

## Therapeutic competence of dried garlic powder (*Allium sativum*) on biochemical parameters in lead (Pb) exposed broiler chickens

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### ABSTRACT

The study was conducted to assess the therapeutic competence of garlic (*Allium sativum*) in lead (Pb) exposed chickens. The experimental birds (n=350) were grouped into T<sub>0</sub> (as control), T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. The birds of group T<sub>1</sub> was provided with lead acetate at 100 mg/kg body weight. Group T<sub>2</sub> had lead acetate at 100 mg/kg b.wt. + 1% garlic supplement, whereas group T<sub>3</sub> was fed with lead acetate at 100 mg/kg b.wt. + 2% garlic supplement, and group T<sub>4</sub> had lead acetate at 100 mg/kg b.wt. + 4% garlic supplement. The mean values (mg/dL) of uric acid, blood urea nitrogen, creatinine, cholesterol, triglycerides, low density lipoprotein, high density lipoprotein, and blood glucose in the birds of group T<sub>1</sub> were significantly increased ( $p < 0.01$ ) on day 42 of post-treatment. Elevation of these parameters was suggestive for the pathological involvement of different organs like liver, kidney, muscles. Statistical analysis of variance indicated that lead acetate at 100 mg/kg b.wt. + 2% garlic supplement (T<sub>3</sub>) resulted significant ( $p < 0.01$ ) ameliorative effect on the biochemical parameters as compared to the group T<sub>2</sub> and T<sub>4</sub>. In conclusion, potency of garlic in reversion of the values of the biochemical properties in Pb exposed chickens was close to the normal levels of the values.

### Keywords

Biochemical parameter, Chelating agents, Chicken, Lead

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### INTRODUCTION

In environmental health and medicine, lead (Pb) exposure is considered as one of the most important problems in terms of prevalence of exposure and public health impact (Hossain et al., 2014). Pb is a potent occupational toxin and its toxicological manifestations are well-known (Gagan et al., 2012). Due to its slow rate of elimination, harmful levels of Pb can accumulate in food chain after prolong exposure to low quantities. Pb produces acute and chronic poisoning, and induces a broad-range of physiological, biochemical and behavioral dysfunctions in animals. Atmospheric and soil Pb can contaminate water and consequently enter the aquatic food chains (Kaste et al., 2003).

The assumption of oxidative stress as a mechanism in Pb toxicity suggests that antioxidants may play a role in the treatment of Pb poisoning (Al-Wabel et al., 2007). Environment has been defined as "the aggregate of all the external conditions that influences the life and its development". Although Pb occurs naturally in the environment, it plays no known beneficial role in biological processes (Gulson, 1996). The low concentration of the Pb significantly decreases egg production and egg weight, and increases percentage of embryonic mortality (Vodola et al., 1997). Young chickens are more susceptible than adult chickens

(Mihalache et al., 2004). The bone marrow contains more than 95% of the total Pb burden, where it may be mobilized and contribute to the blood Pb level (BLL) (Weaver et al., 2003).

The protection action of garlic against Pb toxicity could be attributed by 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 (CaNa<sub>2</sub>-EDTA) to protect both human and laboratory animals from Pb toxicity (Yokoyama et al., 1998). However, very few data are available on natural products therapy like garlic (Ashour, 2002; Yassin, 2005).

Garlic helps to prevent disease, largely due to its high content of organosulfur compounds and antioxidant activity (Borek, 2001). Aged garlic extract (AGE) is richer in antioxidants than other commercial garlic preparations and fresh garlic and it also boosts cellular antioxidants, including glutathione, that helps to maintain a healthy immune system and prevents drug toxicity and peroxidases that eliminate toxic peroxides (Wei and Lau, 1998). Treatment of animals with natural product like garlic improves such toxic effect of Pb. The assumption of oxidative stress as a mechanism in Pb toxicity suggests that antioxidant action of garlic sulfhydryl groups might play a role in the treatment of Pb poisoning. The objectives of this study were to enhance the values of biochemical parameter by the use of garlic powder in Pb exposed chickens.

## MATERIALS AND METHODS

**Place of work and duration:** The experiment was conducted at the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, during the period of July 2009 to June 2012.

**Experimental birds:** A total of 350 day-old commercial broiler chickens (Hubbard Classic) of both sexes were collected and housed in floor pens. Chicks had an *ad libitum* access to feed and water. All chicks were weighed individually at day 1, 7, 14, 21, 28, 35 and 42. The diets for the chicks were formulated according to US National Research Council guidelines. Chickens were reared under standard management conditions throughout the experimental course.

**Use of lead acetate and garlic (*Allium sativum*) in different treatment groups:** The chicks were randomly assigned to five (5) separate pens named Group T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>; in which 70 birds contained in each

group. Group T<sub>0</sub> was kept as control group. Group T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, were treated with only lead acetate (dosed at 100 mg/kg b.wt.), lead acetate (dosed at 100 mg/kg b.wt.) + 1% garlic supplement, lead acetate (dosed at 100 mg/kg b.wt.) + 2% garlic supplement, and lead acetate (dosed at 100 mg/kg b.wt.) + 4% garlic supplement, respectively. The experimental course was operated for 42 uninterrupted days. 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 h, and then ground to become powder. Analytical grade lead acetate used in this study was obtained from Merck Company Ltd. (Germany). Garlic (*Allium sativum*) was locally purchased. The doses of lead acetate and garlic were based on other studies (Hanafy, 1994; Ashour, 2002; Yassin, 2005).

**Collection of blood and sampling procedures:** Blood samples were collected for biochemical analysis at day 1, 7, 14, 21, 28, 35 and 42. 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. The tubes were left for a short time to allow clotting. The blood containing tubes were left in slanting position at room temperature for 4 h. Then the tubes were left over night in the refrigerator. The tube was gently agitated and centrifuged at 3,000 rpm for 20 min to get rid of unwanted blood cells. The serum samples were separated and stored at -20°C for biochemical analysis. The serum samples were examined through standard procedures. In brief, 100 µL ready serum samples were taken in cuvette with the help of micropipette. Then 1,000 µL reagent was taken to each cuvette and mixed thoroughly. The cuvette was incubated at 37°C for 5 min. After incubation, each mixture was placed in the Reflectron® (Imahnheim, Boehringer, Germany) against the blank reagent. All the reagents required for the biochemical analysis were obtained from RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, UK. In case of using of stored serum samples, the samples were allowed for thawing at first and then centrifuged again as previous for proper mixing of serum samples. The considered biochemical parameters operated in the present study were Serum Uric acid (SUA), Blood Urea Nitrogen (BUN), Creatinine, Cholesterol, Triglycerides, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), and Blood Glucose. The results were recorded from Reflectron® display. The results were expressed in mg/dL.

**Statistical analysis:** The statistical analysis of variance was analyzed by Duncan's Multiple Range Test (DMRT). 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 DISCUSSIONS

The average values of SUA level in both control and experimental animals are presented in **Table 1**. The mean values of uric acid with lead acetate (100 mg/kg b.wt.) treatment were significantly increased from  $7.68 \pm 0.13$  to  $11.77 \pm 0.057$  on 42<sup>nd</sup> day of post-treatment. Following the administration of garlic supplementation in Pb-induced chickens, uric acid was significantly ( $p < 0.01$ ) reduced. In the present study, 2% garlic feed supplement in lead acetate (100 mg/kg b.wt.) group (T<sub>3</sub>) registered a significant ( $p < 0.01$ ) ameliorating effect on uric acid values. Statistical analyses revealed significantly ( $p < 0.01$ ) lower level of SUA as  $5.24 \pm 0.122$  in the chickens that was fed on lead acetate dosed at 100 mg/kg b.wt. + 2% garlic supplement (T<sub>3</sub>), and the maximum SUA as  $11.77 \pm 0.057$  was found in lead acetate group (T<sub>1</sub>). The observed elevation of uric acid concentration in response to lead acetate administration is in agreement with previous studies of McBride et al. (1998), Khalil et al. (1992) and El-Ashmawy et al. (2005).

In **Table 2**, the potential therapeutic action of garlic could be attributed to their chelating abilities. Lead acetate provoked a prominent increase in BUN from  $15.24 \pm 1.059$  to  $22.86 \pm 1.318$  as compared to control group ( $p < 0.01$ ) following the treatment of 42 days long experimental course. Statistical analyses revealed significantly ( $p < 0.01$ ) lower level of BUN, which was recorded as  $16.052 \pm 0.489$  in group T<sub>3</sub>. However, the maximum BUN ( $22.86 \pm 1.318$ ) was found in Group T<sub>1</sub>. The observed elevation in BUN in response to lead acetate administration is in agreement with previous studies of Chung (2006). It could be suggested that treatment of animals with garlic might be effective in order to alleviate the toxic effect of Pb. The antioxidant

action of garlic sulfhydryl groups might play a role in the treatment of Pb poisoning. Dietary garlic was not found as an adverse effect on treatment groups of chickens.

The effect of garlic supplementation in lead toxicity induced chickens was investigated to determine the creatinine level. The mean values of creatinine with lead acetate dosed at 100 mg/kg b.wt. treatment were significantly increased from  $1.25 \pm 0.201$  to  $3.07 \pm 0.019$  on 42<sup>nd</sup> day of post-treatment (**Table 3**). Statistical analyses revealed significantly ( $p < 0.01$ ) lower level of creatinine ( $0.91 \pm 0.071$ ), which was recorded in group T<sub>3</sub>. In this study, a significant ( $p < 0.01$ ) increased creatinine level observed in T<sub>1</sub> group could be associated with impaired kidney functions. Increased creatinine concentrations are in agreement with previous reports (Ghorbe et al., 2001; Taha et al., 2013). Significant increase in serum creatinine values in all Pb treated groups was also observed by Sujatha et al. (2011). The effect was compared to controls. The ameliorating effect was more satisfactory with 2% garlic supplement group (T<sub>3</sub>). Therefore, urea, uric acid and creatinine could be considered as suitable prognostic indicators of renal dysfunction in case of Pb exposure (Weaver et al., 2003).

About 50% of kidney function must be lost before a rise in the serum concentration of creatinine (Atef et al., 1994). Lead acetate in the diet significantly increases in serum urea and creatinine (El-Ashmawy et al., 2005). However, there are very few data are available in which natural products like garlic has been used in therapeutic purpose (Ashour, 2002; Yassin, 2005). In this study, the ability of garlic to combat Pb toxicity in chickens was examined, and the findings could be useful to understand Pb toxicity and its' useful protection.

The results of cholesterol levels in lead acetate treatment following the administration of garlic supplement at different doses have been presented in **Table 4**. Garlic has been considered as one of the blood

**Table 1.** Effect of garlic on serum uric acid (SUA; mg/dL) in Pb toxicity induced broiler chickens.

Treatment	(Mean $\pm$ SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T <sub>0</sub>	$6.24 \pm 0.18^a$	$5.62 \pm 0.02^a$	$5.77 \pm 0.02^a$	$5.78 \pm 0.05^a$	$5.86 \pm 0.11^a$	$5.63 \pm 0.01^a$
T <sub>1</sub>	$7.68 \pm 0.13^a$	$8.11 \pm 0.03^a$	$9.217 \pm 0.13^a$	$10.52 \pm 0.04^b$	$10.56 \pm 0.05^c$	$11.77 \pm 0.05^d$
T <sub>2</sub>	$6.11 \pm 0.02^a$	$7.34 \pm 0.02^a$	$8.34 \pm 0.02^a$	$7.84 \pm 0.02^b$	$7.32 \pm 0.12^c$	$7.16 \pm 0.02^c$
T <sub>3</sub>	$5.78 \pm 0.03^a$	$6.68 \pm 0.03^a$	$7.29 \pm 0.02^b$	$7.06 \pm 0.02^b$	$6.57 \pm 0.02^c$	$5.24 \pm 0.12^c$
T <sub>4</sub>	$6.92 \pm 0.02^a$	$7.64 \pm 0.03^a$	$8.69 \pm 0.25^b$	$8.06 \pm 0.02^c$	$7.52 \pm 0.02^c$	$6.24 \pm 0.02^a$
p-value	0.0754 NS	0.001 NS	0.042 *	0.0142 **	0.0315 **	0.032**

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

**Table 2.** Effect of garlic on blood urea nitrogen (mg/dL) in Pb toxicity induced broiler chickens.

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T <sub>0</sub>	13.73±0.86 <sup>a</sup>	13.58±0.71 <sup>a</sup>	13.23±0.50 <sup>a</sup>	14.27±0.59 <sup>a</sup>	13.27±0.56 <sup>a</sup>	14.22±0.64 <sup>a</sup>
T <sub>1</sub>	15.24±1.05 <sup>a</sup>	16.38±1.29 <sup>b</sup>	17.95±1.41 <sup>c</sup>	20.62±1.38 <sup>c</sup>	22.63±1.34 <sup>d</sup>	22.86±1.31 <sup>d</sup>
T <sub>2</sub>	16.41±1.04 <sup>a</sup>	17.28±1.05 <sup>b</sup>	18.65±0.96 <sup>b</sup>	18.96±0.94 <sup>c</sup>	19.37±0.91 <sup>c</sup>	18.19±0.70 <sup>d</sup>
T <sub>3</sub>	15.49±1.04 <sup>a</sup>	16.67±0.99 <sup>a</sup>	18.67±1.02 <sup>b</sup>	17.62±0.64 <sup>b</sup>	17.21±0.63 <sup>b</sup>	16.05±0.48 <sup>c</sup>
T <sub>4</sub>	15.99±1.00 <sup>a</sup>	17.58±0.88 <sup>a</sup>	19.67±0.47 <sup>a</sup>	19.3447±0.53 <sup>b</sup>	18.55±0.46 <sup>b</sup>	17.05±0.44 <sup>b</sup>
<i>p</i> -value	0.0754 <sup>NS</sup>	0.0021 <sup>**</sup>	0.0342 <sup>*</sup>	0.0012 <sup>**</sup>	0.0415 <sup>*</sup>	0.0264 <sup>*</sup>

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

**Table 3.** Effect of garlic on creatinine (mg/dL) in Pb toxicity induced broiler chickens.

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T <sub>0</sub>	0.79±0.01 <sup>a</sup>	0.89±0.09 <sup>a</sup>	0.75±0.01 <sup>a</sup>	0.93±0.06 <sup>a</sup>	0.81±0.01 <sup>a</sup>	0.69±0.08 <sup>a</sup>
T <sub>1</sub>	1.25±0.02 <sup>a</sup>	1.92±0.02 <sup>b</sup>	2.19±0.02 <sup>b</sup>	2.35±0.02 <sup>b</sup>	2.67±0.02 <sup>c</sup>	3.07±0.01 <sup>c</sup>
T <sub>2</sub>	1.05±0.08 <sup>a</sup>	1.52±0.05 <sup>b</sup>	1.63±0.05 <sup>b</sup>	1.76±0.01 <sup>c</sup>	1.95±0.011 <sup>d</sup>	1.62±0.10 <sup>d</sup>
T <sub>3</sub>	1.12±0.01 <sup>a</sup>	1.52±0.01 <sup>b</sup>	1.42±0.03 <sup>b</sup>	1.45±0.03 <sup>b</sup>	1.34±0.06 <sup>c</sup>	0.91±0.07
T <sub>4</sub>	0.95±0.02 <sup>a</sup>	1.35±0.01 <sup>b</sup>	1.55±0.01 <sup>b</sup>	1.64±0.06 <sup>b</sup>	1.54±0.01 <sup>c</sup>	1.21±0.01 <sup>c</sup>
<i>p</i> -value	0.0954 <sup>NS</sup>	0.0221 <sup>*</sup>	0.0342 <sup>*</sup>	0.0142 <sup>**</sup>	0.0415 <sup>*</sup>	0.0241 <sup>*</sup>

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

**Table 4.** Effect of garlic on cholesterol (mg/dL) in Pb toxicity induced broiler chickens.

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T <sub>0</sub>	114.42±1.16 <sup>a</sup>	144.95±234.15 <sup>a</sup>	125.92±1.47 <sup>a</sup>	146.95±2.23 <sup>a</sup>	138.39±1.85 <sup>a</sup>	134.97±2.94 <sup>a</sup>
T <sub>1</sub>	152.34±17.47 <sup>a</sup>	234.15±11.78 <sup>b</sup>	257.64±12.67 <sup>b</sup>	305.62±13.80 <sup>b</sup>	324.15±15.80 <sup>c</sup>	364.28±12.33 <sup>c</sup>
T <sub>2</sub>	132.94±15.63 <sup>a</sup>	186.34±13.51 <sup>b</sup>	234.16±19.5 <sup>b</sup>	258.28±14.94 <sup>b</sup>	262.38±11.30 <sup>b</sup>	253.57±17.42 <sup>c</sup>
T <sub>3</sub>	181.59±14.81 <sup>a</sup>	206.32±13.68 <sup>a</sup>	241.09±13.15 <sup>b</sup>	254.67±12.39 <sup>b</sup>	241.68±12.25 <sup>c</sup>	203.62±12.76 <sup>b</sup>
T <sub>4</sub>	139.13±13.0041 <sup>a</sup>	189.16±11.03 <sup>a</sup>	245.61±12.60 <sup>b</sup>	255.92±19.45 <sup>b</sup>	245.63±16.68 <sup>b</sup>	223.61±15.33 <sup>c</sup>
<i>p</i> -value	0.0824 <sup>NS</sup>	0.0281 <sup>*</sup>	0.0372 <sup>*</sup>	0.0122 <sup>**</sup>	0.0325 <sup>*</sup>	0.0215 <sup>*</sup>

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

**Table 5.** Effect of garlic on triglycerides (mg/dL) in Pb toxicity induced broiler chickens.

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T <sub>0</sub>	45.92±0.21 <sup>a</sup>	39.39±1.12 <sup>a</sup>	52.18±0.77 <sup>a</sup>	41.44±1.77 <sup>a</sup>	39.80±1.06 <sup>a</sup>	42.65±0.61 <sup>a</sup>
T <sub>1</sub>	71.69±0.51 <sup>a</sup>	77.07±3.71 <sup>a</sup>	93.12±5.09 <sup>a</sup>	104.46±6.59 <sup>c</sup>	116.94±6.54 <sup>b</sup>	144.17±9.50 <sup>d</sup>
T <sub>2</sub>	65.28±0.20 <sup>a</sup>	78.25±2.06 <sup>a</sup>	85.62±2.46 <sup>a</sup>	98.67±2.18 <sup>b</sup>	112.54±2.29 <sup>b</sup>	106.37±1.51 <sup>c</sup>
T <sub>3</sub>	51.95±0.96 <sup>a</sup>	64.28±2.06 <sup>a</sup>	75.28±2.28 <sup>b</sup>	66.95±2.20 <sup>b</sup>	76.34±2.67 <sup>c</sup>	64.68±2.99 <sup>b</sup>
T <sub>4</sub>	58.67±0.42 <sup>a</sup>	72.11±2.58 <sup>a</sup>	84.25±2.90 <sup>b</sup>	84.27±3.12 <sup>c</sup>	81.24±2.99 <sup>c</sup>	87.67±2.69 <sup>c</sup>
<i>p</i> -value	0.0754 <sup>NS</sup>	0.0021 <sup>*</sup>	0.0422 <sup>*</sup>	0.0142 <sup>**</sup>	0.0495 <sup>*</sup>	0.0241 <sup>*</sup>

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

lipids lowering agents. The maximum serum cholesterol level as 364.28±12.331 was found in group T<sub>1</sub>. Statistical analyses revealed significantly ( $p < 0.01$ ) lower level of serum cholesterol (203.62±12.762) in group T<sub>3</sub>. On the other hand, serum cholesterol level in lead acetate treatment group significantly ( $p < 0.01$ ) increased. Supplementation of 2% garlic was enough to reduce plasma total cholesterol. This study is in agreement with an earlier study of Konjufca et al. (1991). Also, garlic causes to reduce the triglyceride level significantly, as reported by Chowdhury et al. (2002) and Issa et al. (2012). High levels of triglycerides due to Pb may cause serious illnesses including cardiac failure, renal dysfunction etc. The results of blood triglyceride were presented in Table 5. Statistical

analyses indicated that significantly ( $p < 0.01$ ) minimum level of serum triglycerides as 64.68±2.991 was recorded in group T<sub>3</sub>, and the maximum blood triglyceride as 144.17±9.5022 was found in group T<sub>1</sub>. In our study, garlic reduces the triglyceride level significantly, as reported by Dehkordi et al. (2010) and Issa et al. (2012).

Allicin is a major component of garlic organosulfurs, and its antioxidant properties are well-known. In addition to allicin, other garlic organosulfurs such as alliin, allyl cysteine, allyl disulfide, and diallyl disulfide, possess antioxidant properties; these could neutralize several types of reactive oxygen species in the body (Chung, 2006).

**Table 6.** Effect of garlic on low density lipoprotein (LDL) (mg/dL) in Pb toxicity induced broiler chickens.

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T <sub>0</sub>	158.73±5.38 <sup>a</sup>	173.73±4.45 <sup>a</sup>	169.78±6.63 <sup>a</sup>	161.57±7.36 <sup>a</sup>	165.54±7.31 <sup>a</sup>	175.22±6.17 <sup>a</sup>
T <sub>1</sub>	189.15±9.75 <sup>a</sup>	219.64±7.88 <sup>b</sup>	230.34±8.65 <sup>b</sup>	278.69±12.10 <sup>b</sup>	289.58±10.46 <sup>c</sup>	312.21±7.19 <sup>d</sup>
T <sub>2</sub>	174.42±4.74 <sup>a</sup>	198.64±8.39 <sup>a</sup>	230.34±7.82 <sup>b</sup>	247.16±7.03 <sup>b</sup>	235.82±7.4914 <sup>b</sup>	245.81±4.65 <sup>c</sup>
T <sub>3</sub>	163.07±4.15 <sup>a</sup>	195.21±7.64 <sup>b</sup>	245.85±6.52 <sup>b</sup>	214.17±7.55 <sup>c</sup>	196.57±5.51 <sup>c</sup>	188.62±5.67 <sup>c</sup>
T <sub>4</sub>	170.18±2.97 <sup>a</sup>	231.15±7.74 <sup>b</sup>	214.18±6.82 <sup>c</sup>	226.94±9.52 <sup>c</sup>	229.61±8.10 <sup>c</sup>	223.65±4.81 <sup>c</sup>
<i>p</i> -value	0.0642 <sup>NS</sup>	0.0062 <sup>**</sup>	0.0442 <sup>*</sup>	0.0012 <sup>**</sup>	0.0325 <sup>*</sup>	0.0241 <sup>*</sup>

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

**Table 7.** Effect of garlic on high density lipoprotein (HDL) (mg/dL) in Pb toxicity induced broiler chickens.

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T <sub>0</sub>	23.79±1.30 <sup>a</sup>	23.84±1.67 <sup>a</sup>	22.61±1.61 <sup>a</sup>	24.27±0.74 <sup>a</sup>	22.26±1.08 <sup>a</sup>	23.28±0.79 <sup>a</sup>
T <sub>1</sub>	25.91±0.61 <sup>a</sup>	21.77±0.57 <sup>a</sup>	21.42±0.56 <sup>a</sup>	19.66±0.53 <sup>a</sup>	17.85±0.51 <sup>b</sup>	15.27±0.51 <sup>b</sup>
T <sub>2</sub>	27.96±0.88 <sup>a</sup>	25.17±1.25 <sup>a</sup>	25.94±1.23 <sup>b</sup>	26.33±0.75 <sup>b</sup>	24.38±1.18 <sup>c</sup>	27.92±0.56 <sup>c</sup>
T <sub>3</sub>	29.99±1.04 <sup>a</sup>	27.36±1.06 <sup>a</sup>	31.28±0.76 <sup>a</sup>	30.87±0.71 <sup>a</sup>	34.11±0.58 <sup>b</sup>	39.18±0.49 <sup>c</sup>
T <sub>4</sub>	29.15±0.97 <sup>a</sup>	26.38±0.89 <sup>a</sup>	25.69±0.86 <sup>b</sup>	29.65±0.68 <sup>b</sup>	27.24±0.62 <sup>b</sup>	30.82±0.51 <sup>c</sup>
<i>p</i> -value	0.0954 <sup>NS</sup>	0.0421 <sup>*</sup>	0.0342 <sup>*</sup>	0.0142 <sup>**</sup>	0.0455 <sup>*</sup>	0.0142 <sup>*</sup>

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

**Table 8.** Effect of garlic on serum glucose (mg/dL) in Pb toxicity induced broiler chickens.

Treatment	Mean ± SE					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T <sub>0</sub>	224.66±17.30 <sup>a</sup>	209.62±12.11 <sup>a</sup>	205.84±10.03 <sup>a</sup>	229.15±11.67 <sup>a</sup>	213.95±5.70 <sup>a</sup>	226.05±6.27 <sup>a</sup>
T <sub>1</sub>	258.36±20.19 <sup>a</sup>	264.37±16.55 <sup>b</sup>	315.09±15.04 <sup>b</sup>	320.64±15.60 <sup>c</sup>	326.38±13.21 <sup>c</sup>	356.37±13.44 <sup>c</sup>
T <sub>2</sub>	237.06±20.37 <sup>a</sup>	249.61±16.97 <sup>a</sup>	263.57±16.66 <sup>c</sup>	275.63±13.60 <sup>b</sup>	255.95±12.53 <sup>b</sup>	251.96±12.43 <sup>c</sup>
T <sub>3</sub>	229.73±20.31 <sup>a</sup>	222.51±19.84 <sup>a</sup>	218.83±15.06 <sup>b</sup>	211.24±18.20 <sup>b</sup>	201.56±18.67 <sup>c</sup>	187.88±14.79 <sup>c</sup>
T <sub>4</sub>	234.75±20.20 <sup>a</sup>	245.92±17.28 <sup>c</sup>	276.95±16.52 <sup>b</sup>	298.67±15.91 <sup>d</sup>	246.31±17.43 <sup>c</sup>	235.96±15.53 <sup>b</sup>
<i>p</i> -value	0.0715 <sup>NS</sup>	0.0062 <sup>*</sup>	0.0312 <sup>*</sup>	0.00142 <sup>**</sup>	0.0345 <sup>*</sup>	0.026 <sup>*</sup>

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

Low density lipoprotein (LDL) is the major cholesterol carrier in blood. From the data presented in **Table 6**, it is evident that LDL values in Pb exposed chickens differed significantly ( $p < 0.01$ ) as compared to the only lead acetate group (T<sub>1</sub>). The maximum LDL value as 312.21±7.196 was found in group (T<sub>1</sub>) fed with lead acetate (100 mg/kg b.wt.). Statistical analyses revealed that significantly ( $p < 0.01$ ) minimum level of LDL as 188.62±5.672 was detected in group T<sub>3</sub>. The antioxidant activity of garlic was attributed to biologically active lipophilic sulfur-bearing compounds such as allicin, S-allyl-cysteine (SAC), and diallyl-sulde (DAS) (Amagase et al., 2001).

Statistical analyses revealed that significantly ( $p < 0.01$ ) higher level of high density lipoprotein (HDL) as 39.18±0.493 was recorded in T<sub>3</sub> group, and the minimum level of HDL as 15.27±0.512 was found in lead acetate group (T<sub>1</sub>) (**Table 7**). Garlic could enhance the triglyceride level significantly (Issa et al., 2012). Recent reports demonstrated that garlic powder preparations reduced lipoprotein oxidation susceptibility *in vitro* and *in vivo* (Kourounakis, 1991). However, researchers studied the sulfur compounds of garlic causing inhibition of lipid peroxidation.

The mean values of serum glucose of both control and experimental birds are shown in **Table 8**. Oral administration of lead acetate dosed at 100 mg/kg b.wt. caused a significant ( $p < 0.01$ ) increased value in glucose levels after 42 days of treatment as compared to control group. However, the garlic supplement in Pb toxicity induced chickens provoked a significant interaction in glucose levels in T<sub>3</sub> and T<sub>4</sub> group after 42 days of treatment, respectively. The most significant ( $p < 0.01$ ) ameliorating effect on blood glucose level in lead (Pb) toxicity induced broiler chickens was more pronounced with lead acetate dosed at 100 mg/kg b.wt. + 2% garlic supplement (T<sub>3</sub>) therapy. Analysis of variance statistically revealed that a significant ( $p < 0.01$ ) lower level of blood glucose as 187.88±14.792 was recorded.

Pb toxicity might be associated with a number of physiological changes such as abnormal glucose metabolism, hematological disorders, impairment of liver and kidney dysfunction (Ghorbe et al., 2001). The present data revealed a significant decrease in serum glucose level upon lead acetate administration. In this context, Pb might be regarded as a risk factor in the

abnormal glucose metabolism, as reported by Ahrens (1993) who found neurodegenerative disorders. Treatment with natural garlic revealed improvement in serum glucose level. This result coincided with the report of Ashour (2002). The potential action of garlic in returning serum glucose to about its normal level coincided with their function as chelating agents (Yokoyama et al., 1998; Marija et al., 2004). Garlic has been found to be useful in controlling glucose tolerance and is beneficial for both hypo- and hyperglycemia.

## CONCLUSION

The finding of the present study reveals that garlic (*Allium sativum*) might have therapeutic ability on Pb-exposed broiler chickens. It indicates that garlic might have contained chelating compounds capable of altering the biochemical parameter. Therefore, the findings could be useful to understand its' useful protection. The garlic supplement was found significant, which could be recommended for achieving optimum effects of chelation therapy in Pb-exposed broiler chickens.

## COMPETING INTEREST

The authors declare that they have no competing interest.

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