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THERAPEUTIC COMPETENCE OF DRIED GARLIC POWDER (*Allium sativum*) ON BIOCHEMICAL PARAMETERS IN LEAD (Pb) EXPOSED BROILER CHICKENS

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The study was conducted to estimate the therapeutic competence of garlic (*Allium sativum*) on biochemical properties in lead exposed chickens. The experimental birds (350) were grouped into T_0, T_1, T_2, T_3 and T_4. Group T_0 served as control, group T_1 was provided with lead acetate at 100mg/kg body weight, group T_2 had 100mg/kg lead acetate + 1% garlic supplement, group T_3 was fed with 100mg/kg lead acetate + 2% garlic supplement and group T_4 had 100mg/kg lead acetate + 4% garlic supplement. The mean values (mg/dl) of Uric acid, Blood Urea Nitrogen, Creatinine, Cholesterol, Triglycerides, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Blood Glucose with lead Acetate @ 100mg/kg treatment were significantly increased ($P<0.01$) on 42nd Day of treatment. Elevation of serum biochemical parameters suggested the pathological involvement of liver, kidney, muscles etc. Statistical analysis of variance indicated that Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) group resulted the significant ($P<0.01$) ameliorative effect on biochemical parameters in comparison to the group T_2 and T_4. The potency of garlic towards reverting to the values of biochemical properties close to its normal level in lead (Pb) exposed chickens that coincided with the functions as chelating agents.

Key words: Lead, Chicken, Biochemical parameter, Chelating agents.

In the worlds of environmental health and environmental medicine, lead (Pb) exposure remains one of the most important problems in terms of prevalence of exposure and public health impact. Despite decades of intensive research, lead toxicity also remains one of the most, studied subjects of all within the fields of environmental health and environmental medicine. The main sources of contamination of food by lead (Pb) are soil, industrial pollution, agricultural technology and food processing, Gurer et al, 1998. However, due to its slow rate of elimination, harmful levels of lead (Pb) can accumulate in food chain after prolonged exposure to low quantities. Lead (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. Lead (Pb) is an element of risk for the environment and human health and has harmful effects that may exceed those of other inorganic toxicants. Industries are the major sources of lead (Pb) pollution that contribute toxic metal lead (Pb) to the environment and its entry to the biological systems disturb the hemato-biochemical pathways with cumulative accumulation of lead to the body tissues, in some cases leads to death of the organism. 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, 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 lead (Pb) occurs naturally in the environment, it plays no known beneficial

role in biological processes, Gulson, 1996. Lead (Pb) is classified as a 'toxicant' that can affect a broad spectrum of species and remain in the environment for a long time. High levels of lead (Pb) can cause death in many birds and mammals. Lead (Pb) contamination also affects biological systems by affecting ecosystem productivity (inhibiting plant growth) and nutrient cycling, Gulson, 1996. Lead (Pb) combines with sulfhydryl groups on proteins and interferes with some biochemical processes.

Chickens are susceptible to lead (Pb) intoxication. As little as 1.0 mg/kg lead (Pb) in the diet can cause significant depression in the growth of broiler chickens and consistent reduction in blood d-aminolevulinic acid dehydratase, an erythrocyte enzyme sensitive to lead (Pb) , Bakalli et al, 1995. There was a linear relationship between increasing concentration of lead (Pb) in drinking water and decreasing body weight of hens. The low concentration of the lead (Pb) significantly decreases egg production and egg weight, and increases percentage of embryonic mortality ,Vodela et al, 1997. Lead poisoning is a complex disorder affecting many organs in the body, including developing red blood cells, the kidneys, and the nervous system. Chickens are susceptible to lead intoxication. Young chickens are more susceptible than adult chickens , Mihalache et al, 2004. 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 and Mare, 1974. Lead deposition in the body consists of three major pools: blood, bone, and soft tissues. The blood pool accounts for only 2% of the total body burden, unless there is an acute exposure, but is a rapidly exchangeable component. The bone marrow contains more than 95% of the total lead (Pb) burden, where it may be mobilized and contribute to the blood lead level (BLL) , Weaver et al, 2003.

It was pointed out that garlic contains natural sulfur compounds which act as anti-lead active substances, Attia and Ali, 1993. The protection action of garlic against lead (Pb) 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; Yassin, 2005. Garlic can help to lessen free radical damage because it has the ability to protect against radiation. Garlic also contains a number of amino acids which are required for the formation of an enzymatic antidote to free radical pathology. Cysteine, glutamine, isoleucine and methionine found in garlic help to protect the cells from free radical damage. Lead, mercury, cadmium, arsenic and copper pollutants threaten our health on a daily basis. Treating heavy metal poisoning has involved a process called chelation. Garlic can effectively protect the body from metal toxicity. Garlic can prevent the toxic effect of heavy metals from damaging and destroying erythrocyte membrane. Garlic helps to prevent disease, largely due to its high content of organosulfur compounds and antioxidant activity, Borek, 2001. Garlic contains powerful antioxidant that includes S-allyl mercaptocysteine, and S-allyl cysteine, that has a 98% absorption rate into the blood circulation (high bioavailability). Garlic also contains some oil-soluble organosulfur compounds, flavonoids, a phenol allixin and other beneficial nutrients, including selenium, Borek, 2001. Aged garlic extrct (AGE) is richer in antioxidants than other commercial garlic preparations and fresh garlic and 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 and Lau,

1998. Treatment of animals with natural product like garlic improves such toxic effect of lead. The assumption of oxidative stress as a mechanism in lead (Pb) toxicity suggests that antioxidant action of garlic sulfhydryl groups might play a role in the treatment of lead (Pb) poisoning. Therefore it is needed to examine the ability of natural product garlic to combat lead (Pb) toxicity in chickens and the findings could be useful to understand lead (Pb) toxicity and its' useful protection.

MATERIALS AND METHODS

The present work was operated with the aim for ameliorating effect of lead (Pb) toxicity on biochemical parameters by the use of different doses of garlic supplement in lead toxicity induced broiler chickens. The experimental design and following methodology were adopted for performing the present study:

Rearing of 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 ad libitum access to feed and water. All chicks were weighed individually at day 1, 7, 14, 21, 28, 35 and 42. The diets were formulated according to US National Research Council guidelines. Chickens were reared under standard management conditions throughout the experimental course. 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 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. Each experiment was operated separately. Group T_0 was kept as control group. Group T_1, Group T_2, Group T_3 and Group T_4, was treated with only lead acetate @ 100mg/kg, lead acetate @ 100 mg/kg + 1% garlic supplement, lead acetate @ 100 mg/kg + 2% garlic supplement, and lead acetate @ 100 mg/kg + 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 hours, and then ground to become powder. 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 (Germany) Co. Garlic (Allium sativum) was locally purchased. The doses of lead acetate and garlic were based on other studies, Ashour, 2002, Hanafy, 1994, 39 Yassin, 2005. The garlic powder was not deodorized.

Collection of Blood and Sampling

Procedures to Determine the Effect of Garlic on Biochemical Changes in Lead (Pb)

Toxicity Induced Broiler Chickens:

Blood samples were collected for biochemical analysis at 1^{st}, 7^{th}, 14^{th}, 21^{st}, 28^{th}, 35^{th} and 42^{nd} 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 (Pb) toxicity induced broilers chickens. 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 hours. 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 determined by standard procedures. 100 µl ready serum sample was taken in each cuvette with the help of micropipette. Then 1 000 µl reagent was taken to each cuvatte and mixed thoroughly. The cuvette was incubated at 37^0C for 5 minutes. After incubation, each mixture was placed in the Reflectron® (Imahnheim, Boehringer, Germany) against the blank reagent. All kits 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 was Uric acid, Blood Urea Nitrogen, Creatinine, Cholesterol, Triglycerides, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Blood Glucose. Then result was recorded from Reflectron® display. The result was expressed in mg/dl.

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, SAS, 1985. 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).

Place of work

The experiment was conducted in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh funded by Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka, Bangladesh, University Grants Commission (UGC), Bangladesh and USDA project, Bangladesh.

RESULTS & DISCUSSION

Lead (Pb) is a natural element and widespread in the environment. Irrespective of the exposure pathway, lead (Pb) enters the bloodstream and is primarily distributed among three compartments: blood, soft tissues and mineralizing tissues , Al-Saleh, 2000. Uric acid is the main end-product in nitrogen metabolism in birds. The average values of serum uric acid level in both control and experimental animals are presented in Table 01. The mean values of uric acid with lead Acetate @ 100mg/kg treatment were significantly increased from 7.68±0.13 to 11.77±0.057 on 42nd Day of treatment. Following the administration of garlic supplementation in lead toxicity induced chickens, significantly (P<0.01) reduced uric acid values communicated the potential therapeutic activity of garlic supplementation. Garlic supplemented groups returned the mean values of uric acid toward the normal values. In the present study, 2% garlic feed supplement in lead acetate @ 100 mg/kg group (T_3) registered a significant (P<0.01) ameliorating effect on uric acid values. Statistical analysis revealed significantly (P<0.01) lower level of serum uric acid was 5.24±0.122 that was recorded in the chickens fed on Lead Acetate @ 100mg/Kg + 2% Garlic supplement group (T_3) and the maximum serum uric acid (11.77±0.057) was found in Lead acetate group. The observed elevation in uric acid concentration in response to lead acetate administration is in agreement with previous studies , El-Ashmawy et al, 2005; Khalil et al, 1992. Lead (Pb) intoxication significantly enhances the uric acid concentration. However, such increase may indicate impairment of kidney function. The elevation in uric acid was also reported by other authors, McBride et al, 1998. The elevated serum urea levels may be due to the destruction of RBCs by lead acetate. Treatment of animals with garlic alleviated such toxic effect of lead (Pb). The assumption of oxidative stress as a mechanism in lead toxicity suggests that antioxidant action of garlic sulfhydryl groups might play a role in the treatment of lead (Pb) poisoning. The potential therapeutic action of garlic could be attributed to their chelating ability. Lead acetate provoked a prominent increase in Blood Urea Nitrogen (BUN) from 15.24±1.059 to 22.86±1.318 compared to

Table 01. Effect of Garlic on Uric Acid (mg/dl) in Lead (pb) Toxicity Induced Broiler Chickens

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T_0	6.24±0.18^{a}	5.62±0.02^{a}	5.77±0.02^{a}	5.78±0.05^{a}	5.86±0.11^{a}	5.63±0.01^{a}
T_1	7.68±0.13^{a}	8.11±0.03^{a}	9.217±0.13^{a}	10.52±0.04^{b}	10.56±0.05^{c}	11.77±0.05^{d}
T_2	6.11±0.02^{a}	7.34±0.02^{a}	8.34±0.02^{a}	7.84±0.02^{b}	7.32±0.12^{c}	7.16±0.02^{c}
T_3	5.78±0.03^{a}	6.68±0.03^{a}	7.29±0.02^{b}	7.06±0.02^{b}	6.57±0.02^{c}	5.24±0.12^{c}
T_4	6.92±0.02^{a}	7.64±0.03^{a}	8.69±0.25^{b}	8.06±0.02^{c}	7.52±0.02^{c}	6.24±0.02^{a}
P Values	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 02. Effect of Garlic on Blood Urea Nitrogen (mg/dl) in Lead (pb) Toxicity Induced Broiler Chickens

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T_0	13.73±0.86^a	13.58±0.71^a	13.23±0.50^a	14.27±0.59^a	13.27±0.56^a	14.22±0.64^a
T_1	15.24±1.05^a	16.38±1.29^b	17.95±1.41^c	20.62±1.38^c	22.63±1.34^d	22.86±1.31^d
T_2	16.41±1.04^a	17.28±1.05^b	18.65±0.96^b	18.96±0.94^c	19.37±0.91^c	18.19±0.70^d
T_3	15.49±1.04^a	16.67±0.99^a	18.67±1.02^b	17.62±0.64^b	17.21±0.63^b	16.05±0.48^c
T_4	15.99±1.00^a	17.58±0.88^a	19.67±0.47^a	19.3447±0.53^b	18.55±0.46^b	17.05±0.44^b
P Values	0.0754 ^{NS}	0.0021 **	0.0342 *	0.0012 **	0.0415 *	0.0264*

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

Table 03. Effect of Garlic on Creatinine (mg/dl) in Lead (pb) Toxicity Induced Broiler Chickens

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T_0	0.79±0.01^a	0.89±0.09^a	0.75±0.01^a	0.93±0.06 ^{a}	0.81±0.01 ^{a}	0.69±0.08^a
T_1	1.25±0.02^a	1.92±0.02^b	2.19±0.02^b	2.35±0.02^b	2.67±0.02^c	3.07±0.01^c
T_2	1.05±0.08^a	1.52±0.05^b	1.63±0.05^b	1.76±0.01^c	1.95±0.011^d	1.62.±0.10^d
T_3	1.12±0.01^a	1.52±0.01^b	1.42±0.03^b	1.45±0.03^b	1.34±0.06^c	0.91±0.07
T_4	0.95±0.02^a	1.35±0.01^b	1.55±0.01^b	1.64±0.06^b	1.54±0.01^c	1.21±0.01^c
P Values	0.0954 ^{NS}	0.0221 *	0.0342 *	0.0142 **	0.0415 *	0.0241*

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

control values (P<0.01) following the treatment of 42 days long experimental course. Following the treatment of garlic supplement, lead acetate could not registered a significant increase in blood urea nitrogen. (Table 2). Statistical analysis revealed significantly (P<0.01) lower level of blood urea nitrogen (BUN) and was recorded as 16.052±0.489 in the chickens fed on Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) group. The maximum blood urea nitrogen (BUN) (22.86±1.318) was found in lead acetate group (Group T_1). The observed elevation in blood urea nitrogen (BUN) in response to lead acetate administration is in agreement with previous studies, Al-Saleh, 2000; Chung, 2006. It can be concluded that treatment of animals with garlic was effective in order to alleviate the toxic effect of lead (Pb). The antioxidant action of garlic sulfhydryl groups might play a role in the treatment of lead (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 @ 100mg/kg treatment were significantly increased from 1.25±0.201 to 3.07±0.019 on 42nd Day of treatment (Table 03). Statistical analysis revealed significantly (P<0.01) lower level of Creatinine (0.91±0.071) was recorded in the chickens fed on Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) group. In this study, a significant (P<0.01) increased creatinine level in Lead Acetate @ 100mg/Kg (T_1) group could be a result of impaired kidney functions. Increased creatinine concentrations is in agreement with previous reports, Ghorbe et al, 2001. The effect was compared to controls. The ameliorating effect was more satisfactory with 2% garlic supplement group (T_3). 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. The assumption of oxidative stress as a mechanism in lead toxicity suggests that antioxidant action of garlic sulfhydryl groups might play a role in the treatment of lead (Pb) poisoning. The potential therapeutic action of garlic could be attributed to their chelating ability. About 50% of kidney function must be lost before a rise in the serum concentration of creatinine could be detected,Atef et al, 1994. Lead acetate in the diet significantly increases in serum urea and creatinine , El-,Ashmawy et al, 2005. There are not much data available

Table 04. Effect of Garlic on Cholesterol (mg/dl) in Lead (pb) Toxicity Induced Broiler Chickens

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day42
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±13.0041^{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 Values	0.0824 ^{NS}	0.0281 *	0.0372 *	0.0122 **	0.0325 *	0.0215*

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

Table 05. Effect of Garlic on Triglycerides (mg/dl) in Lead (pb) Toxicity Induced Broiler Chickens

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day42
T_0	45.92±0.21^{a}	39.39±1.12^{a}	52.18±0.77^{a}	41.44±1.77^{a}	39.80±1.06^{a}	42.65±0.61^{a}
T_1	71.69±0.51^{a}	77.07±3.71^{a}	93.12±5.09^{a}	104.46±6.59^{c}	116.94±6.54^{b}	144.17±9.50^{d}
T_2	65.28±0.20^{a}	78.25±2.06^{a}	85.62±2.46^{a}	98.67±2.18^{b}	112.54±2.29^{b}	106.37±1.51^{c}
T_3	51.95±0.96^{a}	64.28±2.06^{a}	75.28±2.28^{b}	66.95±2.20^{b}	76.34±2.67^{c}	64.68±2.99^{b}
T_4	58.67±0.42^{a}	72.11±2.58^{a}	84.25±2.90^{b}	84.27±3.12^{c}	81.24±2.99^{c}	87.67±2.69^{c}
P Values	0.0754 ^{NS}	0.0021 *	0.0422 *	0.0142 **	0.0495 *	0.0241*

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

on natural products therapy like garlic(, Ashour, 2002; Yassin, 2005. However, the ability of natural product garlic to combat lead (Pb) toxicity in chickens was examined and the findings could be useful to understand lead (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 04). Analysis of variance of data on serum total cholesterol level revealed significant difference between treatment groups. Garlic has been considered as one of the blood lipids lowering agent. The maximum serum cholesterol (364.28±12.331) was found in lead acetate group (T_1). Statistical analysis revealed significantly (P<0.01) lower level of serum cholesterol (203.62±12.762) was recorded in the chickens fed on Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) 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. 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. This study is in agreement with an earlier study the previous study reported by Konjufca et al, 1991. Organic tellurium compounds are found in high concentration in garlic buds which may contribute to lower the blood cholesterol levels by inhibiting squalene epoxidase, the penultimate enzyme in the synthetic pathway of cholesterol, Qureshi et al, 1983. Reduction in cholesterol and triglycerides with garlic has been reported previously by several researchers, Jain et al, 1983 , Konjufca, et al, 1997. Apparently, the allicin compounds in garlic help to block the creation of cholesterol. The way in which garlic accomplished this specific action is not totally understood. The presence of garlic provides a simple restriction in the rise of blood cholesterol. It may be concluded from the present study that garlic clearly suppresses cholesterol synthesis by lowering total serum cholesterol levels. It may appear to accomplish this by inhibiting the synthesis of harmful cholesterol which boosts the

Table 06. Effect of Garlic on Low Density Lipoprotein (LDL) (mg/dl) in Lead (pb) Toxicity Induced Broiler Chickens

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T_0	158.73±5.38 [a]	173.73±4.45 [a]	169.78±6.63[a]	161.57±7.36[a]	165.54±7.31[a]	175.22±6.17[a]
T_1	189.15±9.75 [a]	219.64±7.88 [b]	230.34±8.65[b]	278.69±12.10[b]	289.58±10.46[c]	312.21±7.19[d]
T_2	174.42±4.74 [a]	198.64±8.39 [a]	230.34±7.82[b]	247.16±7.03[b]	235.82±7.4914 [b]	245.81±4.65[c]
T_3	163.07±4.15 [a]	195.21±7.64 [b]	245.85±6.52[b]	214.17±7.55[c]	196.57±5.51[c]	188.62±5.67[c]
T_4	170.18±2.97 [a]	231.15±7.74 [b]	214.18±6.82[c]	226.94±9.52[c]	229.61±8.10c	223.65±4.81c
P Values	0.0642 [NS]	0.0062 **	0.0442 *	0.0012 **	0.0325 *	0.0241*

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

Table 07. Effect of Garlic on High Density Lipoprotein (HDL) (mg/dl) in Lead (pb) Toxicity Induced Broiler Chickens

Treatment	(Mean±SE)					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T_0	23.79±1.30[a]	23.84±1.67[a]	22.61±1.61[a]	24.27±0.74[a]	22.26±1.08[a]	23.28±0.79[a]
T_1	25.91±0.61[a]	21.77±0.57[a]	21.42±0.56[a]	19.66±0.53[a]	17.85±0.51[b]	15.27±0.51[b]
T_2	27.96±0.88[a]	25.17±1.25[a]	25.94±1.23[b]	26.33±0.75[b]	24.38±1.18[c]	27.92±0.56[c]
T_3	29.99±1.04[a]	27.36±1.06[a]	31.28±0.76[a]	30.87±0.71[a]	34.11±0.58[b]	39.18±0.49[c]
T_4	29.15±0.97[a]	26.38±0.89[a]	25.69±0.86[b]	29.65±0.68[b]	27.24±0.62[b]	30.82±0.51[c]
P Values	0.0954 [NS]	0.0421 *	0.0342 *	0.0142 **	0.0455 *	0.0142*

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

amount of beneficial cholesterol in the blood. Garlic's ability to affect a significant reduction in cholesterol appears to be dose-dependent. Garlic contains the highest level of the antioxidant selenium, which affords excellent cellular protection. Garlic supplementation significantly decreased 3-hydroxy-3-methylglutaryl reductase activity and cholesterol 7 alpha-hydroxylase activity, Konjufca et al, 1997. The cholerectic function may be caused from the dietary intake of garlic due to having soluble organophosphorous compound called S-allyl disulfide (SAC). This substance is a potent cholesterol synthesis inhibitor , Baccarelli, 1999. High levels of triglycerides can lead (Pb) to serious illnesses including cardiac failure, renal dysfunction etc. The results of blood triglyceride were presented in Table 05. The chicks fed lead acetate @100mg/kg diet with 2% garlic supplementation had significantly less value of blood triglycerides compared with lead acetate @100mg/kg diet group. Statistical analysis indicated that significantly (P<0.01) minimum level of serum Triglycerides (64.68±2.991) was recorded in the chickens fed on Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) group and the maximum blood triglyceride (144.17±9.5022) was found in Lead Acetate group (T_1). Reduction in triglycerides with garlic has also been reported previously, Gurer et al, 1998; Konjufca et al, 1997. Allicin is a major component of garlic organosulfurs and its antioxidant properties has already been confirmed previously. In addition to allicin, other garlic organosulfurs, such as alliin, allyl cysteine, allyl disulfide, and diallyl disulfide, possess antioxidant properties and could neutralize several types of ROS, Chung, 2006. Low density lipoprotein (LDL) is the major cholesterol carrier in the blood. From Table 06, it is evident that Low density lipoprotein (LDL) values in lead (Pb) exposed chickens differed significantly (P<0.01) in compared to the only lead acetate group (T_2). The maximum low density lipoprotein (LDL) value (312.21±7.196) was found in Lead acetate @ 100mg/kg group (T_1). Statistical analysis revealed that significantly (P<0.01) minimum level of Low density lipoprotein (LDL) (188.62±5.672) was detected in the chickens fed on Lead Acetate @ 100mg/Kg + 2% Garlic supplement group (T_3). Increased levels of low density lipoprotein (LDL), cholesterol and triglycerides are

Table 08. Effect of Garlic on Serum Glucose (mg/dl) in Lead (pb) Toxicity Induced Broiler Chickens

Treatment	Mean ± SE					
	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
T_0	224.66±17.30[a]	209.62±12.11[a]	205.84±10.03[a]	229.15±11.67[a]	213.95±5.70[a]	226.05±6.27[a]
T_1	258.36±20.19[a]	264.37±16.55[b]	315.09±15.04[b]	320.64±15.60[c]	326.38±13.21[c]	356.37±13.44[c]
T_2	237.06±20.37[a]	249.61±16.97[a]	263.57±16.66[c]	275.63±13.60[b]	255.95±12.53[b]	251.96±12.43[c]
T_3	229.73±20.31[a]	222.51±19.84[a]	218.83±15.06[b]	211.24±18.20[b]	201.56±18.67[c]	187.88±14.79[c]
T_4	234.75±20.20[a]	245.92±17.28[c]	276.95±16.52[b]	298.67±15.91[d]	246.31±17.43[c]	235.96±15.53[b]
P Values	0.0715 [NS]	0.0062 *	0.0312 *	0.00142 **	0.0345 *	0.026*

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

major risk factors for health soundness. 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. Clinical studies show that S-allyl cysteine from garlic helps to reduce the health risk. Garlic may serve as a safe and effective cholesterol-lowering nutrient without any side effects. Garlic has an abundance of sulfhydryl which is an excellent antioxidant. Cysteine, glutamine, isoleucine and methionine found in garlic help to protect the cells from free radical damage. Garlic or allicin supplement significantly inhibited hypercholesterolemia, lowered low density lipoprotein (LDL) concentrations and raised high density lipoprotein (HDL) concentrations, Jain, 1977. Statistical analysis revealed that significantly (P<0.01) higher level of high density lipoprotein (HDL) (39.18±0.493) was recorded in the chickens fed on Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) group and the minimum level of high density lipoprotein (HDL) (15.27±0.512) was found in Lead acetate group (T_1) (Table 07). Reports from previous studies suggested that oral garlic supplementation may be effective in reducing total lipid levels (Lau et al., 1987; Plengvidhya et al., 1988) Recent reports demonstrated that garlic powder preparations reduced lipoprotein oxidation susceptibility in vitro and in vivo, Kourounakis, 1991. The clinical utility of antioxidant activity is not clear to researchers. However, researchers studied the sulfur compounds of garlic resulting the inhibition of lipid peroxidation. Extensive data strongly suggests that using garlic can decrease the phospholipid content of the blood. The mean values of serum glucose of both control and experimental birds are shown in Table 8. Oral administration of lead acetate @ 100mg/kg caused a significant (P<0.01) increased value in glucose levels after 42 days of treatment compared to control values. However, the garlic supplement in lead (Pb) toxicity induced chickens provoked a significant interaction in glucose levels 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) group after 42 days of treatment, respectively. The most significant (P<0.01) ameliorating effect in blood glucose level in lead (Pb) toxicity induced broiler chickens was more pronounced with Lead Acetate @ 100mg/Kg + 2% Garlic supplement (T_3) therapy. Analysis of variance statistically revealed that significant (P<0.01) lower level of blood glucose was 187.88±14.792. Lead (Pb) toxicity was 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, lead (Pb) might be regarded as a risk factor in the abnormal glucose metabolism seen in some kinds of neurodegenerative disorders, Ahrens, 1993. Many environmental and occupational agents including lead (Pb) have

been shown to cause detrimental effects on endocrine function related to glucose metabolism (Baccarelli, 1999). Treatment with natural garlic revealed improvement in serum glucose level particularly. This result coincided with that reported previously, Ashour, 2002. The protection action of garlic against lead toxicity could be attributed to the antioxidant action of its sulfhydryl groups. The potential action of garlic in returning serum glucose to about its normal level coincided with their function as chelating agents , Marija et al, 2004; Yokoyama et al.,1998. Garlic has been found to be useful in controlling glucose tolerance and is beneficial for both hypo and hyperglycemia. The allicin compounds of garlic have been found to possess a significant blood sugar lowering action. Clinical studies have suggested that these compounds lower glucose levels by competing with insulin sites in the liver.

CONCLUSION

Garlic has an abundance of sulfhydryl which may act as an excellent antioxidant. The present study was undertaken to determine the therapeutic ability of natural substances garlic (*Aullium sativum*) on biochemical parameter in lead (Pb) toxicity induced broiler chickens. It was concluded from the present study that garlic might have some therapeutic effect in reducing toxic effect of lead (Pb) on biochemical processes.

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