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ORIGINAL ARTICLE

Synergistic Effects of Sub-Lethal Concentrations of Deltamethrin on Lead Acetate Toxicity in Japanese Quail (*Coturnix japonica*)

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KEYWORDS

Deltamethrin;
Lead acetate;
Blood biochemical parameters;
Japanese quail

ABSTRACT: The purpose of this study was to investigate the hypothesis weather the combination of lead acetate and deltamethrin can enhance avian toxic effects produced by lead exposure only. Overall, 48 Japanese quails, *Coturnix coturnix Japonica* (15 day old) were randomly divided into 4 experimental groups of 12 birds each and sex ratio of 1:1. Experimental groups consisted of control Japanese quails, birds exposed to a single dose of lead acetate and lead acetate combined with sub-lethal doses of deltamethrin (0.25 and 0.50 mg. kg^{-1} diet) for 21 days. We studied the effects of a single dose of lead acetate, combined with deltamethrin, on survival and blood biochemical parameters of Japanese quails. The results revealed a significant increase in plasma AST and ALT activities, glucose, uric acid and creatinine levels after feeding quails with contaminated diet (P <0.05). There was a significant increase in CPK and LDH activities and triglyceride levels in the blood of quails fed diets contaminated with a combination of lead acetate and deltamethrin (P <0.05). The decrease in AChE and ALP activities, total protein, and globulin was observed in plasma of quails fed contaminated diets. There were no significant changes in albumin levels. Although oral administration of lead acetate combined with 0.25 mg deltamethrin caused a significant increase in cholesterol levels, no significant differences were observed in triglyceride levels in other treatments. The synergic effects of deltamethrin on the alterations in the blood biochemical parameters of quails exposed to lead highly depend on the concentrations of this pesticide in feedstuffs.

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INTRODUCTION

Interaction of pollutants with biotic and abiotic factors in the environment would make it difficult to investigate the sublethal toxicity of heavy metals in animals [1]. Furthermore, most toxicological data are obtained using single pollutants, and it is difficult to predict the effects of mixed toxicants in the natural environments.

There are many biotic and abiotic factors including individual characteristics of each species and sources of heavy metals, physical and chemical conditions of the environment, and types and levels of pollutants that have significant effects on the bioavailability and toxicity of metals in animals [2].

Lead (Pb) is one of the most common poisonings in birds [3, 4]. Birds' exposure to lead can occur via feed, water, soil, dust and air. It is rapidly absorbed through the intestinal epithelium and distributed into fat-rich tissues. Nevertheless, absorption of lead from gastrointestinal tract depends on physicochemical properties of the ingested material. Young birds tend to be more susceptible to lead poisoning than adults are, because they have a higher rate of absorption of lead. However, a large part of this metal may be excreted through urine and feces in the first few days after exposure. The half-life of Pb in the blood and bone is approximately 30 days and 10 to 30 years, respectively [5]. This metal interferes with the embryonic development of mallard resulting in abnormalities and embryonic death [6, 7]. Moreover, lead could lead to dysfunction of the renal [8], endocrine [9], gastrointestinal [10], musculoskeletal [11], immune [12], and reproductive systems [13] and change the blood biochemical parameters [14].

Pb levels can increase in the ecosystems in small localized areas around lead mines, smelters and industrial plants. Increased contamination of the environment through air pollution, acid rain, industrial and municipal discharge, agrochemical application, surface runoff and agricultural drainage may affect the bioavailability of lead and its toxicity.

Deltamethrin, [((S)-Cyano(3-phenoxyphenyl)methyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate)] is a type II synthetic pyrethroid, comprising a wide range of man-made insecticides and based on the chemical structure of pyrethrum, an extract from chrysanthemums (C*hrysanthemum* spp.). In many developing countries, deltamethrin is regarded as one of the authorized active ingredients for post-harvest use to protect stored grains [15-18]. The presence of deltamethrin residues in feedstuffs and crops can be a serious threat to animals and humans [15, 19]. Therefore, residual deltamethrin in crops may affect the bioavailability of lead (Pb) and its toxicity to birds.

Poultry have a special place in Iranian diet. That is why Japanese quail rearing has significantly developed in Iran in the last decade. However, the problem is that in some rearing and breeding farms, low quality cereal grains may be used in order to reduce costs of production.

Therefore, this study was designed to provide information on the health and blood biochemical parameters of quails fed grains contaminated with deltamethrin and lead acetate. In the previous study, we found that deltamethrin had a significant effect upon the plasma biochemical parameters of quails [20]. The objectives of this research were to investigate the effects of different concentrations of deltamethrin on lead acetate toxicity in Japanese quails. Therefore, monitoring blood biochemical parameters may provide more direct information on toxicological impacts of deltamethrin and lead acetate on the health of quails.

MATERIALS AND METHODS

Juvenile Japanese quails (15-day old) were purchased from local breeders. All birds were individually banded with metal bands, housed in indoor aviaries, and provided with feedstuff and water ad libitum at the Behbahan Khatam Alanbia University of Technology, Behbahan, Iran (2015). Birds were allowed to adjust to caging conditions for two weeks before the initial experiment.

Forty-eight Japanese quails were divided into four groups of twelve birds each. Group A served as the normal control and received only water and commercial feed ad libitum throughout the experimental period. Group B served as the toxin control and fed a diet contaminated with 0.4 mg lead acetate per kg diet for 21 days. Group C received 0.25 mg deltamethrin per kg diet and 0.4 mg lead acetate, and group D received 0.50 mg deltamethrin per kg diet and 0.4 mg lead acetate for 21 consecutive days. At the end of the experimental period, the quails were weighed, anesthetized and later sacrificed by cervical dislocation. The blood was collected and stored in sterilized glass vials at 4 °C containing the anticoagulant heparin and centrifuged for 15 min at 6000 g, 4 °C. Plasma samples were immediately stored at -21 °C until biochemical analysis.

Plasma levels of acetylcholinesterase (AChE), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine phosphokinase (CPK), total proteins, albumin, globulin, glucose and cholesterol, triglycerides, creatinine and uric acid were determined using an UV/Visible spectrophotometer (model UNICO 2100) and standard biochemical reagents (Pars Azmoon Company, Tehran, Iran).

Data analysis

The significant difference in the biochemical parameters of birds treated with different concentrations of deltamethrin was examined using one-way ANOVA. All data were checked for normality (Kolmogorov-Smirnov test). Means were compared by Duncan's test and a $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 19 (IBM,) software (Chicago, IL, USA). Data are presented as mean (SD).

RESULTS

There was no mortality throughout the course of the experiment in Japanese quails fed 0.4 mg lead acetate and 0.25 and 0.50 mg. kg-1 deltamethrin combined with lead acetate (0.4 mg. kg-1). Control birds remained constant throughout the course of the experiment.

Our behavior studies showed definite behavioral differences between male and female quails, in that, Pb-exposed females displayed less aggressive behavior than males. These effects were more enhanced in the quails along with an increase in concentrations of deltamethrin. The biochemical parameters of treated and control groups are presented in Figure 1-14. Deltamethrin combined with lead acetate and lead acetate alone caused a significant ($P < 0.05$) increase in the plasma activities of ALT and AST after an oral administration (Figure 1 & 2). Although LDH activity was significantly higher ($P < 0.05$) in birds treated with deltamethrin combined with lead acetate compared with those in the control group, there was no significant difference between LDH activity in plasma of quails treated with lead acetate alone and control group ($P > 0.05$) (Figure 4). CPK activity in plasma of the individuals treated with deltamethrin combined with lead acetate increased significantly compared with the control group ($P < 0.05$). Nevertheless, no significant changes were observed in CPK activity in blood of quails fed diets contaminated with lead acetate when compared with the control group (Figure 5). ALP and AChE activities in plasma of the individuals treated with deltamethrin and lead acetate decreased significantly compared with the control group ($P < 0.05$) (Figure 3 & 6).

In addition, there was a significant decrease in plasma total protein and globulin levels (Figure 7 & 9). Sublethal treatment did not significantly change albumin levels in treated groups as compared to the control group (Figure 8).

Deltamethrin combined with lead acetate had a significant effect in increasing glucose, triglycerides, uric acid and creatinine in plasma of quails (Figure 10-14). However, no significant changes were observed in triglycerides in blood of quails treated with lead acetate alone when compared with the control group. Although cholesterol level increased significantly at 0.25 mg deltamethrin combined with lead acetate on the 21st day, no significant change was observed in birds fed contaminated-diet with 0.5 mg deltametrin combined with lead acetate and lead acetate alone as compared to control group (Figure 11).

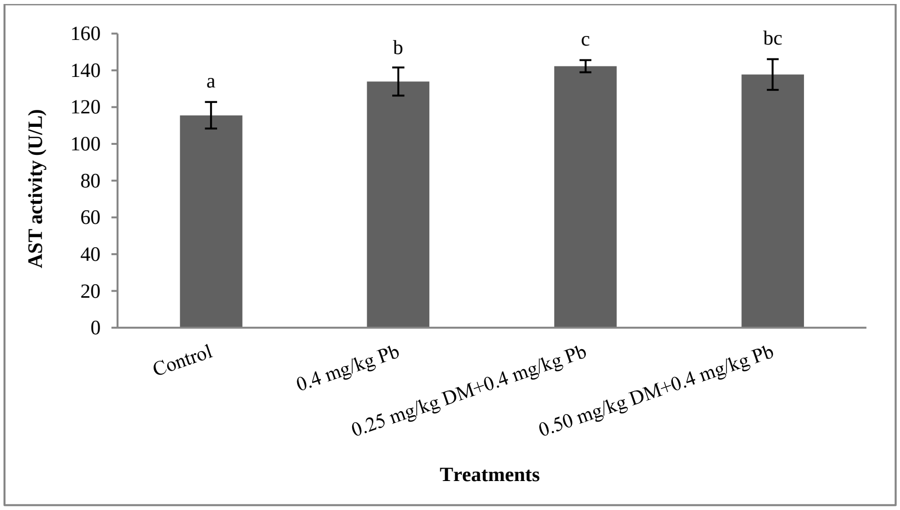

Figure 1. Effect of lead acetate and deltamethrin on AST activity in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

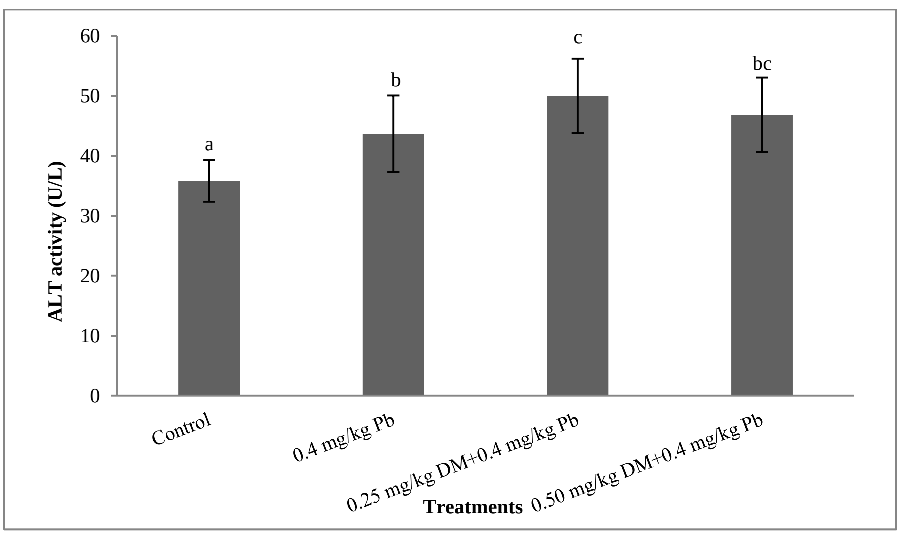

Figure 2. Effect of lead acetate and deltamethrin on ALT activity in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

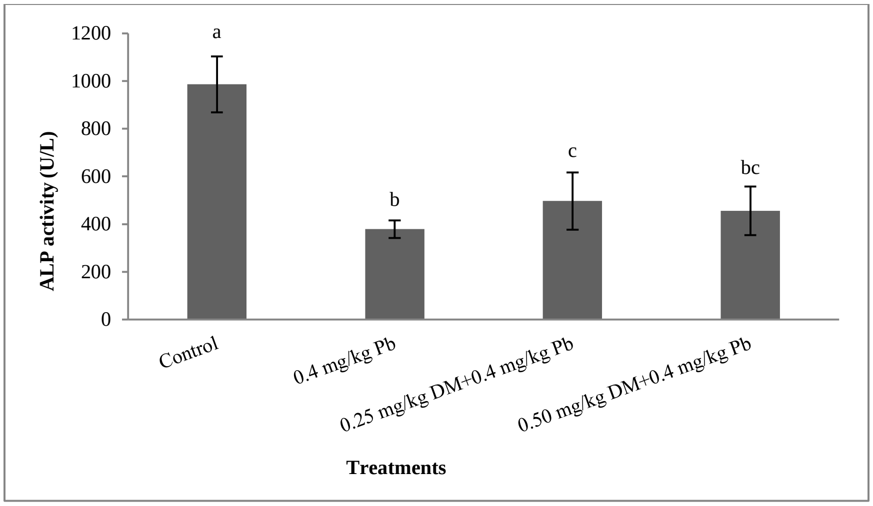

Figure 3. Effect of lead acetate and deltamethrin on ALP activity in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

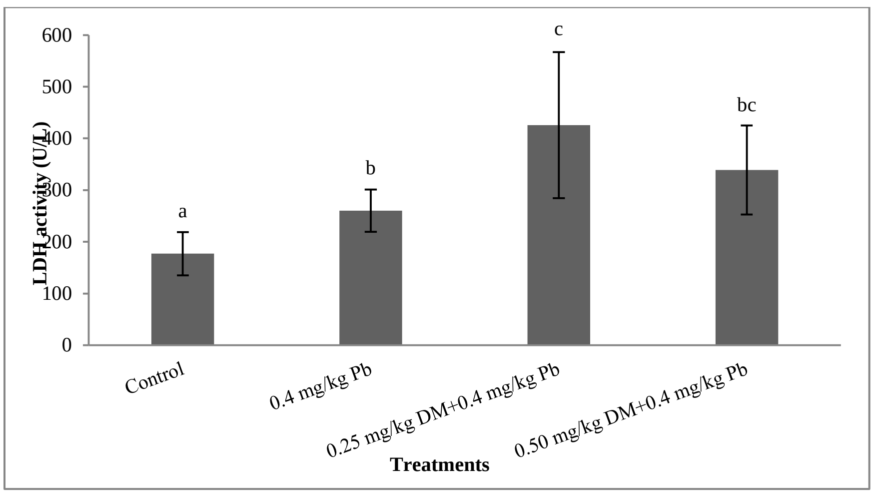

Figure 4. Effect of lead acetate and deltamethrin on LDH activity in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

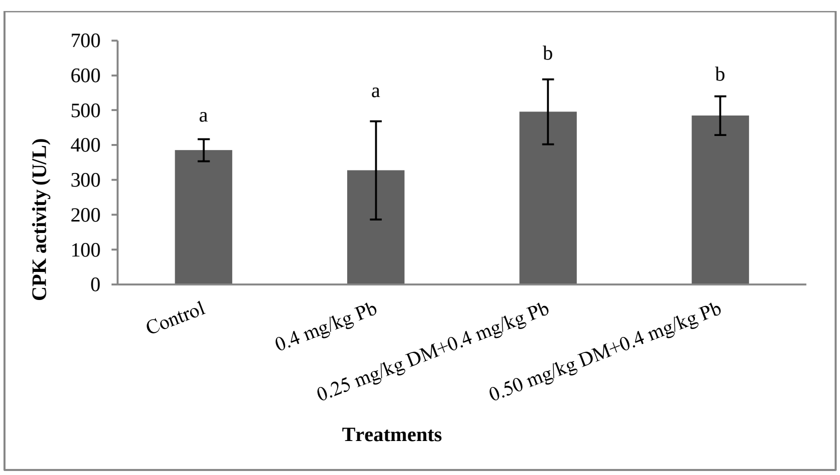

Figure 5. Effect of lead acetate and deltamethrin on CPK activity in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

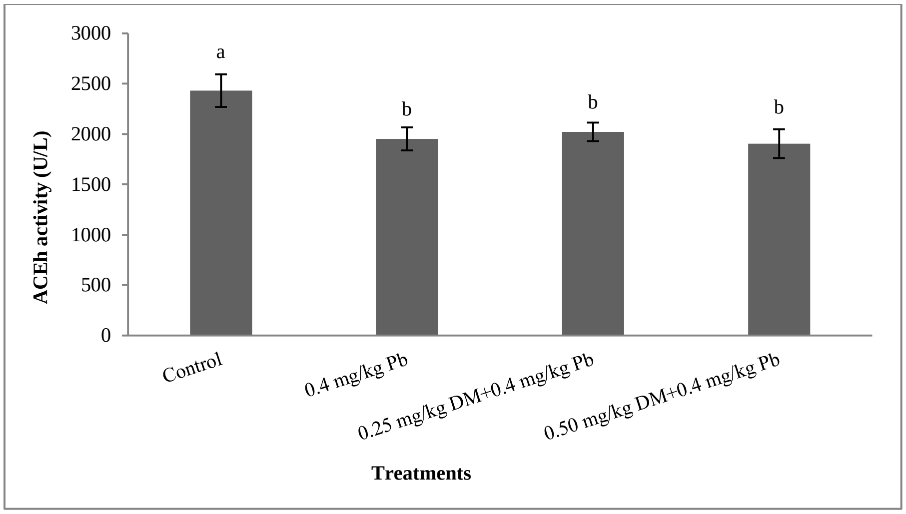

Figure 6. Effect of lead acetate and deltamethrin on ACEh activity in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

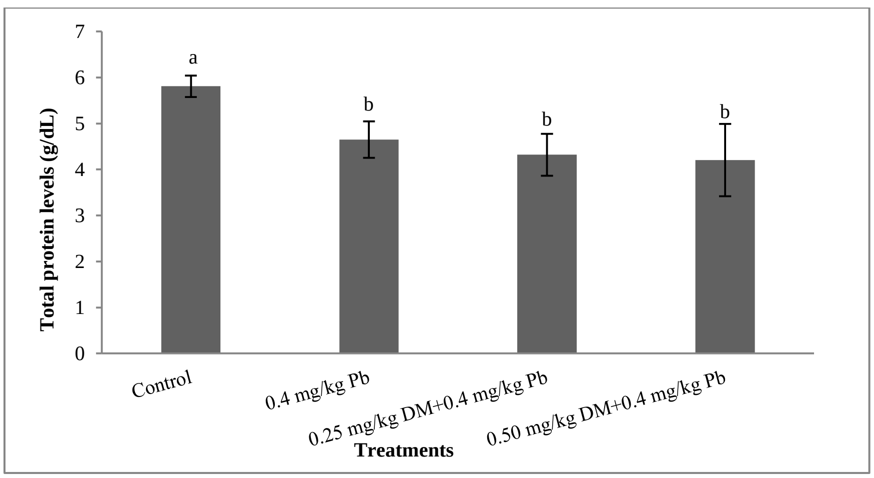

Figure 7. Effect of lead acetate and deltamethrin on Total protein levels in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

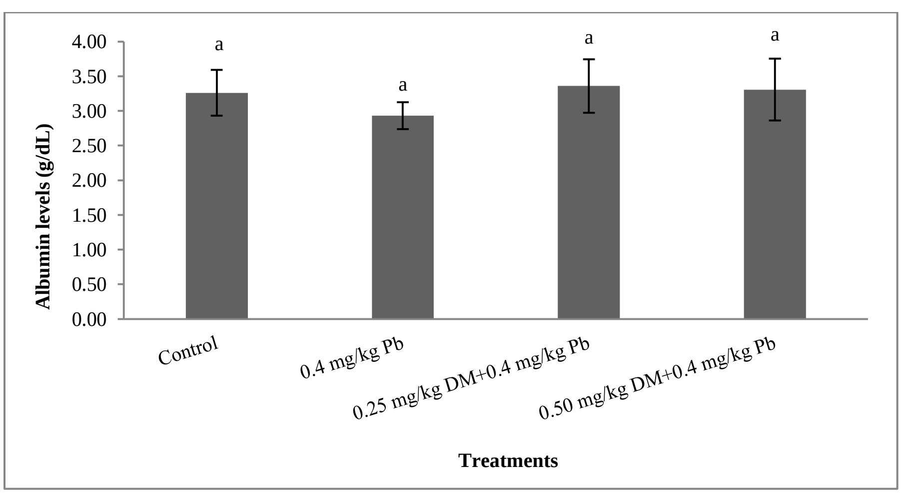

Figure 8. Effect of lead acetate and deltamethrin on Albumin levels in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

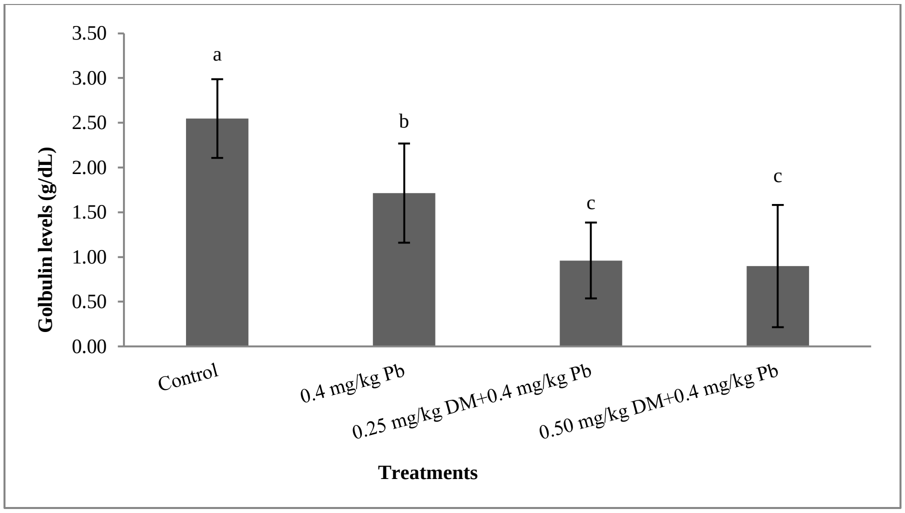

Figure 9. Effect of lead acetate and deltamethrin on Globulin levels in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

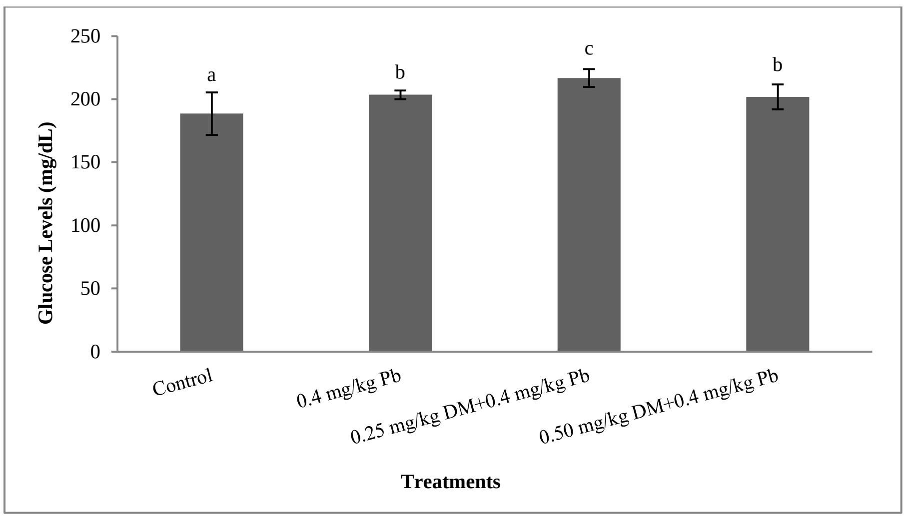

Figure 10. Effect of lead acetate and deltamethrin on glucose level in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

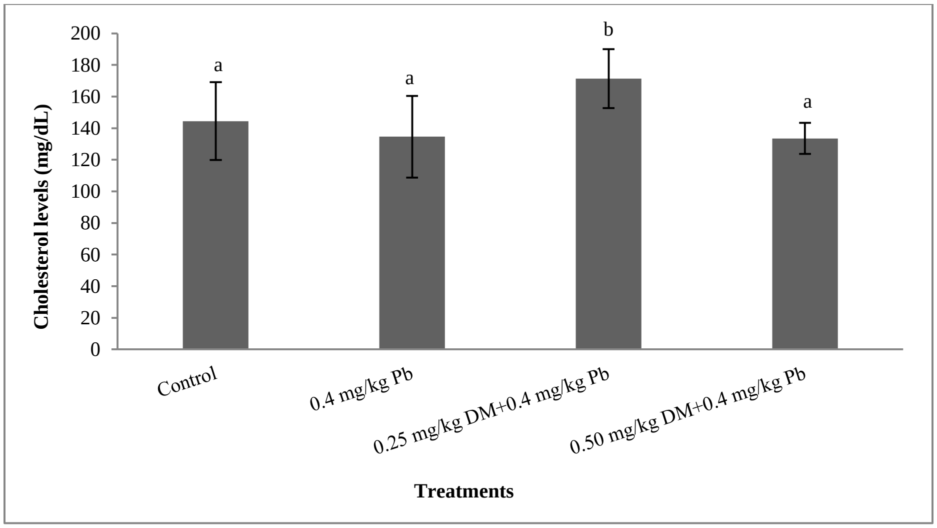

Figure 11. Effect of lead acetate and deltamethrin on Cholesterol levels in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

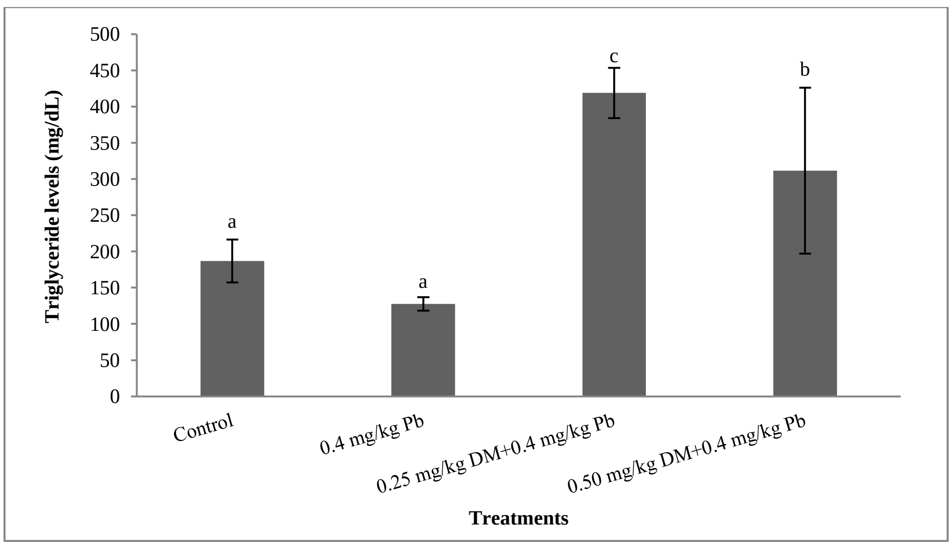

Figure 12. Effect of lead acetate and deltamethrin on Triglyceride levels in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

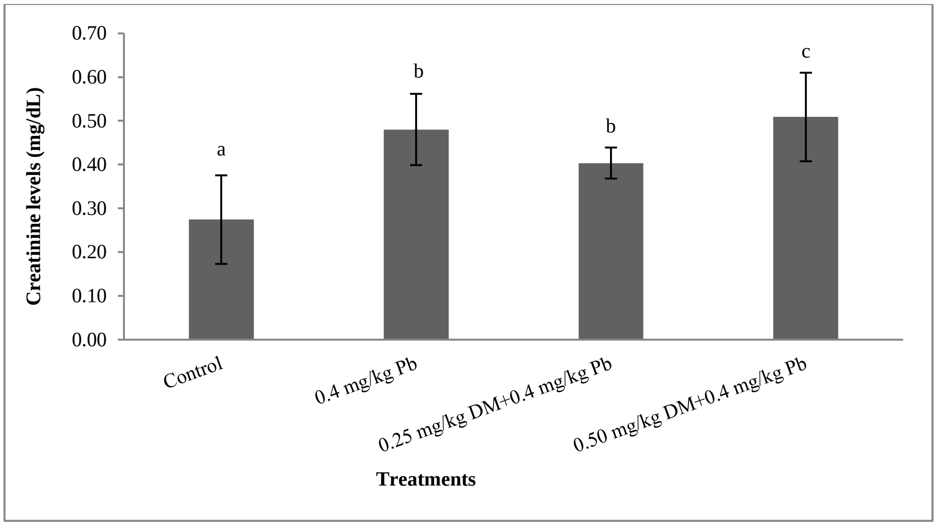

Figure 13. Effect of lead acetate and deltamethrin on Creatinine levels in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

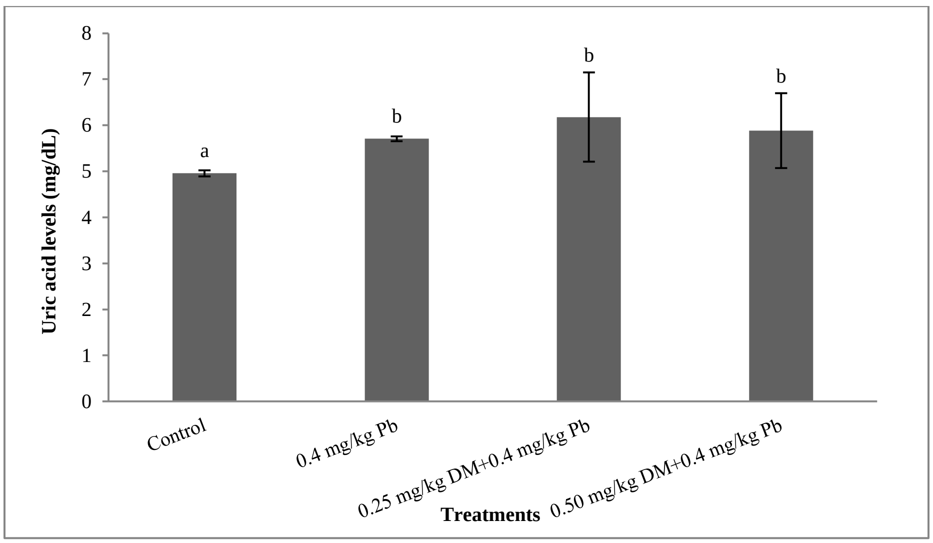

Figure 14. Effect of lead acetate and deltamethrin on Uric acid levels in blood of Japanese quails. Significant differences between values, when compared with the control group, were shown by alphabet symbol ($P < 0.05$). Values represent mean ± S.D.

DISCUSSION

Insecticides and heavy metals can affect bird's health status, populations and their activity by changing physiological and biochemical parameters.

Results indicate that lead acetate differentially affects the behavior of male and female quails in that females did less exploration and the males were selectively more aggressive. The addition of lead acetate in the diet for 3 weeks caused an increase in the aggressive behavior of quails. Lead-exposed males displayed violent behavior towards their cage mates as compare to female quails. The birds treated with deltamethrin and lead exhibited aggressive and nervous behavior, anorexia, sluggishness, lethargy and loss of equilibrium at the third week of experiment. These effects were more enhanced in the quails along with an increase in concentrations of deltamethrin.

Results of the present study indicates that adverse effects of deltamethrin and lead acetate can be more serve in birds following combined exposure. In this study, alterations of the biochemical parameters were observed in Japanese quails used as an avian model species exposed to sub-lethal doses of deltamethrin and lead acetate. The biochemical parameters of treated and control groups are presented in Figure 1-14.

Pb exposure significantly decreased the specific activity of AChE in plasma of quails ($P < 0.05$). In addition, a significant decrease was observed in the plasma AChE activity in birds exposed to deltamethrin and lead acetate ($P < 0.05$) (Figure 6). The decrease in the activity of AChE is in agreement with the observations of previous studies [21-23] in rat, rainbow trout and zebrafish, respectively. AChE activity in plasma of rats was significantly decreased after deltamethrin treatment [24].

AST, ALT, LDH, ALP and CPK are found in cells of different organs such as heart, kidneys, liver, skeletal muscle, brain, erythrocyte, intestine and lungs. The enzymes are indicative of various aspects of metabolism, and they have been used to evaluate the physiological, biochemical and metabolic defects in various tissues of animals [25].

Deltamethrin combined with lead acetate and lead acetate alone caused a significant ($P < 0.05$) increase in the plasma activities of ALT and AST after an oral administration (Figure 1 & 2). Increased in plasma of aspartate aminotransferase and alanine aminotransferase in quails may be as a results of damage to the hepatocyte membranes [28]. These observations were clearly similar to those in quails exposed to butachlor [29], atrazine [30], and malathion [31]. Increased activity of AST and ALT was observed in plasma of rats exposed to deltamethrin [24] and plasma of *Cyprinus carpio* exposed to deltamethrin [32] and chlorpyrifos [33].

ALP activity in plasma of the individuals treated with deltamethrin and lead acetate decreased significantly compared with the control group ($P < 0.05$). The significant decrease in the activity of ALP in the plasma of quails fed contaminated diet with lead acetate and deltamethrin may in part be due to damage to the hepatocyte membranes (Figure 3). This can be associated with the damage in the liver tissue and an imbalance between degradation and synthesis of the enzyme [25]. Furthermore, hemolysis and lipid peroxidation of erythrocytes may be due to decreased ALP activity in the blood of birds treated with deltamethrin and lead acetate. Hemolysis of erythrocytes leads to the release of electrolytes such as magnesium and zinc, which in turn may have an inhibitory effect on ALP activity [26]. In addition, lead acetate and deltamethrin may be inhibited of the ALP activity. Decreased plasma ALP was reported in rat exposed to deltamethrin [27].

Although LDH activity was significantly higher ($P < 0.05$) in birds treated with deltamethrin combined with lead acetate compared with those in the control group, there was no significant difference between LDH activity in plasma of quails treated with lead acetate alone and control group ($P > 0.05$). Increase in the activity of lactate dehydrogenase in plasma as observed in the present study may be a reflection of and elevation in anaerobic metabolism (Figure 4). The observed increase in LDH activity can also be attributed to the conversion of accumulated pyruvate into lactate, transported from the muscles to the liver to supply energy for birds treated with deltamethrin. Increased activity of LDH was observed in blood of *C. japonica* exposed to butachlor [29]. Similar results have been reported already [24, 32, and 33].

CPK activity in plasma of the individuals treated with deltamethrin combined with lead acetate increased significantly compared with the control group ($P < 0.05$). Nevertheless, no significant changes were observed in CPK activity in blood of quails fed diets contaminated with lead acetate when compared with the control group (Figure 5). The increased activity of CPK in plasma of quails fed contaminated diet with lead acetate and deltamethrin may be indicative of a disorder in muscle fibres. These results agree with a previous study carried out on chicks that had been exposed to amitraz [34]. Our results showed that deltamethrin had a significant effect on increasing the toxicity of lead on muscle fibers of quails.

In addition, there was a significant decrease in plasma total protein and globulin levels (Figure 7 & 9). The decrease in protein level might be because of increased utilization of protein to meet the energy demand when the quails were under toxic stress. Furthermore, decreased total protein in quails treated with deltamethrin and lead acetate along with changes in protein patterns might be due to malnutrition, reduced protein synthesis in liver or decreased utilization of dietary protein. A reduction in blood protein level was recorded in American coots (*Fulica americana*) exposed to sub-lethal concentrations of crude oil [35]. Important biochemical parameters that can be used to assess the health status of the liver is the plasma levels of albumin and globulin. Albumin, which is synthesized by the liver, is a major protein that circulates in the bloodstream [22]. Our results showed no significant changes in plasma albumin (Figure 8).

Decreased plasma globulin level as observed in the present study may be an indication of reduced immunity in the quails since the liver will not be able to synthesis enough globulins for immunologic action. The levels of globulin decreased in birds exposed to crude oil [35].

Deltamethrin combined with lead acetate had a significant effect in increasing glucose in plasma of quails (Figure 10). The blood glucose level can be a bio-indicator of stress caused by pollutants such as insecticides and heavy metals [27, 33]. Increase in plasma glucose level of quails under the effect of lead acetate and deltametrin may be due to increased glycogenolysis and decreased glycogen content in the liver and muscle. Increased glycaemia following long-term exposure to deltamethrin and Pb is probably associated with the increased biosynthesis of cortisol. Due to this increase, hepatic glycogen is rapidly broken down into glucose to yield energy for dealing with the toxic effects of deltamethrin and lead acetate. Moreover, decreased insulin synthesis is reported in pancreas of birds exposed to deltamethrin [27], which in turn may play a role in the impairment of glucose regulation. Increased blood glucose in chicks in response to sub-lethal exposure to amitraz was reported by Al-Hammdani and Al-Baggou [34].

Although cholesterol level increased significantly at 0.25 mg deltametrin combined with lead acetate on the 21^{st} day, no significant change was observed in birds fed contaminated-diet with 0.5 mg deltametrin combined with lead acetate and lead acetate alone as compared to control group (Figure 11). Increased blood cholesterol in quails treated with sub-lethal concentrations of the lead acetate and deltametrin, suggests stress induced hyper metabolic state of the birds, mobilization and utilization of cholesterol through blood and also the damage in liver tissue. However, cholesterol levels remained to normal levels in quails fed a diet polluted with 0.5 mg deltamethrin combined with lead acetate. Change in the blood cholesterol level was reported in a number of animals exposed to different concentrations of various pollutants. Peckova et al. [36] reported an increase in blood cholesterol level in the Japanese quails fed contaminated-diet with cyanobacterial biomass. The increase in cholesterol level under toxic stress might be an induction of liver dysfunction [22].

Although, triglyceride levels increased significantly in plasma of quails fed polluted diets with deltamethrin and Pb, no significant changes was observed in quails exposed to lead acetate alone (Figure 12). Significantly, increased triglycerides in blood of quails in response to treatment with deltamethrin and lead acetate might be an energy producing mechanism in detoxification process.

In addition, liver dysfunction may increase blood triglycerides. Disorder in triglyceride uptake by adipose tissue may temporarily increase triglycerides.

Deltamethrin combined with lead acetate had a significant effect in increasing uric acid and creatinine in plasma of quails (Figure 13 & 14). Increased levels of uric acid and creatinine as observed in this study suggest glomerular dysfunction and kidney damage due to its role in excreting these waste compounds. Exposure quails to butachlor can affect excrete of these metabolites [29].

CONCLUSIONS

The synergistic effects of deltamethrin on the changes in the blood biochemical parameters of quails exposed to lead highly depend on the concentrations of this pesticide in feedstuffs. This experiment contributes to the understanding of the pathogenic mechanisms of combined sub-lethal exposure to heavy metals and agrochemicals in birds; also, our results may be used for risk assessment of environmental pollution and investigation of finding of sub-lethal concentrations of pollutants in domestic and wild birds.

CONFLICT OF INTERESTS

Authors have no conflict of interests.

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