



Effect of The Garlic as Chelation Therapy in Reducing Lead and Cadmium Deposition on Suckling Mice

Bidyut Das, Md. Anwar Hossain, Md. Siddiquil Islam, Md. Aktaruzzaman Zullhash, Md. Mowdudul Hasan Talha, Shahab Uddin Munna, Dwija Sutradhar, and Ahasan Ullah Khan*

Received : October 19, 2024

Revised : November 28, 2024

Accepted : December 22, 2024

Online : December 28, 2024

Abstract

The study was conducted on suckling mice to determine the effects of chelation therapy in reducing the deposition of lead (Pb) and cadmium (Cd). A total of 45 suckling mice were randomly divided into three main groups A (control), B, and C. Groups B and C contained Pb and Cd, respectively, at a concentration of 100 mg/kg bwt with 0, 1.70, 3.35, and 6.70% garlic given (B₁, B₂, B₃, B₄, and C₁, C₂, C₃, C₄). Mice exposed to Pb and Cd exhibited pronounced toxic symptoms along with a marked decrease in total erythrocyte and total leukocyte count, hemoglobin levels, and packed cell volume. Additionally, there is a significant increase in serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) levels. The mean body weight of mice of groups B₄ and C₄ was the highest among the treated groups. Groups B₁ and C₁, exposed to Pb and Cd without garlic, showed significant declines in all parameters. Group A (control) shows stable and normal SGOT and SGPT levels. Group C₁, exposed to Cd without garlic, experiences the highest increases in both SGOT (98.53 U/L) and SGPT (132.83 U/L), indicating severe liver damage. The group treated with Pb and Cd showed a significant reduction in total erythrocyte count, packed cell volume, and hemoglobin levels after 42-d of treatment. However, mice treated with a combination of Pb, Cd, and 6.70% garlic exhibited nearly normal levels of hematological and biochemical parameters. SGPT and SGOT levels were significantly decreased in all treated groups along with garlic. This experiment demonstrates that garlic possesses both protective and curative effects against Pb and Cd toxicity.

Keywords: cadmium, chelation, garlic, lead, suckling mice

1. INTRODUCTION

Cadmium (Cd) and lead (Pb) are widely recognized as toxic heavy metals present in the environment. People are commonly exposed to Cd and Pb through various sources, including air, food, industrial materials, drinking water, and consumer goods [1]. Currently, developing countries are experiencing the most severe issues related to Cd and Pb pollution. Cd exposure has been linked to lungs, kidneys, hepatic, as well as skeletal, reproductive, and cardiovascular dysfunctions [2]-[4].

The primary treatment method for heavy metal poisoning is chelation therapy, which enhances the excretion of metals from the body by administering chelating agents [5]. However, no chelation

therapies for Cd toxicity have been clinically approved to date [6]. Chelators like CaNa₂EDTA and meso-2,3-dimercaptosuccinic acid (DMSA) have demonstrated protective effects against Pb toxicity. Nonetheless, CaNa₂EDTA can lead to kidney toxicity, particularly affecting the proximal tubule, especially when used in repeated high doses (over 75 mg/kg) or in individuals with preexisting kidney conditions [7]. As a result, ongoing research is focused on developing safer and more effective treatments for Cd and Pb toxicity.

Recent research has focused on using natural substances such as vitamins, minerals, and herbal remedies to reduce lead absorption and mitigate its toxicity. Garlic (*Allium sativum*) has been found to counteract the harmful effects of lead, suggesting it may contain chelating agents capable of removing lead from the body [8]. Allicin (diallyl thiosulfinate), the primary thiosulfinate in garlic, is produced when the enzyme alliinase interacts with its substrate alliin [9]. Allicin exhibits a wide range of biological activities, including antimicrobial, hypolipidemic, antithrombotic, anticancer, and antioxidant properties [10][11]. This study evaluated the effects of varying doses of allicin on blood Pb and Cd levels, as well as the accumulation of these metals in various tissues in mice exposed to Pb and Cd. The objective was to assess the role

Publisher's Note:

Pandawa Institute stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright:

© 2024 by the author(s).

Licensee Pandawa Institute, Metro, Indonesia. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Table 1. Experimental design to evaluate the effect of garlic concentrations on Pb and Cd-treated rats.

Group	Subgroup	Treatment	Garlic Concentration
A		Control	0%
	B1		0%
B	B2	Lead acetate (100 mg/kg body weight)	1.70%
	B3		3.35%
	B4		6.70%
	C1		0%
C	C2	Cadmium (100 mg/kg body weight)	1.70%
	C3		3.35%
	C4		6.70%

of chelation therapy in reducing Pb and Cd deposition in suckling mice.

2. MATERIALS AND METHODS

2.1. Experimental Site and Period

This research was carried out in the Department of Pharmacology and Toxicology laboratory at Sylhet Agricultural University, Bangladesh during the period from July to August 2018.

2.2. Animals Grouping

The experiment was conducted using 45 Swiss albino mice, each weighing approximately 13–14 g. They were maintained on a standard pellet diet with free access to drinking water. The mice were kept under close observation to ensure they remained in good health throughout the seven-week study period. All animals were randomly assigned into three main groups labeled A, B, and C. Group A served as the control, including 5 mice. Groups B and C were further subdivided into B1, B2, B3, B4 and C1, C2, C3, C4, with each subdivision containing 5 mice. Treatment was administered once daily using a measuring dropper for 7 consecutive weeks. After the experiment, all mice were humanely sacrificed for further examination.

2.3. Preparation of Solution

The required amounts of chemicals (lead acetate, cadmium chloride, and garlic) were accurately measured using an electronic balance. The garlic bulbs were carefully cleaned and stored at temperatures between 0 and 4 °C. Afterward, the garlic was crushed in a mixing machine to create a

slurry, which was then squeezed and filtered through fine cloth; the resulting filtrate was quickly frozen until needed.

2.3.1. Garlic Concentration

The concentrations of 1.70, 3.35, and 6.70% garlic solutions are prepared by dissolving 1.70, 3.35, and 6.70 g of garlic powder in 100 mL of water. These amounts correspond to the desired percentage of garlic powder in the final solution, with each concentration representing the weight of garlic powder per 100 mL of solvent.

2.3.2. Treatment Solutions Preparation

The experimental design consisted of three main groups (Table 1). Group A served as the control, receiving normal feed and water. In group B, mice were administered lead acetate at 100 mg/kg body weight with the following garlic concentrations: B1 received 0% garlic, B2 received 1.70% garlic, B3 received 3.35% garlic, and B4 received 6.70% garlic. Similarly, in group C, mice were given cadmium at 100 mg/kg body weight, with C1 receiving 0% garlic, C2 receiving 1.70% garlic, C3 receiving 3.35% garlic, and C4 receiving 6.70% garlic.

2.4. Toxic Signs and Body Weight

Mice in 3 separate groups were administered lead acetate, cadmium chloride, and garlic over 42 consecutive days. Throughout the experiment, both control and treated mice were closely monitored for any signs of toxicity. Body weight measurements were recorded at the beginning of the experiment and at 14-day intervals throughout the 42-day study.

2.5. Gross Pathological Changes

After the 42-day experimental period, all mice were humanely sacrificed and examined to assess postmortem changes.

2.6. Hematological Parameters

Hematological parameters, including total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin concentration (Hb%), and packed cell volume (PCV), were assessed. Blood samples were collected from the tail and heart of control and treated mice, both before treatment and after 42 d, while under diethyl ether anesthesia. The blood was immediately transferred into sterile test tubes containing anticoagulants in a 1:10 ratio. The collected samples were analyzed for hematological parameters within 2 h. The methods followed for these determinations were based on the procedure described [12]. Hemoglobin levels were measured using the acid hematin method as outlined [13].

2.7. Biochemical Parameters

Biochemical parameters, including serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT), were evaluated. Blood samples were collected on the 42-day treatment from both the control and experimental mice. After 42 d, the mice were fasted overnight. Anesthesia was administered using diethyl ether in a desiccator, and blood was drawn directly from the heart using a disposable syringe and needle after opening the abdominal cavity. The biochemical parameters were measured using Reflodon® (Boehringer Mannheim), following the procedure outlined [14], with enzyme activity expressed in U/L.

2.8. Statistical Analysis

After thorough examination and coding, the information obtained was entered into a Microsoft Excel 2016 database. The mean $\pm$ standard error (SE) was used to express the results. The R program was used for the statistical analysis. Before the official study, the Shapiro-Wilk test was used to verify normality, and Levene's test was utilized to examine for homogeneity of variations. To evaluate differences across groups (A, B₁, B₂, B₃, and B₄) throughout time points (day 0, 14, 28, and 42), a repeated measures analysis of variance (ANOVA) was used. This approach offered a worldwide test for variations and took into consideration the correlation between repeated measures within the same group. Tukey's HSD was utilized for pairwise comparisons to pinpoint certain group-time interactions in instances where ANOVA findings were significant. A student's t-test with Bonferroni correction was used to account for numerous comparisons for each pair of results at particular time points [15]. The ANOVA and Cohen's d for t-tests were used to determine the effect size for group differences. The threshold for statistical significance was set at ($p < 0.05$, <0.01), and superscripts (such as "a," "b," and "c") were used in the tables to denote significant differences.

3. RESULTS AND DISCUSSION

3.1. Effects of Pb on Body Weight and Chelation Therapy

The body weight of mice treated with garlic in the presence of lead acetate was recorded over 42 d (Table 2). At 0-day, initial body weights were relatively similar across the groups, though group

Table 2. Effect of garlic in lead acetate induced toxicities on body weight in mice.

Group	Day 0	Day 14	Day 28	Day 42
A (Control)	13.80 $\pm$ 0.32 ^b	16.48 $\pm$ 0.30 ^b	18.36 $\pm$ 0.11 ^a	22.32 $\pm$ 0.28 ^a
B ₁	14.68 $\pm$ 0.02 ^a	16.30 $\pm$ 0.63 ^c	17.21 $\pm$ 0.09 ^b	17.52 $\pm$ 0.39 ^c
B ₂	14.55 $\pm$ 0.18 ^a	16.58 $\pm$ 0.35 ^b	17.81 $\pm$ 0.37 ^a	18.37 $\pm$ 0.42 ^b
B ₃	14.26 $\pm$ 0.36 ^b	17.25 $\pm$ 0.26 ^a	18.10 $\pm$ 0.77 ^a	19.69 $\pm$ 1.12 ^b
B ₄	14.17 $\pm$ 0.28 ^b	16.72 $\pm$ 0.52 ^b	18.28 $\pm$ 0.37 ^a	21.04 $\pm$ 1.28 ^a
Significance level	*	*	*	*

Note: Body weight in g. A=No treatment, given normal feed and water as per requirement. B₁=Lead acetate 100 mg/kg bwt with 0% garlic. B₂=Lead acetate 100 mg/kg bwt with 1.70% garlic. B₃=Lead acetate 100 mg/kg bwt with garlic 3.35%. B₄=Lead acetate 100 mg/kg bwt with 6.70% garlic. Values above represented as the mean $\pm$ SE for mice in each group, *Significantly increased ($p < 0.05$).

Table 3. Effect of garlic in cadmium chloride-induced toxicities on body weight in mice.

Group	Day 0	Day 14	Day 28	Day 42
A (Control)	14.29±0.17 ^a	17.50±0.11 ^a	19.64±0.50 ^a	22.53±0.45 ^a
C ₁	14.11±0.31 ^a	16.20±0.82 ^b	17.19±0.24 ^b	17.42±0.72 ^b
C ₂	14.36±0.14 ^a	16.57±0.47 ^a	17.76±0.99 ^a	18.87±0.21 ^b
C ₃	13.15±0.10 ^b	16.39±0.17 ^a	17.27±0.17 ^b	18.82±0.96 ^b
C ₄	13.82±0.28 ^a	16.29±0.11 ^b	18.33±0.65 ^a	20.36±0.36 ^a
Significance level	*	*	*	*

Note: Body weight in g. A=No treatment, given normal feed and water as per requirement. C₁=Cadmium chloride 100 mg/kg bwt with 0% garlic. C₂= Cadmium chloride 100 mg/kg bwt with 1.70% garlic. C₃= Cadmium chloride 100 mg/kg bwt with garlic 3.35%. C₄= Cadmium chloride 100 mg/kg bwt with 6.70% garlic. Values above represent the mean ± SE for mice in each group, *Significantly increased (p<0.05).

B1 had the highest weight at 14.68±0.02 g, followed closely by group B2 at 14.55±0.18 g. The control group (A) weighed 13.80±0.32 g, which was slightly lower than groups B1 and B2. While, groups B3 and B4 had body weights of 14.26±0.36 and 14.17±0.28 g, respectively. By 14 days, the control group (A) showed a significant increase in body weight, reaching 16.48±0.30 g. Groups B2 and B4 had similar weights, recording 16.58±0.35 and 16.72±0.52 g, respectively, both of which were comparable to the control group. In contrast, group B1 exhibited a reduction in weight to 16.30±0.63 g, while Group B3 showed the highest weight at this time point, increasing to 17.25±0.26 g (p<0.05). At 28 days, the control group (A) continued to show the highest body weight at 18.36±0.11 g, closely followed by groups B3 and B4, which recorded weights of 18.10±0.77 and 18.28±0.37 g, respectively. Group B1 showed a significantly lower weight compared to the control group, dropping to 17.21±0.09 g (p<0.05), while group B2 maintained a moderate weight at 17.81±0.37 g. By 42 days, the control group reached the highest body weight of 22.32±0.28 g. Group B4 also showed a notable increase, reaching 21.04±1.28 g, which was comparable to the control (p<0.05). Groups B1 and B2, however, had significantly lower weights of 17.52±0.39 and 18.37±0.42 g, respectively. Group B3 reached 19.69±1.12 g, which was higher than Groups B1 and B2 but still lower than the control and B4 groups. This improvement in body weight may be a result of garlic supplements during the toxicity period [15]. Rahman also reported significantly reduced body weight due to Pb toxicity in rats on 60, 75, and 90 days of treatment but the Pb with garlic group didn't show significant weight

gain [16]. The decrease in body weight might be due to the disruption in the interest and metabolism of food nutrients crucial for fitness [17]. Dhar and Banerjee reported no significant effect on body weight in lead acetate-treated rats at 3.4 mg/100kg b.wt. for 4 d which differs from our findings [18]. This difference in result might be due to the shorter treatment period.

3.2. Effects of Cd on Body Weight and Chelation Therapy

The impact of garlic on body weight in mice exposed to cadmium chloride was observed over 42 day period (Table 3). On 0 day, there were no significant differences in the initial body weights across groups. By 14 days, the control group (A) showed a notable increase in weight, reaching 17.50±0.11 g, whereas group C1, treated with cadmium chloride, exhibited significantly lower weight gain (16.20±0.82 g). Groups C2 and C3 demonstrated slight improvements in weight gain, recording 16.57±0.47 and 16.39±0.17 g, respectively. By 28 days, the control group continued to gain weight (19.64±0.50 g), while Cd-treated groups (C1, C2, and C3) showed reduced weight gains compared to the control. Mice in group C4, which received garlic, showed a more substantial increase in weight (18.33±0.65 g) compared to the other Cd-treated groups. By 42 days, the control group reached a final weight of 22.53±0.45 g, significantly higher than Cd-treated groups. Group C1 maintained the lowest weight (17.42±0.72 g), while groups C2 and C3 had moderate weight gains, reaching 18.87±0.21 and 18.82±0.96 g, respectively. Group C4, treated with garlic, demonstrated a substantial protective effect,

Table 4. Hematological parameters of mice treated with Pb and/or garlic.

Group	TEC (million/mm ³)		Hb (gm%)		PCV (%)		TLC (thousand/mm ³)	
	Day 0	Day 42	Day 0	Day 42	Day 0	Day 42	Day 0	Day 42
A (Control)	8.78±0.56 ^a	8.96±0.40 ^a	9.69±0.74 ^a	9.78±0.15 ^a	38.71±0.35 ^a	38.30±0.41 ^a	9.16±0.85 ^a	8.89±0.59 ^a
B ₁	8.14±0.23 ^b	6.07±0.66 ^b	9.63±0.69 ^a	7.22±0.32 ^c	37.86±0.79 ^b	29.11±0.64 ^c	9.21±0.67 ^a	6.24±0.55 ^c
B ₂	8.06±0.02 ^b	7.45±0.51 ^{ab}	9.40±0.12 ^b	8.59±0.41 ^{bc}	39.57±0.63 ^a	33.89±0.32 ^b	9.06±0.74 ^a	6.13±0.17 ^{cd}
B ₃	8.27±0.71 ^b	7.32±0.30 ^b	9.50±0.58 ^a	8.66±0.14 ^{ab}	38.41±0.18 ^a	32.78±0.58 ^b	9.28±0.38 ^a	7.70±0.35 ^{bc}
B ₄	9.18±0.07 ^a	8.50±0.45 ^a	9.30±0.55 ^b	8.82±0.20 ^a	38.20±1.02 ^a	35.78±1.40 ^b	8.87±0.93 ^b	7.45±0.23 ^b
Significance level	*	*	*	**	*	**	*	**

Note: A=No treatment, given normal feed and water as per requirement. B₁=Lead acetate 100 mg/kg bwt with 0% garlic. B₂=Lead acetate 100 mg/kg bwt with 1.70% garlic. B₃=Lead acetate 100 mg/kg bwt with garlic 3.35%. B₄=Lead acetate 100 mg/kg bwt with 6.70% garlic. Values above represent the mean ± SE for mice in each group. *Significantly decreased (p<0.01), **Significantly increased (p<0.05).

with a final weight of 20.36±0.36 g, indicating that garlic helped mitigate Cd toxicity. These changes in body weight across the groups were statistically significant (p<0.05) at all time points. The current study shows similarity with previous studies which reported improved body weight gain due to garlic supplementation followed by Cd toxicity in mice [19] and rats [20]. A decrease of body weight strength can be due to the interference of Cd with the preoccupation and metabolism of feed nutrients essential for health and the improvement in body weight might be the result of the protective effects of garlic against Cd toxicity [17][21][22].

3.3. Hematological Parameter of Pb-Induced Mice and Chelation Therapy

Table 4 represents the TEC, Hb, PCV, and TLC content of different groups of mice due to daily administration of lead acetate. At 0-d, the TEC was comparable among the groups, with group A (control) showing a TEC of 8.78±0.56 million/mm³, which was significantly higher than groups B₁ and B₂, which had TECs of 8.14±0.23 and 8.06±0.02 million/mm³, respectively (p<0.05). By 42 days, notable reductions in TEC were observed in the Pb-treated groups. Group B₁ exhibited a significant decrease to 6.07±0.66 million/mm³ (p<0.05), while group A maintained a stable TEC at 8.96±0.40 million/mm³. Groups B₂ and B₃ showed moderate decreases to 7.45±0.51 and 7.32±0.30 million/mm³, respectively, but were not as severely affected as group B₁. In contrast, group B₄ showed a TEC of 8.50±0.45 million/mm³, which was not significantly different from the control.

In the case of Hb levels, at 0-d, Hb levels did not differ significantly among the groups, with values ranging from 9.30±0.55 gm% in group B₄ to 9.69±0.74 gm% in the control group (A). By 42-d, a significant change in Hb levels was noted. Group B₁ saw a substantial decline to 7.22±0.32 gm% (p<0.01), while the control group maintained a stable Hb level of 9.78±0.15 gm%. Groups B₂ and B₃ also experienced decreases in Hb, with levels of 8.59±0.41 and 8.66±0.14 gm%, respectively. Group B₄ exhibited a stable Hb level at 8.82±0.20 gm%, indicating lesser impact compared to the other groups.

In the case of PCV, all groups exhibited similar PCV values, ranging from 37.86±0.79% to

39.57±0.63% at 0-d. By 42-d, significant reductions in PCV were observed across the treatment groups. Group B1 recorded a PCV of 29.11±0.64% ($p < 0.01$), a substantial decrease from the control's 38.30±0.41%. Groups B2 and B3 also showed declines in PCV, with values of 33.89±0.32% and 32.78±0.58%, respectively ($p < 0.05$). Group B4 maintained a relatively higher PCV at 35.78±1.40%.

On the other hand, at 0-d, TLC values were similar across groups, with group A showing 9.16±0.85 thousand/mm³. By 42-d, significant reductions in TLC were evident among the Pb-treated groups. Group B1 had the lowest TLC, dropping to 6.24±0.55 thousand/mm³ ($p < 0.01$), compared to the control group's 8.89±0.59 thousand/mm³. Groups B2 and B3 had TLC values of 6.13±0.17 and 7.70±0.35 thousand/mm³, respectively, indicating significant declines, while group B4 showed a TLC of 7.45±0.23 thousand/mm³. Previous studies reported the reduction of TEC, Hb, TLC, and PCV values due to Pb toxicity and improved TEC, Hb, TLC, and PCV values after providing garlic supplements [15][16].

3.4. Hematological Parameter of Cd-Induced Mice and Chelation Therapy

Table 5 represents the TEC, Hb, PCV, and TLC content of different groups of mice due to daily administration of cadmium chloride. In the case of TEC, there were minor variations in TEC among the groups, but these changes were statistically significant ($p < 0.05$) at 0-d. Group C2 had the highest TEC at 9.32±1.09 million/mm³, while the control group (A) recorded a TEC of 8.04±0.65 million/mm³. By 42-d, significant reductions in TEC were observed in the experimental groups exposed to cadmium, particularly in group C1, which showed the most pronounced decrease to 4.79±0.42 million/mm³ ($p < 0.01$). In contrast, group A (control) maintained a relatively stable TEC of 7.98±0.26 million/mm³. Groups C2, C3, and C4 demonstrated moderate reductions, with TEC values of 6.05±0.39, 6.18±1.59, and 6.81±0.45 million/mm³, respectively ($p < 0.05$).

In the case of Hb levels, Hb levels ranged from 8.73 to 10.33 gm% at 0-d, with group C4 showing the highest value (10.33±0.58 gm%) and group C3 is the lowest (8.73±1.49 gm%). By 42-d, significant

Table 5. Hematological parameters of mice treated with Cd and/or garlic.

Group	TEC (million/mm ³)		Hb (gm%)		PCV (%)		TLC (thousand/mm ³)	
	Day 0	Day 42	Day 0	Day 42	Day 0	Day 42	Day 0	Day 42
A (Control)	8.04±0.65 ^{bc}	7.98±0.26 ^a	9.57±0.68 ^b	10.28±0.08 ^a	37.54±0.49 ^b	38.19±0.25 ^a	9.21±0.77 ^a	9.18±0.39 ^a
C ₁	8.19±0.73 ^b	4.79±0.42 ^d	9.04±0.76 ^{bc}	5.51±0.09 ^c	38.67±0.96 ^a	23.08±0.23 ^d	9.52±0.57 ^a	5.57±0.27 ^d
C ₂	9.32±1.09 ^a	6.05±0.39 ^{bc}	9.84±0.27 ^a	8.28±0.11 ^b	38.93±0.47 ^a	32.27±0.36 ^b	9.34±0.88 ^a	5.28±0.45 ^d
C ₃	8.74±0.81 ^a	6.18±1.59 ^b	8.73±1.49 ^c	8.20±0.07 ^b	37.66±0.82 ^b	31.12±0.09 ^c	8.71±0.21 ^b	6.36±0.31 ^c
C ₄	8.27±0.42 ^b	6.81±0.45 ^a	10.33±0.58 ^a	9.67±0.07 ^a	38.43±0.18 ^a	33.85±0.51 ^b	9.66±0.06 ^a	7.14±0.34 ^b
Significance level	*	**	*	*	*	*	*	**

Note: A=No treatment, given normal feed and water as per requirement. C₁=Cadmium chloride 100 mg/kg bwt with 0% garlic. C₂=Cadmium chloride 100 mg/kg bwt with 1.70% garlic. C₃=Cadmium chloride 100 mg/kg bwt with 3.35% garlic. C₄=Cadmium chloride 100 mg/kg bwt with 6.70% garlic. Values above represent the mean ± SE for mice in each group. *Significantly decreased ($p < 0.01$), **Significantly increased ($p < 0.05$).

changes were observed. Group C1 showed the largest decrease in Hb, dropping to 5.51 ± 0.09 gm% ($p < 0.05$), whereas the control group (A) maintained a stable Hb level of 10.28 ± 0.08 gm%. Group C4 showed a lesser decline to 9.67 ± 0.07 gm%, while Groups C2 and C3 had Hb levels of 8.28 ± 0.11 and 8.20 ± 0.07 gm%, respectively.

In the case of PCV, all groups had similar PCV levels, with values ranging from $37.54 \pm 0.49\%$ to $38.93 \pm 0.47\%$, indicating no significant differences at 0-d. By 42-d, significant decreases were observed in all experimental groups. Group C1 experienced the most dramatic reduction in PCV, falling to $23.08 \pm 0.23\%$ ($p < 0.05$), while the control group (A) maintained a steady level at $38.19 \pm 0.25\%$. Groups C2, C3, and C4 showed declines to $32.27 \pm 0.36\%$, $31.12 \pm 0.09\%$, and $33.85 \pm 0.51\%$, respectively.

The TLC were relatively consistent across all groups, with values ranging from 8.71 to 9.66 thousand/mm³ at 0-d. By 42-d, significant reductions in TLC were observed in the Cd-treated groups. Group C1 had the largest drop, with TLC decreasing to 5.57 ± 0.27 thousand/mm³ ($p < 0.01$). In contrast, group A (control) showed no significant change, maintaining a TLC of 9.18 ± 0.39 thousand/mm³. Group C4 showed a moderate reduction in TLC to 7.14 ± 0.34 thousand/mm³ ($p < 0.01$), while groups C2 and C3 recorded lower TLC levels at 5.28 ± 0.45 and 6.36 ± 0.31 thousand/mm³, respectively. Cadmium chloride caused changes in the blood indices of rats [17]. The decrease in Hb satisfied may be due to an enlarged rate of devastation or a decrease in the rate of construction of erythrocytes which is apparent by the low of

TEC in the preserved groups in the present study. The deduction in the blood parameters (PCV, RBC, and Hb) may be qualified to hyperactivity of the bone heart that leads to the construction of red blood cells with decreased integrity that easily destructed in circulation [23]. However, garlic supplements showed improved hematological parameters than only Cd-treated group which is similar to the results reported Eteng, et al. which found that garlic succeeded in improving the biochemical parameters of the kidney and liver towards regular values [19].

3.5. Biochemical Parameters of Pb-Induced Mice and Chelation Therapy

The effect of garlic on Pb-induced toxicities was assessed by measuring SGOT/ALT and SGPT/AST in mice on day 0 to 42 (Table 6). In the case of SGOT (ALT) levels, at 0-d, no significant differences were observed in SGOT (ALT) levels between groups A, B1, B2, B3, and B4, with values ranging from 63.12 to 64.91 U/L, indicating that all groups had similar baseline levels. By 42-d, significant differences were observed across the groups. The control group (A) maintained a stable SGOT level at 63.82 ± 1.2 U/L. However, group B1 showed a significant increase, with SGOT levels rising to 108.22 ± 0.43 U/L ($p < 0.05$), the highest among the groups. Group B2 also exhibited a significant increase, reaching 96.50 ± 0.57 U/L ($p < 0.05$). Groups B3 and B4 showed moderate but significant increases in SGOT levels, recording 89.97 ± 0.57 and 90.36 ± 0.46 U/L, respectively ($p < 0.05$).

Table 6. Effect of garlic in Pb-induced toxicities on SGOT and SGPT in mice.

Group	SGOT (U/L)		SGPT (U/L)	
	Day 0	Day 42	Day 0	Day 42
A (Control)	64.46 ± 0.33^a	63.82 ± 1.20^d	94.58 ± 0.74^a	92.33 ± 0.47^d
B ₁	63.67 ± 0.13^a	108.22 ± 0.43^a	90.20 ± 0.28^b	116.90 ± 0.92^a
B ₂	64.82 ± 0.72^a	96.50 ± 0.57^b	93.85 ± 0.81^a	105.05 ± 0.72^b
B ₃	64.91 ± 0.45^a	89.97 ± 0.57^c	93.68 ± 0.59^a	108.23 ± 0.54^b
B ₄	63.12 ± 0.88^a	90.36 ± 0.46^b	91.27 ± 0.63^b	101.45 ± 0.17^c
Significance level	NS	*	*	**

Note: A=No treatment, given normal feed and water as per requirement. B₁=Lead acetate 100 mg/kg bwt with 0% garlic. B₂=Lead acetate 100 mg/kg bwt with 1.70% garlic. B₃=Lead acetate 100 mg/kg bwt with garlic 3.35%. B₄=Lead acetate 100 mg/kg bwt with 6.70% garlic. Values above represent the mean $\pm$ SE for mice in each group, **Significantly decreased ($p < 0.01$), *Significantly increased ($p < 0.05$).

Table 7. Effect of garlic in Cd-induced toxicities on SGOT and SGPT in mice.

Group	SGOT (U/L)		SGPT (U/L)	
	Day 0	Day 42	Day 0	Day 42
A (Control)	63.82±0.57 ^a	62.86±0.07 ^d	89.06±0.74 ^a	89.21±0.31 ^d
C ₁	62.02±1.23 ^a	98.53±0.62 ^a	92.23±0.05 ^a	132.83±0.42 ^a
C ₂	63.65±0.54 ^a	89.62±0.56 ^b	90.52±0.45 ^a	120.45±0.44 ^b
C ₃	63.88±0.87 ^a	84.71±0.17 ^b	90.67±0.57 ^a	118.58±0.74 ^b
C ₄	62.19±0.62 ^b	82.34±0.22 ^c	92.44±0.11 ^a	111.44±0.57 ^c
Significance level	*	*	*	**

Note: A=No treatment, given normal feed and water as per requirement. C₁=Cadmium chloride 100 mg/kg bwt with 0% garlic. C₂= Cadmium chloride 100 mg/kg bwt with 1.70% garlic. C₃= Cadmium chloride 100 mg/kg bwt with garlic 3.35%. C₄= Cadmium chloride 100 mg/kg bwt with 6.70% garlic. Values above represent the mean ± SE for mice in each group, **Significantly decreased (p<0.01), *Significantly increased (p<0.05).

In the case of SGPT (AST) levels, at 0-d, SGPT (AST) levels were relatively similar across the groups, with no significant differences observed, and values ranging from 90.20 to 94.58 U/L. By 42-d, significant elevations in SGPT levels were noted in all investigational groups associated with the control. Group A (control) maintained an SGPT level of 92.33±0.47 U/L, while Group B1 showed the highest increase to 116.90±0.92 U/L, which was significantly greater than all other groups (p < 0.01). Group B2 also demonstrated a significant rise to 105.05±0.72 U/L (p < 0.05), followed by Group B3 at 108.23±0.54 U/L (p < 0.05) and Group B4 at 101.45±0.17 U/L (p < 0.01).

3.6. Biochemical Parameters of Cd-Induced Mice and Chelation Therapy

The effect of garlic supplementation on Cd-induced toxicity was assessed by measuring SGOT and SGPT in mice from day 0 to 42 (Table 7). In the case of SGOT levels, at 0-d, there were significant differences (p<0.05) in SGOT levels between any of the groups A, C₁, C₂, C₃, and C₄, with values ranging from 62.02 to 63.88 U/L. Previously, Arfat, et al. reported significant advancement in SGOT and SGPT's next cadmium chloride administration at diverse doses [24]. By 42-d, significant changes in SGOT levels were observed. The control group (A) maintained a steady level of 62.86±0.07 U/L. However, Group C₁ exhibited the highest increase, with SGOT levels rising significantly to 98.53±0.62 U/L (p < 0.05). Group C₂ also showed a significant rise to 89.62±0.56 U/L (p < 0.05). Groups C₃ and C₄ had

lower SGOT levels, recorded at 84.71±0.17 and 82.34±0.22 U/L, respectively, but these increases were still statistically significant compared to the control (p < 0.05).

In the case of SGPT levels, at 0-d, no significant variances were started between the groups for SGPT levels, with values ranging from 89.06 to 92.44 U/L. By 42-d, significant rises in SGPT levels were noted in all experimental groups compared to the control. Group A (control) maintained an SGPT level of 89.21±0.31 U/L, while group C₁ showed the highest increase, reaching 132.83±0.42 U/L, which was significantly higher than all other groups (p < 0.01). Groups C₂, C₃, and C₄ also showed elevated SGPT levels at 120.45±0.44, 118.58±0.74, and 111.44±0.57 U/L, respectively, with significant differences compared to the control (p < 0.05). Aspartate transaminases (AST) and alanine transaminases (ALT) are crucial for evaluating liver function. While ALT is primarily a cytoplasmic enzyme, AST is found in both the cytoplasm and mitochondria. Elevated ALT levels are commonly seen in acute hepatitis, whereas increased AST levels may indicate conditions such as myocardial infarction, cirrhosis, and liver diseases. Pb can accumulate in numerous body parts, including the bone, liver, kidney, heart, and spleen. Notably, group C₄ showed the lowest SGOT and SGPT levels, suggesting that garlic offers protective effects against Cd toxicity. Similarly, in groups C₂ and C₃ SGOT and SGPT levels were also low but higher than the 6.7% garlic-treated group [25][26].

4. CONCLUSIONS

Exposure to Pb and Cd demonstrated altered doings of exact enzymes, and hematological and biochemical directories in mice. These accounts Pb and Cd were sufficient enough to produce toxicity in hematopoietic tissues and different organs. Chelation therapy with garlic was able to reverse the variations in hematological and biochemical strictures and thus perfect the toxic effects of Pb and Cd. From the present study, it is settled that the chelation with garlic was active in reducing Pb and Cd toxicity and restoring the different values of biochemical and hematological parameters.

AUTHOR INFORMATION

Corresponding Author

Ahasan Ullah Khan — Department of Entomology, Sylhet Agricultural University, Sylhet-3100 (Bangladesh);

Email: ahasanullahsau@gmail.com

 orcid.org/0000-0002-7029-8215

Authors

Bidyut Das — Department of Pharmacology and Toxicology, Sylhet Agricultural University, Sylhet-3100 (Bangladesh);

 orcid.org/0009-0000-7318-4574

Md. Anwar Hossain — Department of Pharmacology and Toxicology, Sylhet Agricultural University, Sylhet-3100 (Bangladesh);

 orcid.org/0009-0008-2550-077X

Md. Siddiquil Islam — Department of Pharmacology and Toxicology, Sylhet Agricultural University, Sylhet-3100 (Bangladesh);

 orcid.org/0009-0000-4036-7809

Md. Aktaruzzaman Zullhash — Department of Pharmacology and Toxicology, Sylhet Agricultural University, Sylhet-3100 (Bangladesh);

 orcid.org/0009-0000-1366-1498

Md. Mowdudul Hasan Talha — Department of Pharmacology and Toxicology, Sylhet Agricultural University, Sylhet-3100 (Bangladesh);

 orcid.org/0009-0004-6605-1350

Shahab Uddin Munna — Department of Microbiology and Immunology, Sylhet Agricultural University, Sylhet-3100 (Bangladesh);

 orcid.org/0009-0004-1161-6908

Dwija Sutradhar — Department of Microbiology and Immunology, Sylhet Agricultural University, Sylhet-3100 (Bangladesh);

 orcid.org/0009-0003-7593-7551

Author Contributions

Conceptualization, B. D., M. A. H. and M. S. I.; Methodology, B. D. and M. A. Z., Software, M. H. T., S. U. M. and D. S.; Validation, B. D.; Analysis and Interpretation of Data, B. S., and A. U. K.; Data Curation, B. D., A. U. K., and S. U. M., Writing – Original Draft Preparation, B. D.; Writing – Review & Editing, M. A. H., M. S. I., M. A. Z., and A. U. K.; All authors have read and approved to the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

- [1] Z. Rahman and V. P. Singh. (2019). "The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr) (VI), mercury (Hg), and lead (Pb)) on the total environment: an overview". *Environmental Monitoring and Assessment*. **191** (7): 419. [10.1007/s10661-019-7528-7](https://doi.org/10.1007/s10661-019-7528-7).
- [2] F. Hong, T. Jin, and A. Zhang. (2004). "Risk assessment on renal dysfunction caused by co-exposure to arsenic and cadmium using benchmark dose calculation in a Chinese population". *Biometals*. **17** (5): 573-80. [10.1023/b:biom.0000045741.22924.d8](https://doi.org/10.1023/b:biom.0000045741.22924.d8).
- [3] A. Koyu, A. Gokcimen, F. Ozguner, D. S. Bayram, and A. Kocak. (2006). "Evaluation of the effects of cadmium on rat liver". *Molecular and Cellular Biochemistry*. **284** (1-2): 81-5. [10.1007/s11010-005-9017-2](https://doi.org/10.1007/s11010-005-9017-2).
- [4] M. Tellez-Plaza, A. Navas-Acien, C. M. Crainiceanu, and E. Guallar. (2008). "Cadmium exposure and hypertension in the 1999-2004 National Health and Nutrition

- Examination Survey (NHANES)". *Environmental Health Perspectives*. **116** (1): 51-6. [10.1289/ehp.10764](https://doi.org/10.1289/ehp.10764).
- [5] J. J. Kim, Y. S. Kim, and V. Kumar. (2019). "Heavy metal toxicity: An update of chelating therapeutic strategies". *Journal of Trace Elements in Medicine and Biology*. **54** 226-231. [10.1016/j.jtemb.2019.05.003](https://doi.org/10.1016/j.jtemb.2019.05.003).
- [6] M. F. McCarty. (2012). "Zinc and multi-mineral supplementation should mitigate the pathogenic impact of cadmium exposure". *Medical Hypotheses*. **79** (5): 642-8. [10.1016/j.mehy.2012.07.043](https://doi.org/10.1016/j.mehy.2012.07.043).
- [7] S. Porru and L. Alessio. (1996). "The use of chelating agents in occupational lead poisoning". *Occupational Medicine (London)*. **46** (1): 41-8. [10.1093/occmed/46.1.41](https://doi.org/10.1093/occmed/46.1.41).
- [8] A. M. Massadeh, S. A. Al-Safi, I. F. Momani, A. A. Alomary, Q. M. Jaradat, and A. S. AlKofahi. (2007). "Garlic (*Allium sativum* L.) as a potential antidote for cadmium and lead intoxication: cadmium and lead distribution and analysis in different mice organs". *Biological Trace Element Research*. **120** (1-3): 227-34. [10.1007/s12011-007-8017-3](https://doi.org/10.1007/s12011-007-8017-3).
- [9] T. Miron, T. Bercovici, A. Rabinkov, M. Wilchek, and D. Mirelman. (2004). "[3H] Allicin: preparation and applications". *Analytical Biochemistry*. **331** (2): 364-9. [10.1016/j.ab.2004.03.054](https://doi.org/10.1016/j.ab.2004.03.054).
- [10] L. Y. Chung. (2006). "The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide". *Journal of Medicinal Food*. **9** (2): 205-13. [10.1089/jmf.2006.9.205](https://doi.org/10.1089/jmf.2006.9.205).
- [11] K. Kumari, P. Adhikari, A. Pandey, S. S. Samant, M. Lal, and V. Pande. (2024). "Influence of Solvent Polarity on Phytochemicals, Antioxidants, and Antimicrobial Properties of *Delphinium denudatum*: A Medicinal Herb from Sainj Valley, Himachal Pradesh, India". *Bioactivities*. **2** (1): 30-40. [10.47352/bioactivities.2963-654X.214](https://doi.org/10.47352/bioactivities.2963-654X.214).
- [12] B. P. Williams. (1982). "Hematology and urinalysis". *Journal of Chemical Education*. **59** (9). [10.1021/ed059p805.4](https://doi.org/10.1021/ed059p805.4).
- [13] D. J. Meuten, F. M. Moore, T. A. Donovan, C. A. Bertram, R. Klopffleisch, R. A. Foster, R. C. Smedley, M. J. Dark, M. Milovancev, P. Stromberg, B. H. Williams, M. Aubreville, G. Avallone, P. Bolfa, J. Cullen, M. M. Dennis, M. Goldschmidt, R. Luong, A. D. Miller, M. A. Miller, J. S. Munday, P. Roccabianca, E. N. Salas, F. Y. Schulman, R. Laufer-Amorim, M. G. Asakawa, L. Craig, N. Dervisis, D. G. Esplin, J. W. George, M. Hauck, Y. Kagawa, M. Kiupel, K. Linder, K. Meichner, L. Marconato, M. L. Oblak, R. L. Santos, R. M. Simpson, H. Tvedten, and D. Whitley. (2021). "International Guidelines for Veterinary Tumor Pathology: A Call to Action". *Veterinary Pathology*. **58** (5): 766-794. [10.1177/03009858211013712](https://doi.org/10.1177/03009858211013712).
- [14] A. S. Naylor, B. J. Edwards, and C. M. Robertson. (2023). "Effects of treatment dosage of whole-body cryotherapy upon post-match recovery of endocrine and biochemical markers in elite rugby league players: An experimental study". *Health Science Reports*. **6** (4): e1227. [10.1002/hsr2.1227](https://doi.org/10.1002/hsr2.1227).
- [15] M. A. Hossain, M. A. Sayed, M. S. Jahan, M. Mostofa, and M. S. H. Khan. (1970). "Effect of garlic and vitamin B-complex in lead acetate induced toxicities in mice". *Bangladesh Journal of Veterinary Medicine*. **6** (2): 203-210. [10.3329/bjvm.v6i2.2337](https://doi.org/10.3329/bjvm.v6i2.2337).
- [16] S. Rahman and S. Sultana. (2006). "Chemopreventive activity of glycyrrhizin on lead acetate mediated hepatic oxidative stress and its hyperproliferative activity in Wistar rats". *Chemico-Biological Interactions*. **160** (1): 61-9. [10.1016/j.cbi.2005.12.003](https://doi.org/10.1016/j.cbi.2005.12.003).
- [17] J. L. Young, X. Yan, J. Xu, X. Yin, X. Zhang, G. E. Arteel, G. N. Barnes, J. C. States, W. H. Watson, M. Kong, L. Cai, and J. H. Freedman. (2019). "Cadmium and High-Fat Diet Disrupt Renal, Cardiac and Hepatic Essential Metals". *Scientific Reports*. **9** (1): 14675. [10.1038/s41598-019-50771-3](https://doi.org/10.1038/s41598-019-50771-3).
- [18] A. Dhar and P. K. Banerjee. (1983). "Impact of lead on nucleic acids and incorporation of leveled amino acid into protein". *International Journal of Vitamin and Nutrition Research*. **53** (3): 349-354.

- [19] M. U. Eteng, F. C. Onwuka, E. O. Akpanyung, N. C. Osuchukwu, S. C. Bassey, and P. Nwankpa. (2012). "Reversal of cadmium-induced toxicity following dietary supplementation with garlic, ginger and cabbage in male Wistar rats". *Journal of Natural Products and Plant Resources*. **2** (1): 169-174.
- [20] C. W. Cha. (1987). "A study on the effect of garlic to the heavy metal poisoning of rat". *Journal of Korean Medical Science*. **2** (4): 213-24. [10.3346/jkms.1987.2.4.213](https://doi.org/10.3346/jkms.1987.2.4.213).
- [21] S. J. Flora, A. Mehta, and R. Gupta. (2009). "Prevention of arsenic-induced hepatic apoptosis by concomitant administration of garlic extracts in mice". *Chemico-Biological Interactions*. **177** (3): 227-33. [10.1016/j.cbi.2008.08.017](https://doi.org/10.1016/j.cbi.2008.08.017).
- [22] M. Marija, R. Zeljko, J. Vilma, and O. Zorana. (2004). "Population dynamics and *Borrelia burgdorferi* infection rate of ixodes ricinus ticks in the Belgrade area". *Acta Veterinaria*. **54** (2-3): 219-225. [10.2298/avb0403219m](https://doi.org/10.2298/avb0403219m).
- [23] H. T. Tung, F. W. Cook, R. D. Wyatt, and P. B. Hamilton. (1975). "The anemia caused by aflatoxin". *Poultry Science*. **54** (6): 1962-9. [10.3382/ps.0541962](https://doi.org/10.3382/ps.0541962).
- [24] Y. Arfat, N. Mahmood, M. U. Tahir, M. Rashid, S. Anjum, F. Zhao, D. J. Li, Y. L. Sun, L. Hu, C. Zhihao, C. Yin, P. Shang, and A. R. Qian. (2014). "Effect of imidacloprid on hepatotoxicity and nephrotoxicity in male albino mice". *Toxicology Reports*. **1** : 554-561. [10.1016/j.toxrep.2014.08.004](https://doi.org/10.1016/j.toxrep.2014.08.004).
- [25] S. Mumtaz, S. Ali, R. Khan, H. A. Shakir, H. M. Tahir, S. Mumtaz, and S. Andleeb. (2020). "Therapeutic role of garlic and vitamins C and E against toxicity induced by lead on various organs". *Environmental Science and Pollution Research*. **27** (9): 8953-8964. [10.1007/s11356-020-07654-2](https://doi.org/10.1007/s11356-020-07654-2).
- [26] F. M. El-Demerdash, M. I. Yousef, F. S. Kedwany, and H. H. Baghdadi. (2004). "Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and beta-carotene". *Food and Chemical Toxicology*. **42** (10): 1563-71. [10.1016/j.fct.2004.05.001](https://doi.org/10.1016/j.fct.2004.05.001).