyses of variance of data on PCV values revealed significant (P<0.01) difference among treatment groups. There were no significant changes in control group (T₀). Broilers given only lead acetate @ 100mg/kg, PCV values were decreased from 25.18±0.81 to 23.53±1.01 after 42 days observation that was significantly (P<0.01), different in comparison to the control group. The mean PCV values were significantly increased from 26.35±0.72 to 28.07±1.27, 23.64±0.90 to 30.68±0.75 and 28.24±1.01 to 29.74±0.63 in the treatment groups of Lead Acetate @ 100mg/Kg + 1% Garlic supplement (T₂), Lead Acetate @ 100mg/Kg +2% Garlic supplement (T₃) and Lead Acetate @ 100mg/Kg + 4% Garlic supplement (T₄), respectively at 42days of observation.

Effect of Garlic on serum ALT and AST in Lead Toxicity Induced Broiler Chickens

Dietary application of lead acetate at a dose of 100 mg/kg daily for 42 days induced a significant increase in the levels of ALT and AST. The results of ALT and AST were presented in Table 04 and 05, respectively. Analysis of variance of data on serum ALT and AST level revealed significant difference between treatment groups. The mean values of ALT and AST with Lead Acetate @ 100mg/kg treatment were significantly increased from 30.11±1.13 to 36.27±1.19 and 51.16±1.42 to 103.61±2.02 respectively on 42nd Day of treatment. However, following the application of dietary garlic in lead toxicity induced broiler chickens; the mean values of ALT were significantly decreased from 29.35±1.05 to 27.32±0.93, 26.57±0.04 to 24.51±0.63 and 27.54±0.92 to 25.64±0.71 in Lead Acetate @ 100mg/Kg + 1 % Garlic supplemented group, Lead Acetate @ 100mg/Kg + 2 % Garlic supplemented group; Lead Acetate @ 100mg/Kg + 4 % Garlic supplemented group, respectively (Table 04). Similarly the mean values of AST were significantly decreased from 54.32±0.37 to 77.68±1.26, 47.39±0.29 to 57.95±1.68 and 42.82±1.53 to 75.68±1.70 in Lead Acetate @ 100mg/Kg + 1 % Garlic supplemented group (T₂) Lead Acetate @ 100mg/Kg + 2 % Garlic supplemented group (T₃) and Lead Acetate @ 100mg/Kg + 4 % Garlic

supplemented group (T₄), respectively (Table 05). Analysis of variance statistically revealed significant (P<0.01) lower level of serum ALT and AST. The lowest mean values of ALT and AST was detected as 24.51±0.63 and 57.95±1.68, respectively in the chickens fed on Lead Acetate @ 100mg/Kg + 2% Garlic supplement group (T₃). However, the maximum levels of ALT and AST were recorded as 36.27±1.19 and 103.61±2.02, respectively in Lead acetate group (T₁). The serum level of ALT and AST were increased significantly at lead acetate group (T₁) in comparison to the control group (T₀). The most significant response was recorded in 2% garlic supplemented diet with lead acetate.

Table 2. Effect of Garlic on Hemoglobin Content (gm %) in Lead Toxicity Induced Broiler Chickens

Treatment	Mean ± SE					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T ₀	7.971±0.102 ^a	10.01±0.10 ^a	9.95±0.10 ^a	10.02±0.09 ^a	10.016±0.08 ^a	9.86±0.09 ^a
T ₁	7.697±0.241 ^a	7.29±0.22 ^b	7.06±0.24 ^b	6.84±0.13 ^b	6.68±0.12 ^c	6.17±0.19 ^c
T ₂	7.418±0.166 ^a	7.52±0.10 ^b	7.58±0.13 ^b	7.59±0.13 ^b	8.35±0.22 ^c	8.49±0.22 ^c
T ₃	8.362±0.262 ^a	8.42±0.21 ^a	8.71±0.21 ^b	9.29±0.36 ^b	9.65±0.22 ^c	10.44±0.26 ^d
T ₄	8.044±0.381 ^a	8.21±0.27 ^b	8.25±0.19 ^b	8.39±0.272 ^c	8.57±0.20 ^c	8.88±0.25 ^c
P-Value	0.1542 ^{NS}	0.0035 ^{**}	0.0035 ^{**}	0.0025 ^{**}	0.037 [*]	0.042 [*]

T₀, T₁, T₂, T₃ and T₄ indicates Control, Lead Acetate @ 100mg/Kg, Lead Acetate @ 100mg/Kg + 1 % Garlic supplement, Lead Acetate @ 100mg/Kg + 2 % Garlic supplement and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement groups, respectively. Values in the figures indicate mean ±Standard error (SE). Means belonging the same superscripts did not differ significantly. Each value is the mean of 10 birds. Data were calculated at 99% level of significance (P< 0.01). * = Significant, ** = Highly Significant, NS = Non-significant.

Table 3. Effect of Garlic on Packed Cell Volumes (%) in Lead Toxicity Induced Broiler Chickens

Treatment	Mean ± SE					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T ₀	33.31±0.65 ^a	33.08±0.50 ^a	32.53±0.50 ^a	31.31±0.26 ^a	31.84±0.22 ^a	31.77±0.23 ^a
T ₁	25.18±0.81 ^a	26.85±0.68 ^a	26.12±0.55 ^b	25.08±0.52 ^b	24.01±0.56 ^c	23.53±1.01 ^c
T ₂	26.35±0.72 ^a	28.68±1.11 ^b	27.13±1.17 ^b	29.08±0.85 ^b	28.45±1.54 ^c	28.07±1.27 ^c
T ₃	23.64±0.90 ^a	24.51±1.32 ^a	27.54±1.22 ^b	27.51±0.99 ^b	28.57±0.89 ^b	30.68±0.75 ^c
T ₄	28.24±1.01 ^a	29.68±1.07 ^a	28.09±0.85 ^b	29.68±0.74 ^c	28.54±1.04 ^b	29.74±0.63 ^b
P Values	0.1623 ^{NS}	0.0374 [*]	0.0425 [*]	0.0032 ^{**}	0.0271 [*]	0.0241 ^{**}

T₀, T₁, T₂, T₃ and T₄ indicates Control, Lead Acetate @ 100mg/Kg, Lead Acetate @ 100mg/Kg + 1 % Garlic supplement, Lead Acetate @ 100mg/Kg + 2 % Garlic supplement and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement groups, respectively. Values in the figures indicate mean ±Standard error (SE). Means belonging the same superscripts did not differ significantly. Each value is the mean of 10 birds. Data were calculated at 99% level of significance (P< 0.01). * = Significant, ** = Highly Significant, NS = Non-significant.

Effect of Garlic on serum Cholesterol in Lead Toxicity Induced Broiler Chickens

The results of cholesterol levels in lead acetate treatment following the administration of garlic supplement at different doses have been presented in Table 06. Analysis of variance of data on serum total cholesterol level revealed significant difference between treatment groups. After 42 days of garlic supplement the mean values of serum cholesterol ($P < 0.01$) were reduced significantly.

Table 4. Effect of Garlic on serum ALT (alanine aminotransferase, (U/L) in Lead Toxicity Induced Broiler Chickens

Treatment	Mean \pm SE					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T ₀	24.97 \pm 1.12 ^a	24.05 \pm 0.617 ^a	24.818 \pm 0.69 ^a	26.25 \pm 0.83 ^a	23.14 \pm 0.53 ^a	23.54 \pm 1.20 ^a
T ₁	30.11 \pm 1.13 ^a	31.247 \pm 1.42 ^b	34.21 \pm 1.43 ^c	33.62 \pm 1.23 ^c	35.62 \pm 1.18 ^c	36.27 \pm 1.19 ^d
T ₂	29.35 \pm 1.05 ^a	30.218 \pm 0.92 ^b	27.91 \pm 1.01 ^b	28.12 \pm 1.07 ^c	29.11 \pm 1.02 ^c	27.32 \pm 0.93 ^d
T ₃	26.57 \pm 0.04 ^a	28.97 \pm 1.01 ^a	26.93 \pm 0.73 ^b	29.17 \pm 0.62 ^a	27.58 \pm 0.88 ^b	24.51 \pm 0.63 ^c
T ₄	27.54 \pm 0.92 ^a	27.77 \pm 0.85 ^b	28.34 \pm 0.86 ^b	27.38 \pm 0.96 ^c	26.68 \pm 0.70 ^c	25.64 \pm 0.71 ^c
P Values	0.0794 ^{NS}	0.0321 [*]	0.0021 ^{**}	0.0034 ^{**}	0.0355 [*]	0.0038 ^{**}

T₀, T₁, T₂, T₃ and T₄ indicates Control, Lead Acetate @ 100mg/Kg, Lead Acetate @ 100mg/Kg + 1 % Garlic supplement, Lead Acetate @ 100mg/Kg + 2 % Garlic supplement and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement groups, respectively. Values in the figures indicate mean \pm Standard error (SE). Means belonging the same superscripts did not differ significantly. Each value is the mean of 10 birds. Data were calculated at 99% level of significance ($P < 0.01$). * = Significant, ** = Highly Significant, NS = Non-significant.

Table 5. Effect of Garlic on serum AST (Aspartate Aminotransferase, (U/L) in Lead Toxicity Induced Broiler Chickens

Treatment	Mean \pm SE					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T ₀	55.68 \pm 1.85 ^a	51.67 \pm 1.92 ^a	56.67 \pm 1.94 ^a	52.95 \pm 1.47 ^a	57.95 \pm 0.18 ^a	56.96 \pm 1.71 ^a
T ₁	51.16 \pm 1.42 ^a	56.05 \pm 1.21 ^b	73.60 \pm 1.55 ^c	86.61 \pm 1.14 ^c	91.66 \pm 1.50 ^d	103.61 \pm 2.02 ^d
T ₂	54.32 \pm 0.37 ^a	58.92 \pm 1.97 ^a	51.58 \pm 1.56 ^b	50.21 \pm 0.83 ^c	96.22 \pm 0.97 ^c	77.68 \pm 1.26 ^d
T ₃	47.39 \pm 0.29 ^a	54.56 \pm 0.27 ^a	87.68 \pm 0.75 ^b	75.95 \pm 0.92 ^c	78.84 \pm 0.53 ^c	57.95 \pm 1.68 ^d
T ₄	42.82 \pm 1.53 ^a	45.67 \pm 1.02 ^b	86.34 \pm 0.62 ^b	96.35 \pm 1.46 ^b	85.47 \pm 1.29 ^c	75.68 \pm 1.70 ^c
P Values	0.0654 ^{NS}	0.0531 ^{NS}	0.0332 [*]	0.0042 ^{**}	0.0415 ^{**}	0.0025 ^{**}

T₀, T₁, T₂, T₃ and T₄ indicates Control, Lead Acetate @ 100mg/Kg, Lead Acetate @ 100mg/Kg + 1 % Garlic supplement, Lead Acetate @ 100mg/Kg + 2 % Garlic supplement and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement groups, respectively. Values in the figures indicate mean \pm Standard error (SE). Means belonging the same superscripts did not differ significantly. Each value is the mean of 10 birds. Data were calculated at 99% level of significance ($P < 0.01$). * = Significant, ** = Highly Significant, NS = Non-significant.

However, following the application of dietary garlic in lead toxicity induced broiler chickens, the mean values of cholesterol were ranged from 132.94 \pm 15.63 to 253.57 \pm 17.42, 181.59 \pm 14.81 to

203.62±12.76 and 139.13±13.0041 to 223.61±15.33 in Lead Acetate @ 100mg/Kg + 1 % Garlic supplemented group; Lead Acetate @ 100mg/Kg + 2 % Garlic supplemented group, and Lead Acetate @ 100mg/Kg + 4 % Garlic supplemented group, respectively. The maximum serum cholesterol (364.28±12.33) was observed in Lead acetate group (T₁). Statistical analysis revealed significantly (P<0.01) lower level of serum cholesterol was 203.62±12.76 that was recorded in the chickens fed on Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T₃) group. Whereas lead acetate treatment group significantly (P<0.01) increased the cholesterol level. However, the difference among the treatment of different doses of garlic feed supplement was statistically non-significant.

Table 6. Effect of Garlic on serum Cholesterol (mg/dl) in Lead Toxicity Induced Broiler Chickens

Treatment	Mean ± SE					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T ₀	114.42±1.16 ^a	144.95±234.15 ^a	125.92±1.47 ^a	146.95±2.23 ^a	138.39±1.85 ^a	134.97±2.94 ^a
T ₁	152.34±17.47 ^a	234.15±11.78 ^b	257.64±12.67 ^b	305.62±13.80 ^b	324.15±15.80 ^c	364.28±12.33 ^c
T ₂	132.94±15.63 ^a	186.34±13.51 ^b	234.16±19.5 ^b	258.28±14.94 ^b	262.38±11.30 ^b	253.57±17.42 ^c
T ₃	181.59±14.81 ^a	206.32±13.68 ^a	241.09±13.15 ^b	254.67±12.39 ^b	241.68±12.25 ^c	203.62±12.76 ^b
T ₄	139.13±13.41 ^a	189.16±11.03 ^a	245.61±12.60 ^b	255.92±19.45 ^b	245.63±16.68 ^b	223.61±15.33 ^c
P-Value	0.0824 ^{NS}	0.0281 [*]	0.0372 [*]	0.0122 ^{**}	0.0325 [*]	0.0215 [*]

T₀, T₁, T₂, T₃ and T₄ indicates Control, Lead Acetate @ 100mg/Kg, Lead Acetate @ 100mg/Kg + 1 % Garlic supplement, Lead Acetate @ 100mg/Kg + 2 % Garlic supplement and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement groups, respectively. Values in the figures indicate mean ±Standard error (SE). Means belonging the same superscripts did not differ significantly. Each value is the mean of 10 birds. Data were calculated at 99% level of significance (P< 0.01). * = Significant, ** = Highly Significant, NS = Non-significant.

The development of anemia resulted from lead poisoning in this study that may result from various inhibitory effects of lead on heme biosynthesis. Lead acetate treated group (T₁) significantly (P<0.001) decreased red blood cell counts, hemoglobin levels and hematocrit values. Similar findings have been previously reported by Redig (1991). Development of anemia in lead toxicity may be attributed to the decreased red blood cell survival time because of the increased membrane fragility, reduced RBC count, decreased hemoglobin content or summation of all these factors (ATSDR, 1993). The decrease in RBC count observed in this study is in agreement with Solliway et al., (1996). A shortening of erythrocyte survival time was observed in rats exposed to lead (Terayama, 1993). Lead may induce oxidative stress in RBCs. Blood lead concentration at low levels (30-35 µg/dl) inhibits the enzyme, d-amino levulonic acid dehydratase (ALAD) which is responsible for coupling of two molecules of amino levulonic acid (ALA) to form porphobilinogen and further synthesis of hemoglobin. The observed decreased values of RBC count in the present study indicated the toxic effect of lead intake. It can be concluded that garlic supplement significantly suppressed the hemolysis rate and protects erythrocyte membranes. Terayama et al., (1993) pointed out different types of anemia developed in animals exposed to lead. Treatment of animals with different doses of garlic resulted in some improvement in the RBC count, hemoglobin concentration and hematocrit value. However, the prophylactic effect of garlic supplement on blood parameters was more pronounced at 2% garlic supplement plus Lead Acetate 100mg/kg. It was pointed out that garlic contains natural sulfur compounds which act as anti-lead active substances (Attia et al., 1993). This implies the antioxidant action of garlic sulfhydryl groups on RBCs. The

present study also correlates with the findings reported by Tandon et al. (2001). It has been reported that garlic markedly increases erythrocyte membrane rigidity and decreases cellular deformability (Chiu *et al.*, 1979). Garlic can provide glutathione, biosynthesize metallothionein or similar protein, and its antioxidant properties appear to protect against potential oxidative damage of cellular membrane of erythrocytes by lead. (Jain et al., 1983)

The assumption of oxidative stress as a mechanism in lead toxicity in this study may suggest that antioxidant action of garlic sulfhydryl groups might play a role in the treatment of lead poisoning. The potential therapeutic action of garlic could be attributed to their chelating activity. Garlic can stimulate the production of glutathione, an amino acid which is known to be a very potent antioxidant and de-toxifier and the smooth muscle relaxant. The present study revealed that administration of garlic supplement induced significant increases of hematocrit values in treated chickens which agrees with the results of Martins et al. (2002) who verified that the addition of garlic increased erythrocytes number, hemoglobin content, and hematocrit values. Garlic has some constituents that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow. Antioxidant micronutrients are known to ameliorate this adverse health outcome (Mahaffey et al., 1979). Mihalache et al., 2004 reported that lead intoxication favour the occurrence of anemia and liver cytolytic syndrome resulting the increased serum ALT and AST level which is inconformity with present study. Khan et al. (1996) also reported that lead toxicity increased serum ALT and AST activity. Bordia et al. (1975) reported a marked reduction of serum cholesterol levels in rats fed a diet supplemented with 2% or 3% garlic powder. Supplementation of 2% garlic was enough to reduce plasma total cholesterol but in this study addition of 4% garlic powder did not further affect plasma cholesterol levels significantly. All the serum cholesterol levels in treated broilers decreased linearly compared to control. This study is in agreement with an earlier study of Konjufca et al., (1997). Reduction in cholesterol and triglycerides with garlic has been reported previously by two researchers (Jain, 1986; Konjufca et al., 1997) which is similar with present findings.

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