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EFFECTS OF DRIED GARLIC POWDER (*ALLIUM SATIVUM*) AGAINST THE SUBCLINICAL LEAD (Pb) POISONING IN BROILER CHICKENS

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A study was conducted to determine the effects of dried garlic powder (*Allium sativum*) against subclinical lead (Pb) poisoning in broiler. 350 day-old broiler chickens were grouped into five as T_0, T_1, T_2, T_3 and T_4 consisting of 70 birds each where T_0 served as control. Group T_1 was provided with lead acetate at 100mg/kg body weight, T_2 had 100mg/kg lead acetate + 1% garlic supplement, T_3 was fed with 100mg/kg lead acetate + 2% garlic supplement and T_4 had 100mg/kg lead acetate + 4% garlic supplement for 42 consecutive days. Lead acetate resulted a prominent increase ($P<0.001$) of lead (Pb) concentration in bone, brain, kidney, liver, muscles, spleen, and blood from 0.042±0.007 to 5.362±0.217, 0.051±0.006 to 4.125±0.125, 0.081±0.002 to 3.890±0.200, 0.084±0.004to 3.385±0.111, 0.070±0.002 to 3.085±0.151, 0.068±0.002to 2.253±0.026 and 0.072±0.005 to 5.965±0.121mg/kg respectively. Reduction in tissue lead concentrations was significantly ($P<0.05$) higher in the group fed with lead acetate and 2% garlic supplemented group (T_3) in comparison to the group T_2 and group T_4.
Key words: Lead (Pb), Garlic, Chelation, Chicken.

There are numerous types of environmental pollution, which constitute a potential danger to humanity (Khan et al. 1996). Chickens are susceptible to lead intoxication. Young chickens are more susceptible than adult chickens (Simpson et al. 1970). Long-term lead intoxication of chicken's results in degeneration of motor nerves in the spinal cord and loss of axons in peripheral nerves without demyelination. In addition, muscles show atrophy and degeneration of fibers. Attempts to measure the effects of lead on the chicken's cell-mediated immune response, humoral immune response and interferon production have yielded inconsistent results (Vengris et al. 1974). Lead deposition in the body consists of three major pools: blood, bone and soft tissues (Rabinowitz et al. 1976). The blood pool accounts for only 2% of the total body burden, unless there is an acute exposure, but is a rapidly exchangeable component.

Lead is a dense bluish-grey metallic element which occurs in nature. In general, lead compounds are insoluble in water, although many are readily soluble in acidic solutions, which is the chemical characteristic that most allows lead to cause physiological harm. Lead (Pb) is a natural element and widespread in the environment. The two major routes of lead (Pb) entry into the body are the alimentary and respiratory tracts (Fischbein 1992). Irrespective of the exposure pathway, lead (Pb) enters the bloodstream and is primarily distributed among three compartments: blood and soft tissues and mineralizing tissues (Gerhardsson et al. 1995). Lead (Pb) is known to be a toxic agent, and blood lead level (BLL) is a convenient and direct indicator of such toxicity. Lead (Pb) poisoning may affect numerous organ systems and is associated with a number of morphological, biochemical and physiological changes including kidney dysfunction, abnormal glucose metabolism, nervous system disturbances, impairment of

liver function and hematological disorders (Ghorbe et al. 2001). Exposure to lead (Pb) significantly decreased red blood cell counts, hemoglobin levels and hematocrit values (Terayama 1993). Anemia accompanying lead (Pb) poisoning has the inhibitory effects of lead (Pb) on heme biosynthesis. Exposure to lead (Pb) 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 the rats exposed to lead (Terayama 1993). The precise mechanism underlying lead (Pb) toxicity on RBC is still to be defined. However, lead (Pb) could affect the erythrocyte membrane and decrease their mobility (Terayama et al. 1993). The decrease in hemoglobin concentration may coincide with higher BLLs. Lead (Pb) may inhibit the ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Lead (Pb) inhibits haem biosynthesis and causes a decline in red cell δ-aminolevulinic acid dehydrogenase activity and an increase in urinary δ- aminolevulinic acid, urinary coproporhyrin and red cell zinc protoporphyrin (Kumar et al. 1995).

Lead is not only metabolized in the body, but it may be conjugated with glutathione and excreted primarily in the urine (EPA 1986); (ATSDR 1993). Exposure to lead (Pb) is primarily evidenced by elevated blood lead (Pb) levels. Lead (Pb) is distributed to liver tubular epithelium and to kidney and redistributed to bone, teeth, and hair; the long bones contain more lead and about 95% of the body total lead (Pb) load is stored in the skeleton with a half life greater than 20years (EPA 1986). The half-life of lead (Pb) in erythrocytes is 35 days; in soft tissues (kidney, liver and nervous tissue) the half-life is 40 days; the half-life in bone is 20 to 30 years (Ellenhorn 1988). The rate of excretion of lead (Pb) is low. Renal clearance of lead (Pb) essentially occurs by active tubular transport from glomerular filtration. Urinary excretion accounts for 76% of daily losses, while gastrointestinal secretions for 16% and hair, nails, sweat and other routes for 8% (WHO 1995); (Ellenhorn 1988).The comparative toxic effects of oral and intra-peritoneal administration of garlic extracts on lung and liver tissue of rats were studied by (Alnaqeeb et al. 1996). Administration of low doses of garlic (50 mg/kg) to rats either orally or intraperitoneally had little effect on lung and liver tissues as compared to control animals. In contrast, administration of high doses of garlic (500 mg/kg) resulted in profound changes in lung and liver tissues of rats. Intraperitoneal administration of the high dose of garlic was more damaging to lung and liver tissue of rats than oral administration (Alnaqeeb et al. 1996). Lead (Pb) has been indicted to be involved in the aetiology of human and animal diseases. Garlic antagonized lead (Pb) toxicity to clean up lead contents from chickens which had been exposed to natural or experimental lead pollution and consequently eliminate one of the sources of lead (Pb) (Hanafy et al. 1994).Therefore, the present study was carried out to evaluate the protective ability of garlic to ameliorate the deposition of lead (Pb) in major organs in chickens.

MATERIALS AND METHODS

Administration of Lead Acetate and Garlic (Allium Sativum) in Different Treatment Groups:

The chicks were randomly assigned to five (05) separate pens named Group T_0, Group T_1, Group T_2, Group T_3 and Group T_4, and 70 birds in each group. Group T_0 was kept as control group. Group T_1 was given only lead acetate @ 100mg/kg. Group T_2 was treated with lead acetate @ 100 mg/kg + 1% garlic supplement. Group T_3 was treated with lead acetate @ 100 mg/kg + 2% garlic supplement and Group T_4 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 (Allium sativum) powder for GroupT_2, Group T_3 and Group T_4, respectively. Control diet was free from both dietary garlic (Allium sativum) and lead acetate. Diets were formulated from the locally commercially available ingredients. Garlic was prepared without skin and dried in a Freeze Drier Model (LABCONCO) for

72 hours, and then ground to become powder. 10 birds were sacrificed from each group on every week at Day1, 7, 14, 21, 28, 35 and 42. Analytical grade lead acetate that used in this study was obtained from Merck (Germany). Garlic (Allium sativum) was locally purchased. The doses of lead acetate and garlic were based on other studies (Hanafy et al. 1974); (Yassin et al. 2005).

Determination of Accumulation of Lead (Pb) in Lead (Pb) Toxicity Induced Broiler Chickens:

Blood, bone, brain, kidney, liver, muscles and spleen were collected aseptically from the experimental broiler chickens. Tissue samples were placed in the laboratory as soon as possible in an unchanged condition and stored at - 20^0 C until analyzed.

Analytical Procedures of Tissue Samples to Determine the Effect of Garlic in Lead (Pb) Toxicity Induced Broiler Chickens:

The sample preparations were performed by using a wet digestion procedure. 3-5 gm of tissue samples were digested with ultrapure grade concentration Nitric Acid (5ml), Perchloric Acid (2.5ml) and 30% Hydrogen Peroxide (2.5 ml) and left overnight in tissue digestion chamber. The predigested tissue samples were heated step by step up to a final temperature at 120^0C for complete digestion according to the methods as described by (Alonso et al. 2000) for lead analysis. Then the samples preparation and detection were conducted according to the standard procedures as described by (Tessier et al. 1999); (Jacobson et al. 1991). All the samples were detected by flame atomic absorption spectrophotometer (AAS) with Zeeman background correction by subtracting the mean values of blank samples.

Analytical Procedures of Blood Samples to Determine the Effect of Garlic in Lead (Pb) Toxicity Induced Broiler Chickens:

At each sampling date, blood was collected from each bird into screw cap acid–washed test tubes containing EDTA. Blood samples were analyzed for lead (Pb) on wet-weight basis using flame atomic absorption spectrophotometer. 2-3 ml of blood samples were digested with ultrapure grade concentration Nitric Acid (5.0 ml), Perchloric Acid (2.5ml) and 30% Hydrogen Peroxide (2.5 ml) and left overnight in tissue digestion chamber. The predigested blood samples were heated step by step up to a final temperature at 120^0C for complete digestion according to the methods as described by (Alonso et al. 2000) for lead analysis. The sample digestion procedures were operated in a digestion chamber for the determination of lead (Pb) using an atomic absorption spectrophotometer (AAS, Model-990, UK) with Zeemen background correction and calculated against a standard curve. The digests were subsequently filtered through Whatmanfiltre No 1 and diluted to 25 ml volumetric flask. All glassware was carefully cleaned with a solution of 1% nitric acid for 48 h followed by rinsing with deionized water. Then the samples preparation and detection were conducted according to the standard procedures as described by (Tessier et al. 1999); (Jacobson et al. 1991). All the samples were detected by flame atomic absorption spectrophotometer (FAAS) with Zeeman background correction by subtracting the mean values of blank samples.

Statistical Analysis

The statistical analysis of variance was 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 ± standard error (SE).

RESULTS AND DISCUSSION

Effect of Garlic on Deposition of Lead (Pb) in Bone in Lead (Pb) Toxicity Induced Broiler Chickens:

The results of lead (Pb) level in bone have been presented in Table: 01. In this study Lead Acetate treatment group @ 100mg/Kg (T_1) resulted the significant ($P<0.001$) increased accumulation of lead (Pb) in bone. Following the treatment with dietary garlic in lead (Pb) toxicity induced broiler chickens, all the treatment groups for detection of residual deposition of lead (Pb) in bone decreased significantly ($P<0.05$) compared to the Lead Acetate group (T_1).

Table 1. Effect of Garlic on Deposition of Lead (Pb, ppm) in Bone in Lead Toxicity Induced Broiler Chickens:

Treatment	Mean ±SE						
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day42**
T_0	0.053± 0.001^{a}	0.096± 0.007^{a}	0.052± 0.006^{a}	0.072± 0.005^{a}	0.060± 0.005^{a}	0.064± 0.005^{a}	0.072± 0.005^{a}
T_1	0.042± 0.007^{a}	0.856± 0.008^{b}	1.895± 0.169^{c}	2.695± 0.315^{d}	3.261± 0.200^{d}	4.682± 0.230^{e}	5.362± 0.217^{e}
T_2	0.063± 0.003^{a}	0.692± 0.004^{b}	2.0361± 0.018^{c}	2.328± 0.173^{c}	2.412± 0.082^{d}	2.163± 0.071^{c}	2.067± 0.033^{b}
T_3	0.053± 0.003^{a}	0.421± 0.018^{b}	0.813± 0.045^{c}	1.012± 0.043^{d}	1.234± 0.062^{d}	1.235± 0.093^{c}	1.085± 0.092^{b}
T_4	0.053± 0.002^{a}	0.523± 0.005^{b}	1.426± 0.096^{c}	1.956± 0.094^{c}	1.895± 0.041^{d}	1.856± 0.091^{c}	1.751± 0.011^{b}
P Values	0.192^{NS}	0.038*	0.001**	0.001**	0.0034**	0.026*	0.042*

Statistical analysis revealed significantly (P<0.001) lower lead (Pb) level in bone (1.085±0.092) in chickens fed Lead Acetate @ 100mg/Kg and 2% garlic feed supplement (T_3) in comparison to the Lead Acetate @ 100mg/Kg group (T_1) (Table: 01). The results indicate that garlic might have contained chelating compounds capable of enhancing elimination of lead (Pb). Garlic feeding can be exploited to safeguard human consumers by minimizing lead (Pb) concentrations in bones of food animals (Hanafy et al. 1994). The ability of garlic to provide glutathione, biosynthesize metallothionein or similar protein, and its antioxidant properties appear to protect against potential oxidative damage by lead (Pb) (Tandon et al. 2001). This result is in agreement to the study reported by several researchers (Bakalli et al. 1995). Besides chelation, other compounds of garlic (S-allylcystine, S-allylmercaptocystein and some micronutrients) also prevent absorption of lead (Pb) from the gastro-intestinal tract (Pourjafar et al. 1996). Garlic also contains some oil-soluble organosulfur compounds, flavonoids, a phenol allixin and other beneficial nutrients, including selenium. (Amagase et al. 2001); (Kasuga et al. 2001). It also boosts cellular antioxidants, including glutathione, that helps maintain a healthy immune system and prevents drug toxicity, and peroxidases that eliminate toxic peroxides (Wei et al. 1998).

Effect of Garlic on Deposition of Lead (Pb) in Brain in Lead (Pb) Toxicity Induced Broiler Chickens:

Experiments were conducted to evaluate the potential for dietary garlic to influence the residual deposition of lead (Pb) in brain in lead toxicity induced broiler chickens. Table 2 shows the residual deposition of lead (Pb) in brain tissues in control and experimental animal groups. The concentrations of lead (Pb) in brain tissues from experimental birds were also measured on days 1, 7, 14, 21, 28, 35 and 42 days. Garlic (Allium Sativum) was also investigated for its potential to prevent the accumulation of lead (Pb) to reduce their residual deposition of lead (Pb) in brain in chickens. The significant change in brain tissues was recorded between treatment groups. Lead acetate provoked a prominent increase (P<0.001) of lead (Pb) concentration from 0.051±0.006 to 4.1257±0.120 compared to control levels following the treatment of 42 days long experimental course (Table 03). Following the treatment with garlic supplement, lead acetate could not register a significant increase in the accumulation of lead (Pb) in brain. The mean values of lead (Pb) were significantly (P<0.001) reduced (1.025±0.054 mg/kg) in Lead Acetate @ 100mg/Kg + 2% Garlic supplement group (T_3) in comparison to T_3 and T_4 (Table 03). The concomitant use of garlic supplement and lead acetate in chickens was found to reduce tissue (Pb) lead burden, considerably indicating the potential therapeutic activity

Table 2. Effect of Garlic on Deposition of Lead (Pb, ppm) in Brain in Lead Toxicity Induced Broiler Chickens:

Treatment	(Mean±SE)						
	Day1	Day 7	Day 14	Day 21	Day 28	Day 35	Day42
T_0	0.063± 0.001^a	0.052± 0.006^a	0.043± 0.006^a	0.052± 0.006^a	0.043± 0.006^a	0.033± 0.008^a	0.072± 0.006^a
T_1	0.051± 0.006^a	0.956± 0.091^b	1.854± 0.273^c	2.165± 0.191^c	2.685± 0.163^c	3.625± 0.211^d	4.125± 0.125^d
T_2	0.044± 0.001^a	0.865± 0.071^b	1.245± 0.071^c	1.835± 0.072^d	2.132± 0.042^d	2.145± 0.031^c	1.962± 0.034^c
T_3	0.052± 0.001^a	0.502± 0.062^b	1.210± 0.051^c	2.061± 0.072^d	1.893± 0.071^c	1.632± 0.061^c	1.025± 0.052^b
T_4	0.043± 0.002^a	0.671± 0.113^b	1.420± 0.101^c	1.854± 0.131^d	2.061± 0.121^c	1.852± 0.114^c	1.522± 0.114^b
P Values	0.0942^{NS}	0.0328*	0.0021**	0.0013**	0.0034**	0.031*	0.0024**

Data were calculated at 99% level of significance (P< 0.01). * = Significant, ** = Highly Significant, NS = Non significant.

of garlic against lead toxicity, which is very concomitant to the results from rats reported previously by (Senapati 2001). Moreover, a study conducted by (Hanafy et al. 1994) reported that lead (Pb) burden was reduced in muscle and liver tissues of chickens given both lead and garlic simultaneously. According to the results of the present study, it can be concluded that garlic is a safe supplement in reducing the lead toxicity in chickens.

Effect of Garlic on Deposition of Lead (Pb) in Kidney in Lead (Pb) Toxicity Induced Broiler Chickens:

The effect of garlic supplement in lead (Pb) toxicity induced broiler chickens was also investigated to determine the accumulation of lead (Pb) in kidney (Table 3). In this study, a significant (P<0.001) increase of accumulation of lead (Pb) levels in kidney in Lead Acetate @ 100mg/Kg (T_1) group could be a result from impaired kidney functions resulted from lead toxicity. The present study revealed reduced level of the residual deposition of lead in kidney in Lead Acetate @ 100mg/Kg + 1% Garlic supplement (T_2), Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) and Lead Acetate @ 100mg/Kg + 4% Garlic supplement (T_4), respectively (Table 04). This ameliorating effect was more noticeable (0.914±0.009) with Lead Acetate @ 100mg/Kg + 2%

Table3. Effect of Garlic on Deposition of Lead (Pb, ppm) in Kidney in Lead Toxicity Induced Broiler Chickens:

Treatment	(Mean±SE)						
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day42
T_0	0.060± 0.002^a	0.072± 0.005^a	0.094± 0.003^a	0.081± 0.005^a	0.084± 0.005^a	0.074± 0.011^a	0.089± 0.005^a
T_1	0.081± 0.002^a	1.084± 0.051^b	2.097± 0.232^c	2.384± 0.252^c	2.785± 0.166^c	3.491± 0.133^d	3.890± 0.200^d
T_2	0.091± 0.001^a	0.794± 0.051^b	1.546± 0.154^c	1.854± 0.144^d	2.025± 0.124^d	1.895± 0.155^c	1.580± 0.133^b
T_3	0.064± 0.002^a	0.521± 0.032^b	1.114± 0.132^c	1.524± 0.131^d	1.584± 0.131^c	1.328± 0.110^b	0.914± 0.009^b
T_4	0.070± 0.002^a	0.634± 0.021^b	1.286± 0.224^c	1.674± 0.221^d	1.865± 0.201^d	1.623± 0.199^c	1.257± 0.119^b
P Values	0.1851 NS	0.0294*	0.0031**	0.0018**	0.0013**	0.0043**	0.0013**

Data were calculated at 99% level of significance (P< 0.01). * = Significant, ** = Highly Significant, NS = Non significant.

Garlic supplement (T_3) treatment compared to the lead acetate group. However, such increase may indicate impairment in kidney function. Therefore, urea, uric acid and creatinine could be considered as suitable prognostic indicators of renal dysfunction in case of lead exposure (Weaver et al. 2003). (Franson and Custer 1982) reported in exposed chickens that whereas kidney residues were about three times than liver residues.

Effect of Garlic on Deposition of Lead (Pb) in Liver in Lead (Pb) Toxicity Induced Broiler Chickens:

There was a significant elevation ($P<0.05$) in lead (Pb) concentration in the liver of chickens treated with Lead Acetate @ 100mg/Kg (T_1) in comparison to the chickens of the control group. Treatment of chickens with garlic feed supplement lowered residual deposition of lead (Pb). After 42 days of garlic supplementation the mean values of lead (Pb) were reduced significantly ($P<0.05$). The reduced levels of lead concentration were generally reached its significance ($P < 0.05$) after 30 days of garlic feed supplement. The therapeutic effects of garlic to combat lead poisoning in chickens were assessed. Oral administration of lead acetate compared to the control and treatment groups. The results of gradual accumulation of lead (Pb) in liver were presented in Table 05. Analysis of variance of data on liver tissues revealed significant difference between treatment groups. The lead (Pb) levels detected in liver were 0.061±0.001, 0.084±0.004, 0.052±0.002, 0.063±0.004 and 0.071±0.003 at day 01 in Control group (T_0), Lead Acetate @ 100mg/Kg group (T_1), Lead Acetate @ 100mg/Kg + 1 % Garlic supplement group (T_2), Lead Acetate @ 100mg/Kg + 2 % Garlic supplement group (T_3) and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement group (T_4), respectively (Table 05). However, following the treatment for 42 days long experimental course with different therapeutic doses of garlic in lead toxicity induced broiler chickens; the residual deposition of lead (Pb) was detected as 0.084±0.005, 3.385±0.112, 1.286±0.019, 0.782±0.021 and 1.062±0.029 at day 42 in Control (T_0) group, Lead Acetate @ 100mg/Kg group (T_1), Lead Acetate @ 100mg/Kg + 1 % Garlic supplement group (T_2), Lead Acetate @ 100mg/Kg + 2 % Garlic supplement group (T_3) and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement group (T_4), respectively (Table 04). However, the significant ($p<0.01$) reduced level of lead (Pb) concentration was recorded in 2% Garlic supplemented group in compared to the Lead Acetate @ 100mg/Kg group. Moreover these chelators in turn are potentially toxic and often fail to remove lead (Pb) from all body tissues (Hanafy et al. 1994); (Osweiler 1999). (Senapati et al.2001) reported that garlic can increase lead (Pb) concentration in urine and feces of rats. Other reports revealed that

Table 4. Effect of Garlic on Deposition of Lead (Pb, ppm) in Liver in Lead Toxicity Induced Broiler Chickens:

Treatment	(Mean±SE)						
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day42
T_0	0.061± 0.001^{a}	0.092± 0.03^{a}	0.091± 0.033^{a}	0.081± 0.004^{a}	0.084± 0.005^{a}	0.054± 0.007^{a}	0.084± 0.005^{a}
T_1	0.084± 0.004^{a}	0.965± 0.01^{a}	2.117± 0.159^{b}	2.742± 0.133^{b}	3.073± 0.039^{c}	3.284± 0.067^{c}	3.385± 0.111^{d}
T_2	0.052± 0.002^{a}	0.987± 0.007^{b}	1.494± 0.054^{c}	1.842± 0.022^{c}	1.924± 0.042^{c}	1.752± 0.002^{c}	1.286± 0.015^{b}
T_3	0.063± 0.004^{a}	0.843± 0.005^{b}	1.053± 0.053^{b}	1.362± 0.083^{c}	1.284± 0.0222^{c}	1.244± 0.0442^{b}	0.782± 0.022^{b}
T_4	0.071± 0.003^{a}	0.862± 0.004^{b}	1.252± 0.033^{b}	1.554± 0.074^{c}	1.524± 0.033^{b}	1.426± 0.033^{b}	1.062± 0.024^{b}
P Values	0.0621^{NS}	0.0027**	0.0257*	0.0047**	0.0042**	0.0061**	0.0031**

Data were calculated at 99% level of significance ($P< 0.01$). * = Significant, ** = Highly Significant, NS = Non significant.

administration of garlic and lead acetate is effective in lead reduction (Hanafy et al.1994; (Senapati et al. 2001). (Pourjafar et al. 2007) reported that garlic is most effective on liver detoxification. Moreover, a study conducted by (Hanafy et al. 1994) reported that lead burden was reduced in muscle and liver tissues of chickens given both lead and garlic simultaneously.

Effect of Garlic on Deposition of Lead (Pb) in Muscles in Lead (Pb) Toxicity Induced Broiler Chickens:

The present study is aimed to through the light to examine the ability of natural substance garlic to combat lead toxicity in chickens. Lead (Pb) was significantly ($P<0.001$) accumulated in the muscles of lead acetate @100mg/kg group (T_1) compared to the control group (T_0). Because of the potential health risk for lead (Pb) exposure to people from consuming chickens' meat, the muscle tissues were measured for deposition of lead (Pb) in experimentally lead (Pb) induced chickens (Table 06). The lead (Pb) levels detected in muscles were 0.061±0.001, 0.070±0.002, 0.053±0.004, 0.054±0.003 and 0.063±0.004 at day 01 in Control group (T_0), Lead Acetate @ 100mg/Kg group (T_1), Lead Acetate @ 100mg/Kg + 1 % Garlic supplement group (T_2), Lead Acetate @ 100mg/Kg + 2 % Garlic supplement group (T_3) and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement group (T_4), respectively (Table 5). However, following the treatment for 42 days long experimental course with different therapeutic doses of garlic in lead toxicity induced broiler chickens; the residual deposition of lead (Pb) in muscles was detected as 0.093±0.002, 3.085±0.111, 1.152±0.034, 0.790±0.035 and 0.895±0.043 at day 42 in Control (T_0) group, Lead Acetate @ 100mg/Kg group (T_1), Lead Acetate @ 100mg/Kg + 1 % Garlic supplement group (T_2), Lead Acetate @ 100mg/Kg + 2 % Garlic supplement group (T_3) and Lead Acetate @ 100mg/Kg + 4 % Garlic supplement group (T_4), respectively (Table 06). The garlic supplementation provoked highly significant decreased values ($P<0.05$) in residual accumulation of lead (Pb) in muscles. This ameliorating effect was more significantly ($P<0.001$) pronounced with Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) therapy. Garlic feeding can be exploited to safeguard human consumers by minimizing lead (Pb) concentrations in meat of food animals which had been grown in a lead (Pb) polluted environment (Hanafy et al. 1994). Organosulfur components (as diallyl sulfide) present in garlic exhibit protective effects against toxicants (Fatma et al. 2009). Further support for our findings comes from (Khan et al. 1994), who reported that toxic doses of lead (Pb) administrated orally accumulated in the liver, muscles and kidney. (Hanafy et al. 1994) reported garlic antagonized lead (Pb) toxicity and have investigated the possible use of garlic feeding to clean up

Table 5. Effect of Garlic on Deposition of Lead (Pb, ppm) in Muscles in Lead Toxicity Induced Broiler Chickens:

Treatment	(Mean±SE)						
	Day1	Day 7	Day 14	Day 21	Day 28	Day 35	Day42
T_0	0.061± 0.001^{a}	0.082± 0.004^{a}	0.074± 0.005^{a}	0.095± 0.004^{a}	0.082± 0.006^{a}	0.084± 0.006^{a}	0.093± 0.002^{a}
T_1	0.070± 0.002^{a}	0.715± 0.07^{b}	1.993± 0.133^{b}	2.313± 0.142^{c}	2.994± 0.04^{c}	3.093± 0.109^{d}	3.085± 0.151^{d}
T_2	0.053± 0.004^{a}	0.754± 0.052^{b}	1.632± 0.015^{b}	1.756± 0.067^{c}	1.657± 0.036^{d}	1.452± 0.073^{c}	1.152± 0.034^{b}
T_3	0.054± 0.003^{a}	0.526± 0.058^{b}	1.063± 0.025^{c}	1.263± 0.082^{d}	1.241± 0.081^{d}	1.114± 0.094^{c}	0.790± 0.071b
T_4	0.063± 0.004^{a}	0.985± 0.09^{b}	1.354± 0.039^{c}	1.426± 0.028^{d}	1.574± 0.046^{d}	1.362± 0.084^{c}	0.895± 0.063^{b}
P Values	0.0751^{NS}	0.0014**	0.0254*	0.0015**	0.0034**	0.0061**	0.0035**

Data were calculated at 99% level of significance ($P< 0.01$). * = Significant, ** = Highly Significant, NS = Non significant.

lead contents from chickens which had been exposed to natural or experimental lead (Pb) pollution.

Effect of Garlic on Deposition of Lead (Pb) in Spleen in Lead (Pb) Toxicity Induced Broiler Chickens:

The results of lead (Pb) deposition in spleen in lead toxicity induced broiler chickens were presented in Table 06. The concentrations of lead (Pb) in spleen tissues from experimental birds were also measured on days 1, 7, 14, 21, 28, 35 and 42 days. Lead Acetate @100mg/kg group (T1) increased the lead (Pb) deposition in spleen significantly (P<0.01). The minimum lead (Pb) accumulation in spleen (0.480±0.037) was found in chickens fed ration containing Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3). The chickens revealed significantly (P<0.001) less value of lead (Pb) levels in Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) group compared with the other doses of garlic supplemented treatment groups. The chickens under garlic treatment had 0.8961±0.0205, 0.4806±0.0373 and 0.6721±0.0463 lead levels (mg/kg) in Lead Acetate @ 100mg/Kg + 1% Garlic supplement (T_2), Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) and Lead Acetate @ 100mg/Kg + 4% Garlic supplement (T_4), respectively (Table 07). Following the treatment with garlic supplement in lead (Pb) toxicity induced broiler chickens; the residual accumulation of lead (Pb) was significantly reduced in Lead Acetate @ 100mg/Kg + 1% Garlic supplement (T_2), Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) and Lead Acetate @ 100mg/Kg + 4% Garlic supplement (T_4), respectively (Table 6). There was no significant change in lead (Pb) levels in spleen in control group. Administration of 100 mg/kg lead acetate provoked a significant increase of lead (Pb) deposition, with recorded increased values on 42days of treatment. 2% garlic feed supplement and lead acetate registered a significant (P<0.01) ameliorating decreased effect in the deposition of lead (Pb) concentration in spleen (0.480±0.093) in lead toxicity induced broiler chickens. This result is in agreement to the study reported by (Senapati et al. 2001) in rats. Lead ingested by chickens is deposited in bones, soft tissues, and eggs and produces elevated blood lead levels (Bakalli et al. 1995). Concomitant use of garlic supplements at the three different doses was found to reduce lead concentration considerably indicating the potential therapeutic activity of garlic against lead (Pb) as reported by (Senapati et al. 2001). Garlic contains sulfur-containing amino acids, like S-allylcystine, S-allylmercaptocystein and alliin (Hanafy et al. 1994); (Horie et al. 1992). Use of garlic dry powder following subclinical lead poisoning in goats was found to reduce tissue lead concentration considerably indicating the potential therapeutic activity of garlic

Table 6. Effect of Garlic on Deposition of Lead (Pb, ppm) in Spleen in Lead Toxicity Induced Broiler Chickens:

Treatment	(Mean±SE)						
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day42
T_0	0.051± 0.006^{a}	0.033± 0.003^{a}	0.052± 0.003^{a}	0.043± 0.005^{a}	0.031± 0.002^{a}	0.026± 0.004^{a}	0.036± 0.005^{a}
T_1	0.068± 0.002^{b}	0.592± 0.009^{b}	1.150± 0.01^{b}	1.522± 0.09^{c}	1.783± 0.025^{c}	2.093± 0.106^{d}	2.253± 0.026^{d}
T_2	0.093± 0.002^{a}	0.568± 0.004^{b}	0.963± 0.004^{b}	1.345± 0.05^{c}	1.359± 0.04^{d}	1.290± 0.033^{c}	0.896± 0.042^{b}
T_3	0.086± 0.006^{a}	0.546± 0.009^{b}	0.856± 0.009^{b}	1.965± 0.05^{c}	1.117± 0.04^{d}	0.968± 0.041^{c}	0.480± 0.093^{b}
T_4	0.068± 0.002^{a}	0.526± 0.006^{b}	0.822± 0.006^{b}	1.095± 0.05^{c}	1.205± 0.05^{d}	1.056± 0.052^{c}	0.672± 0.024^{b}
P Values	0.0634^{NS}	0.0024**	0.028*	0.0018**	0.0017**	0.0017**	0.0017**

Data were calculated at 99% level of significance (P< 0.01). * = Significant, ** = Highly Significant, NS = Non significant.

against lead (Pb) toxicity in goats (Badiei et al. 2005). (Hanafy et al. 1994) reported that garlic antagonized lead toxicity and have investigated the possible use of garlic feeding to clean up lead (Pb) contents from chickens which had been exposed to natural or experimental lead (Pb) pollution. Lead (Pb) is known to be a toxic agent, and blood lead level (BLL) is a convenient and direct indicator of such toxicity (Gerhardsson et al.1995).

Effect of Garlic on Deposition of Lead (Pb) in Blood in Lead (Pb) Toxicity Induced Broiler Chickens:

Significant increased values in the blood lead (Pb) levels (5.965±0.121) were found in the lead acetate group compared to the control group. The prophylactic efficacy of garlic (Allium sativum) supplement to reduce blood lead (Pb) concentration was also evaluated experimentally in broiler chickens in this study. Following the administration of lead acetate @100 mg/kg blood lead (Pb) levels was significantly ($P<0.01$) increased from 0.072±0.003 to 5.965±0.117 on 42 days long experimental course. Blood lead (Pb) level values corresponding to the different garlic supplemented treatment groups with only lead acetate group were differed significantly. It can be inferred that garlic supplement reduced the concentration of lead (Pb) level significantly ($P<0.01$). On 42 days of the treatment, blood lead concentration values were significantly reduced in the garlic supplemented groups in Lead Acetate @100mg/Kg +1% Garlic supplement (T_2) group, Lead Acetate @ 100mg/Kg +2% Garlic supplement (T_3) group and Lead Acetate @ 100mg/Kg +4% Garlic supplement (T_4) group. Garlic supplement possibly provoked remarkable Pb excretion from blood in this study. Following the treatment with garlic supplement, lead (Pb) burden in tissues decreased in comparison to the only lead treated group, but decreased value was significantly ($P<0.001$) recorded (1.223 ±0.082) in 2% garlic supplemented lead toxicity induced group (Table 7). The treatment with garlic supplement efficiently ameliorated the toxic effect of lead. This result is in agreement to the study reported by (Senapati et al. 2001) in rats. Lead ingested by chickens is deposited in bones, soft tissues, and eggs and produces elevated blood lead levels (Bakalli et al. 1995). Garlic leads to remarkable lead (Pb) excretion from blood in the present study. The concentration of lead (Pb) levels in different tissues decreased in comparison with garlic supplemented groups. Garlic contains sulfur-containing amino acids, like S-allylcystine, S-allylmercaptocystein and alliin (Hanafy et al. 1994); (Horie et al. 1992). Lead (Pb) is known to be a toxic agent, and blood lead

Table 7. Effect of Garlic on Deposition of Lead (pb, ppm) in Blood in Lead Toxicity Induced Broiler Chickens:

Treatment	(Mean±SE)						
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day42
T_0	0.071± 0.003^{a}	0.077± 0.003^{a}	0.056± 0.007^{a}	0.092± 0.004^{a}	0.083± 0.006^{a}	0.064± 0.008^{a}	0.086± 0.006^{a}
T_1	0.072± 0.005^{a}	1.965± 0.02^{a}	2.652± 0.039^{b}	3.285± 0.155^{c}	3.895± 0.155^{c}	4.953± 0.122^{d}	5.965± 0.121^{d}
T_2	0.067± 0.002^{a}	1.623± 30.009^{a}	2.526± 0.022^{b}	2.845± 0.012^{c}	3.567± 0.024^{c}	3.118± 0.082^{c}	2.634± 0.072^{b}
T_3	0.057± 0.003^{a}	1.258± 0.004^{a}	1.684± 0.074^{b}	2.412± 0.073^{c}	2.268± 0.031^{c}	1.985± 0.073^{c}	1.223± 0.082^{a}
T_4	0.072± 0.003^{a}	1.264± 0.005^{a}	2.429± 0.017^{b}	3.165± 0.097^{b}	2.849± 0.053^{b}	2.117± 0.066^{b}	1.695± 0.055^{b}
P Values	0.0841$^{NS=}$	0.00358**	0.0024*	0.0011**	0.0084**	0.0024**	0.0022**

Data were calculated at 99% level of significance ($P< 0.01$). * = Significant, ** = Highly Significant, NS = Non significant.

level (BLL) is a convenient and direct indicator of such toxicity (Gerhardsson et al. 1995). The present results indicated that garlic contain chelating compounds are also capable of enhancing elimination of lead.

CONCLUSIONS

The present study was undertaken to investigate lead (Pb) impact on body tissues to examine the ability of natural garlic to combat lead toxicity in chickens. The results suggest that garlic could help for protection against organ damage caused by this toxic heavy metal, lead (Pb). It indicate that garlic might have contained chelating compounds capable of enhancing elimination of lead (Pb).Therefore, the findings could be useful to understand its' useful protection. The garlic supplement was found significant and that could be recommended for achieving optimum effects of chelation therapy for the reducing the residual deposition of lead (Pb) in chickens.

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