Assessment of toxic interaction of lead and chromium metals in binary mixture in quails: A hematological study

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ABSTRACT

INTRODUCTION Overpopulation and global trends toward industrialization are continuously affecting the environment in terms of pollution. The heavy metals in industrial effluents are considered the prime environmental toxicants, which are regarded as harmful to the surrounding organisms. The current research aims to investigate the co-exposure effects of the two most harmful heavy metals of lead and chromium, on the hematological indices of common quails.

METHODS An experimental animal study was carried out to evaluate the hematological parameters such as hematocrit, hemoglobin content, RBC count, WBC count, ESR, MCV, MCH, and MCHC. In addition, the behavioral and body weight changes due to these metals were also considered in this study. For the experiment, 12 healthy, adult, common quails (Coturnix coturnix) of 100–120 g body weight were used. Animals were classified into three groups (4 birds/group). Group 1 was the control group which was provided only with tap water and standard pellet feed. Group 2 was the low dose experimental group, provided with lead (1 mg/kg body weight) and chromium (2 mg/kg body weight). Group 3 was the high dose experimental group with administration of high doses of lead (10 mg/kg body weight) and chromium (8 mg/kg body weight).

RESULTS The study revealed a significant ($p<0.05$) rise in the values of WBC (109/L) count (high dose, 21.37 ± 0.55; low dose, 17.05 ± 0.43; control, 13.12 ± 0.43), platelets (109/L) count (high dose, 298.7 ± 7.46; low dose, 231.0 ± 10.85; control, 181.5 ± 8.35), ESR (high dose, 7.87 ± 0.38; low dose, 3.95 ± 0.32; control, 1.82 ± 0.13), MCV (fL) (high dose, 147.65 ± 9.40; low dose, 147.52 ± 1.49; control=116.00 ± 2.04), and MCH (pg) (high dose, 37.35 ± 1.66; low dose, 40.46 ± 0.35; control=33.50 ± 0.65) in the experimental groups. However, a significant ($p<0.05$) decline was observed in the number of total RBCs (1012/L) (high dose, 1.90 ± 0.19; low dose, 2.72 ± 0.11; control=4.50 ± 0.12), hematocrit (%) (high dose, 40.25 ± 2.02; low dose, 40.25 ± 2.02; control=51.25 ± 1.10), hemoglobin(g/dL) (high dose, 7.00 ± 0.40; low dose, 11.02 ± 0.42; control=15.35 ± 0.37), and MCHC (g/dL) (high dose, 25.55 ± 0.59; low dose, 27.43 ± 0.40; control=29.00 ± 0.41) in the blood of the experimental groups. In the experimented groups, behavioral variations were also observed.

CONCLUSIONS The co-exposure to lead and chromium induced potential toxic effects on the hematopoietic system in quails alongside unusual behavior and decline in body weight.

INTRODUCTION

Industrialization and urbanization have changed the standard of life of humans, but they are a serious concern to the scientific community in terms of pollution. Due to its highly toxic nature, industrial effluent has become the prime source of environmental pollution. Heavy metals in these effluents are considered the prime environmental toxicants[1]. Heavy metals with comparably high density

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(i.e. 5 g/cm^3) are generally correlated with the discharge of industrial pollutants into the environment via water, air, and soil. Some of the heavy metals (zinc, copper, cobalt, manganese, selenium, and magnesium) play crucial roles in the physiological and biochemical pathways in living organisms in small amounts. They act as trace elements inside the body and are termed micronutrients[1,2].

The biotoxic effects of heavy metals refer to their adverse effects on the body when taken in excess of biologically recommended levels. The possible outcomes might be reflected in the form of toxic (acute, chronic, or subchronic), neurotoxic, carcinogenic, mutagenic, or teratogenic effects. Heavy metals poisoning can arise due to their reaction with the normal biochemistry of the exposed organisms. When consumed, they become altered into their more stable oxidation states (Pb^{+2}, Cd^{+2}, As^{+2}, As^{+3}, Hg^{+2}, and Ag^{+}). They become joined with the cellular biomolecules, like proteins and enzymes, to establish more stable and stronger bonding. In the case as mentioned earlier, the poison metal induces the replacement of the hydrogen atoms and/or metal particles and inhibits the enzyme's action. These metals in their ionic forms are considered the most poisonous variants[3,4].

Lead (Pb) is considered among the most toxic heavy metals, with a longer lifespan and high persistence in the environment. It is tasteless, with no distinguishing color or smell. It is found abundantly in both natural and industrial reservoirs. It is thought to be a non-essential element in terms of nutritional value inside organisms[5]. Living organisms can be exposed to lead through industrial products such as batteries, pipes, glazes, paints, pigments, toys, furniture, and many others[6]. Lead contamination can take place via the respiratory or gastrointestinal tract. Lead can exert adverse effects even at low concentrations, i.e. 10 µg/dL. Only 30–40% of the inhaled or ingested lead enters the bloodstream, where it is absorbed and remains there for 30–35 days. Afterward, the absorbed lead is circulated and accumulated in other body tissues like liver tissues, renal cortex, brain, aorta, lungs, and bones. The estimated and observed half-life of lead in different organs varies greatly. For instance, it can persist in brain tissues for about two years and 20–30 years in bones. The liver is considered the largest reservoir of lead intoxication, approximately 33% of the total intake[5,7].

Inorganic forms of lead are absorbed through food, water intake, and breathing. Lead intoxication can cause harmful effects in birds and other organisms such as rats and fishes. It has been revealed that lead poisoning can damage the hepatic[8], renal[9], and reproductive and nervous functionality. Aside from other damages, one of the significant harmful effects of lead on the gastrointestinal system is epithelial necrosis. Administration of lead can also cause hematological changes (increased erythrocyte fragility and the suppression of bone marrow) which may lead to the impairment of erythrocyte synthesis and their proper functioning in birds[10].

Chromium (Cr) is a trace element that is usually considered an essential micronutrient in terms of animal nutrition. It is generally in two oxidation states (trivalent and hexavalent chromium). Divalent chromium cannot exist in the environment independently since it is readily transformed into trivalent chromium after oxidation. Trivalent and hexavalent chromium represent distinctive toxicity and bioaccumulation based on the oxidation state. The absorption rate and biological toxicity of Cr^{+6} are higher than those of Cr^{+3} because they can easily cross the plasma membrane and immediately convert to trivalent chromium. This trivalent chromium performs hazardous functions inside the cell[11]. Hexavalent chromium is readily reduced into other chromium derivatives (Cr^{+5}, Cr^{+4}) and free radicals (thiol and hydroxyl radicals) by cumulative action of glutathione reductase, hydrogen peroxide, and vitamin C. Both of these products may attack the cellular DNA, membrane lipids, and cell proteins to disturb the overall function and stability of the cells. The overall damages can be described in the form of low levels of hemoglobin and hematocrit, and high levels of WBCs and plasma hemoglobin. This condition indicates intravascular hemolysis. Hexavalent chromium can also hinder the proper functioning of muscles and kidneys[12].

Birds are thought to be excellent bio-indicators in terms of environmental contamination since they are widely distributed, more in number, feed on different trophic levels, and have longer lifespan in comparison to other organisms[13]. So, we can quickly assess the harmful environmental toxicants by observing birds' physiology. Quails are considered one of the major and valuable model organisms to study environmental toxicants' effects on the physiological mechanisms of specific organs. This study aimed to find the outcomes of co-exposure effects of lead and chromium in vertebrates. The outcomes from this research will provide new insights into the harmful effects of co-exposure of different heavy metals in animals living in different environments like water, wetland and dry land.

METHODS

Study design

This research used adult male healthy common quails (Coturnix coturnix) of 100–120 g body weight. After purchasing them, all the animals were acclimatized for one week before the experimental trials. Trials were executed in an animal facility center at the University of Agriculture Faisalabad for a period of 30 days (as per previous literature 21–30 days is enough time to see the effects of heavy metals in animals). Twelve birds were equally divided into three groups. Group 1 was the control group and the remaining two groups were treated as experimental groups. The animals were kept in wooden cages at room temperature with tap water and standard bird pellet feed with twelve-hours of dark and light cycles. All the animal rights as set by the ethical committee, University of Agriculture Faisalabad, were strictly followed throughout the experiments.

All the chemicals (lead and chromium) used were purchased from Sigma-Aldrich Germany and the solutions and doses prepared according to previous studies and literature. Twelve healthy and adult common quails (Coturnix coturnix) of 100–120 g body weight were selected for the experiments. Animals were distributed into three groups (4 birds/group). Group 1 was the control group. It was provided only with tap water and standard pellet feed. Group 2 was the low dose group and was provided with low doses of lead (1 mg/kg body weight) and chromium (2 mg/kg body weight) in addition to standard pellet feed and tap water. Group 3 was the high dose group with high doses of lead (10 mg/kg body weight) and chromium (8 mg/kg body weight) in addition to tap water and standard pellet feed.

Lead dose was prepared by homogenizing 100 mg of lead chloride into 100 mL of distilled water. Low and high doses of lead were administered once a day at 1 mg/kg of body weight and 10 mg/kg body weight concentrations, respectively. Chromium solution was also prepared in the same way, and its low and high doses were 2 mg/kg of body weight and 8 mg/kg of body weight, respectively. Both the lead and chromium doses were injected orally at a time with the help of a syringe (with a small tube, Supplementary file Figure 1).

The initial and final body weight of the birds were measured with the help of digital weight balance in order to detect variations in body weight after the experiment. The overall behavioral alterations (alertness, crowing, feeding and drinking, foamy droppings, and mating) in the birds of the control and experiment groups were recorded regularly on a scale 0–4, for the period of 30 days by using video cameras.

Sampling

After the successful completion of the experimental trial, all the birds were sacrificed (slaughtered) and dissected, keeping in view all the Prevention of Cruelty to Animals Act (1890), Punjab Wildlife Act (1974) and all the hygiene and safety measures. Blood from the birds was collected in the 3 mL EDTA (an anticoagulant) test tubes and labelled. Prior to hematological testing, one or two drops of blood were taken on a fresh and clean glass slide to examine the presence of any pathogen. Infection-free blood was used for testing to avoid variations. Samples were collected at a particular time in the early hours of the day. All the blood samples were placed in the freezer immediately to prevent them from contamination and disintegration. One of the basic objectives of the research was the calculation of hematological profiles. Hematological parameters encompassed the determination of red blood cell (RBC) count, white blood cell (WBCs) count, platelets (thrombocytes) count, and the total numbers of monocytes, lymphocytes, basophils, neutrophils, and eosinophils. Moreover, the evaluation of hematocrit, hemoglobin content, mean cell volume (MCV), erythrocyte sedimentation rate (ESR), mean cell hemoglobin concentration (MCHC), and mean cell hemoglobin (MCH) was also performed.

Hematological analysis

RBCs and WBCs counts were estimated by using the Neubauer Crystalline counting chamber, which was described by Todd et al.[14]. To calculate the leukocytes and other types of WBCs, the Hunter and Bonford[15] method was used. ESR was calculated by using the most commonly used Westergren method to estimate the hematocrit and hemoglobin in the blood samples; the cyanmethemoglobin method of Dacie and Lewis[16] was employed.

Statistical analysis

After careful testing and investigation, all the derived data were analyzed statistically and expressed as mean ± standard error of the mean (SEM). It was further analyzed by using a basic statistical technique ANOVA, followed by Tukey's t-test. Both the analyses were conducted using the SPSS statistical Software. In order to compare the control versus experimental groups, graph pad Prism 5 software were used. Significance level was set at $p<0.05$ [17].

RESULTS

Hematological variations

Hematological variations are shown in Table 1. Significant ($p<0.05$) decline in values of hematocrit was detected in both the high- and low-dose groups in comparison with the control group. A significant ($p<0.05$) difference in the level of hemoglobin was observed between both experimental and control groups after 30 days of the research trial. Hemoglobin was down-regulated significantly ($p<0.05$) in both high- and low-dose groups than in the control group. Lead and chromium intoxication resulted in a measurable increase in the rate of ESR in both the high- and low-dose experimental groups, compared to that of the control group, whereas the opposite trend was observed in the RBCs count.

The number of WBCs was significantly ($p<0.05$) enhanced in both the high- and low-dose experimental groups, than in the control group. Leukocyte differential count was elaborated in the form of a total number of all types of leukocytes, such as monocytes, lymphocytes, basophils, eosinophils, and neutrophils. A high percentage of monocytes was observed in the birds of the high-dose group in comparison to the control group. However, no significant difference was found in birds of the low-dose group with respect to that of the control group. The study also demonstrated a significant ($p<0.05$) increase in the number of lymphocytes in the birds treated with high and low doses, compared to the control group. A significant rise in the number of basophils and eosinophils was detected in the high-dose group in comparison to both the low-dose and control groups. However, there was no significant difference between the low-dose group and the control group. Neutrophils were found to be increased significantly

Table 1. Hematological profile of quails after co-exposure to lead and chromium for 30 days

Measurements	Control Mean ± SEM	Pb + Cr (Low dose) Mean ± SEM	Pb + Cr (High dose) Mean ± SEM	p
Hematocrit (%)	51.25 ± 1.10[a]	40.25 ± 2.02[b]	40.25 ± 2.02[b]	<0.05
Hemoglobin (g/dL)	15.35 ± 0.37[a]	11.02 ± 0.42[b]	7.00 ± 0.40[c]	<0.05
Erythrocyte sedimentation rate (ESR)	1.82 ± 0.13[c]	3.95 ± 0.32[b]	7.87 ± 0.38[a]	<0.05
RBCs (1012/L)	4.50 ± 0.12[a]	2.72 ± 0.11[b]	1.90 ± 0.19[c]	<0.05
WBCs (10^9/L)	13.12 ± 0.43[c]	17.05 ± 0.43[b]	21.37 ± 0.55[a]	<0.05
Monocytes (10^9/L)	0.19 ± 0.04[b]	0.18 ± 0.06[b]	0.42 ± 0.04[a]	<0.05
Lymphocytes (10^9/L)	2.05 ± 0.11[c]	2.83 ± 0.09[b]	3.50 ± 0.05[a]	<0.05
Basophils (10^9/L)	0.06 ± 0.02[b]	0.10 ± 0.05[b]	0.26 ± 0.01[a]	<0.05
Neutrophils (10^9/L)	2.12 ± 0.46[c]	4.27 ± 0.53[b]	6.95 ± 0.56[a]	<0.05
Eosinophils (10^9/L)	0.35 ± 0.08[b]	0.41 ± 0.13[b]	0.93 ± 0.01[a]	<0.05
Platelets (10^9/L)	181.5 ± 8.35[c]	231.0 ± 10.85[b]	298.7 ± 7.46[a]	<0.05
MCV (fL)	116.00 ± 2.04[b]	147.52 ± 1.49[a]	147.65 ± 9.40[a]	<0.05
MCH (pg)	33.50 ± 0.65[b]	40.46 ± 0.35[a]	37.35 ± 1.66[a, b]	<0.05
MCHC (g/dL)	29.00 ± 0.41[a]	27.43 ± 0.40[a]	25.55 ± 0.59[b]	<0.05

Explanation what **a, b** and **c** represent

Table 2. Body weight (BW) changes and average weight gain of quails after co-exposure to lead and chromium for 30 days

Groups	Initial BW (g) Mean ± SEM	Final BW (g) Mean ± SEM	Weight gain (g) Mean ± SEM	p
Control	104.25 ± 1.10	164.25 ± 5.39	60.0 ± 4.29[a]	<0.05
Pb + Cr (Low dose)	103 ± 1.08	139.50 ± 4.05	36.5 ± 2.97[b]	<0.05
Pb + Cr (High dose)	104.25 ± 1.10	147.25 ± 5.54	43.0 ± 4.44[a,b]	<0.05

Explanation what **a, b** and **c** represent

Table 3. Behavioral changes* in quails after co-exposure to lead and chromium for 30 days

Week	Range	Control	Low dose	High dose
1	0–20	8	7	7
2	0–20	10	9	10
3	0–20	14	10	13
4	0–20	15	8	11
Total	0–80	47	34	41

*On a scale 0–4, for each of the 5 attributes: alertness, crowing, feeding and drinking, foamy droppings, and mating.

by administering both the high and low doses of lead and chromium, with respect to that of the control group. MCV was almost the same in the low- and high-dose groups; however, a significant increase was observed in comparison with that of the control group. A significant ($p<0.05$) increase in mean cell hemoglobin (MCH) was detected in the low-dose experimental group when compared with that of the control group. However, no significant difference in the value of MCH was observed when comparing the high-dose group with the low-dose group and control group. High doses of lead and chromium resulted in a significant ($p<0.05$) decrease in MCHC, whereas low amounts of lead and chromium did not show any significant difference with respect to the control group. The complete hematological profiles of exposed quail

blood samples in the low- and high-dose groups versus the control group are shown in Supplementary file Figure 2.

Body weight

Significant ($p<0.05$) decrease in the mean ± SEM of body weight gain was detected in the low-dose group with respect to the control group. High doses of Pb and Cr did not show significant difference in body weight with respect to the low-dose and control groups. The control group showed a significant increase in total weight gain compared to the low-dose group (Table 2).

Behavioral variations

Weekly behavioral variations (alertness, feeding and drinking, crowing, foamy droppings, and mating) in the control and experimental groups were observed. After 30 days of observation and recording their behavior, the overall behavioral patterns and attributes seem to be decreased in both the low- (34 points) and high-dose (41 points) experimental groups, compared to the control group (47 points). These variations are given in Table 3.

DISCUSSION

The present study revealed the potential variations in the hematological parameters of the experimental groups with respect to the control group, when treated with low and high doses (co-exposure) of lead and chromium.

A significant reduction in mean ± SEM body weight gain was detected in the low-dose experimental group with respect to the control group. High doses of lead and chromium did not show a significant difference in weight gain for the low-dose and control groups. The control group showed a significant increase in total weight gain compared to the low-dose group. The decrease in weight gain may be due to the decrease in feeding after the co-exposure of lead and chromium. Similar results of decreased body weight in experimental groups were also reported by Ibrahim et al.[18].

A significant decline in hemoglobin was observed in both the low-dose and high-dose experimental groups in comparison with the control group. It could be due to the inhibition of heme synthesis caused by the activity of lead, as reported by earlier researchers. Lead hinders the functionality of three essential enzymes which are considered responsible for heme biosynthesis, i.e. ferro chelatase, delta-aminolaevulinic acid dehydratase (ALAD) and delta-aminolaevulinic acid synthase. Ferro chelatases are involved in the addition of iron into the porphyrin ring, but lead can interrupt its pathway. Consequently, suppression of heme synthesis degrades circulating hemoglobin in the blood[19]. Reduction in the hemoglobin due to lead-induced toxicity was also observed in previous studies[18,20-22]. A similar decline in the hemoglobin content was observed after the administration of a lethal dose of chromium in humans[23]. Similarly, a significant down-regulation of hemoglobin concentration was also seen in common carp and Labeo rohita exposed to trivalent chromium[24,25]. The same trend with anemic condition was also observed in the case of lead chloride intoxication in Labeo rohita24.

A significant decline in the hematocrit was detected in both the low-dose group and high-dose group concerning the control group after 30 days of the experiment. It might be due to the impaired hematopoietic system, which ultimately results in the overall decrease in the number of red blood cells of treated groups. Similar results were obtained with the administration of lead acetate (1000 ppm) to male albino mice for a period of 120 days[21]. A significant decrease in the hematocrit was previously reported in lead-intoxicated children and rats[20,26]. Sublethal and lethal concentrations of chromium were also reported to cause a significant decline in the percentage of hematocrit in exposed women[23].

After the treatment of lead and chromium, the experimental groups showed a significant up-regulation of (ESR) with respect to that of the control group. This decline is possibly due to the increase in the number of some particular blood proteins known as acute phase reactants, such as fibrinogen and C reactive protein (CRP). An increase in the number of these proteins expresses the degree of inflammation inside the body in case of certain immune responses. ESR may show an inverse relation with RBCs. As the number of RBCs decreases, the level of ESR increases. Similar results and mechanisms for the increase of ESR were also reported in Wister rats[22].

Further, lead and chromium-experimental groups represented a significant fall in the number of RBCs in comparison with the control group. These changes demonstrate the anemic condition in birds after their exposure to heavy metals. The possibility for this decline may be associated with the failure of the hematopoietic mechanism in the bone marrow of affected birds[27]. In previous studies, the decline in erythrocyte count and reduction in the life span of RBCs was associated with the inhibition of the heme-producing enzyme ALAD[22,28]. Similar results were also revealed after the exposure of hexavalent and trivalent chromium and lead in women and grass carp, respectively[23,24,27].

White blood cells (WBCs) exhibited a significant up-regulation in their concentration in both the low-dose and high-dose treated groups than in the control group. Since the WBCs are considered as the body's first line of defence against foreign substances, their increase may represent the resistive condition in the immune system of birds against lead and chromium toxicity. Previous studies showed that lead intoxication increased the WBC count in male rats and mice, which resulted in a form of leukocytosis[22,28]. A significant increase in the WBCs was also reported in lead-exposed rats[29]. One of the studies revealed a decrease in WBCs against chromium exposure, which could be associated with the apoptosis of leukocytes due to chromium ions. However, our research was not only based on the administration of chromium. It involved the binary

mixture of both lead and chromium. The elevation of WBCs can possibly be induced by lead and chromium rather than chromium itself[30].

A high percentage of monocytes was observed in the birds of the high-dose group in comparison to the control group. However, no significant difference was found in birds of the low-dose group with respect to that of the control group. Results also demonstrated a significant rise in the number of lymphocytes in the birds treated with high and low doses, compared to the control group. A similar trend in terms of monocytes and lymphocytes was also reported by Nisar et al.[22]. Both the monocytes and lymphocytes are thought to play roles in inflammatory and infectious conditions in birds, which could be the reason for their increase in heavy metal-treated groups[31]. This seems to resemble our results.

A significant up-regulation in the number of basophils and eosinophils was exhibited in the high-dose group in comparison to both the low-dose and control groups. However, there was no significant difference between the low-dose group and the control group. Neutrophils were found to be increased significantly after the intake of both the high and low doses of lead and chromium, compared to the control group. This increase may play a role in the inflammation and delayed hypersensitivity in birds, as reported by Mitchell and Johns[31].

Platelet (thrombocytes) count in the experimental groups was increased significantly with respect to the control group. This condition is medically described as thrombocytosis. The possible cause for the elevation of platelets in treated birds is metal-induced toxicity. They are involved in the phagocytic activity to remove foreign substances from the blood of birds. Platelet counts are not usually performed due to their abrupt clumping. However, they are generally elaborated as increased, decreased, or adequate[31].

Mean cell volume (MCV) was almost the same in the low- and high-dose groups; however, a significant increase was observed in comparison with the control group. A slight increase in MCV was also described in chromium-intoxicated rats by Dworzański et al.[30].

A significant increase in mean cell hemoglobin (MCH) was shown by both experimental groups when compared to the control group. However, no comparable difference was seen in the values of MCH in the treated groups. Dworzański et al.[30] also reported a lower rise in the value of MCH after exposure of rats to different doses of chromium compounds. High doses of lead and chromium exhibited a significant fall in MCHC, whereas low amounts of lead and chromium did not show any significant difference with respect to the control group. Our findings are similar to those of El-Boshy et al.[32].

Behavioral changes due to lead and chromium intoxication were assessed by varying behavior such as; an increase in alertness, a decrease in feeding and drinking, and foamy droppings. Alertness caused by these toxicants was attributed to the generation of neurotoxicity in birds. Exposure of birds to these metals can cause the impairment of neuronal ion channels (particularly sodium ions) and disability of basic enzymes involved in the functioning of neurons. Dysfunction of enzymes such as acetylcholine esterase, monoamine oxidase, and Na+ / K+ ATPase can cause neuronal disorder. This may lead to the increased activity of acetylcholine and, consequently response in the form of overstimulation of the cholinergic nerve. Hence, the birds may show imbalance, unstable behavior, or alertness[33]. The same behavioral alterations (increased alertness, instability, and imbalance) were also detected in quails exposed to different pesticides[34,35].

Limitations

As this study is based on an animal in vivo experiment to elucidate the hematological and behavioral changes after the co-exposure of lead and chromium, this research is limited in the context that it can be further extended towards certain molecular aspects of all the obtained results.

CONCLUSIONS

The binary mixture of lead and chromium in low and high doses, posed detrimental effects on the hematopoietic system of quails, which lead to changes in their body weight and caused significant behavioral and physiological variations.

REFERENCES

1. Xu H, Jia Y, Sun Z, et al. Environmental pollution, a hidden culprit for health issues. Eco Environ Health. 2022;1(1):31-45. doi:10.1016/j.eehl.2022.04.003
2. Singh R, Gautam N, Mishra A, Gupta R. Heavy metals and living systems: an overview. Indian J Pharmacol. 2011;43(3):246-253. doi:10.4103/0253-7613.81505
3. Duruibe JO, Ogwuegbu MOC, Egwurugwu JN. Heavy metal pollution and human biotoxic effects. Int J Phys Sci. 2007;2(5):112–118.
4. Collin MS, Venkatraman SK, Vijayakumar N, et al. Bioaccumulation of lead (Pb) and its effects on human: a review. J Hazard Mater Adv. 2022;7:100094. doi:10.1016/j.hazadv.2022.100094
5. Wu X, Cobbina SJ, Mao G, Xu H, Zhang Z, Yang L. A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. Environ Sci Pollut Res Int. 2016;23(9):8244-8259. doi:10.1007/s11356-016-6333-x
6. van der Kuijp TJ, Huang L, Cherry CR. Health hazards of China's lead-acid battery industry: a review of its market drivers, production processes, and health impacts. Environ Health. 2013;12:61. doi:10.1186/1476-069X-12-61
7. Mudipalli A. Lead hepatotoxicity & potential health effects. Indian J Med Res. 2007;126(6):518-527.
8. Hegazy AMS, Fouad UA. Evaluation of lead hepatotoxicity; histological, histochemical and ultrastructural study. Forensic Med Anat Res. 2014;2(3):70-79. doi:10.4236/fmar.2014.23013
9. Borges LP, Brandão R, Godoi B, Nogueira CW, Zeni G. Oral

administration of diphenyl diselenide protects against cadmium-induced liver damage in rats. Chem Biol Interact. 2008;171(1):15-25. doi:10.1016/j.cbi.2007.09.005
10. Suljević D, Hodžić-Klapuh L, Handžić N, Fočak M. Morpho-functional alterations in lymphocytes and erythrocytes of Japanese quail due to prolonged in vivo exposure to heavy metal complexes. J Trace Elem Med Biol. 2020;59:126472. doi:10.1016/j.jtemb.2020.126472
11. Ahmad F, Javed MT, Sandhu MA, Kausar R. Effects of higher levels of chromium and copper on broiler health and performance during the peak tropical summer season. Vet Arh. 2004;74(5):395-408.
12. Alijagic A, Islamagić E, Fočak M, Suljevic D. Effects of trivalent and hexavalent dietary chromium on blood biochemical profile in Japanese quails. Bulgar J Vet Med. 2018;21:470-477. doi:10.15547/bjvm.1095
13. Egwumah F, Egwumah P, Tyowua B. Evaluation of Black-Headed Oriole Oriolus brachyrhynchus (Swainson, 1837) as bioindicator of arsenic contamination using atomic absorption spectrometry (AAS). J Agric Ecol Res Int. 2017;12(3):1-9. doi:10.9734/JAERI/2017/35338
14. Todd JC, Sanford AH, Davidson I, Henry JB. Todd-Sanford clinical diagnosis by laboratory methods. 14th ed. W. Saunders; 1969.
15. Hunter D, Bonford RR. Hutchinson Clinical Methods. 14th ed. Cassell; 1963.
16. Dacie JV, Lewis SM. Practical Haematology. Longman; 1975.
17. Inkielewicz-Stepniak I, Radomski MW, Wozniak M. Fisetin prevents fluoride- and dexamethasone-induced oxidative damage in osteoblast and hippocampal cells. Food Chem Toxicol. 2012;50(3-4):583-589. doi:10.1016/j.fct.2011.12.015
18. Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE. Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed. 2012;2(1):41-46. doi:10.1016/S2221-1691(11)60187-1
19. Suljević D, Handžić N, Fočak M, et al. Lead exposure influences serum biomarkers, hepatocyte survival, bone marrow hematopoiesis, and the reproductive cycle in Japanese Quails. Biol Trace Elem Res. 2021;199(4):1574-1583. doi:10.1007/s12011-020-02272-y
20. Othman AI, al Sharawy S, el-Missiry MA. Role of melatonin in ameliorating lead induced haematotoxicity. Pharmacol Res. 2004;50(3):301-307. doi:10.1016/j.phrs.2004.01.013
21. Çetin N, Eşsiz D, Eraslan G, Saltaş H. Effects of chronic lead intake via drinking water on some hematologic parameters in mice. EÜ J Health Sci. 2004;13(3):59-63.
22. Nisar NA, Sultana M, Baba NA, et al. Ameliorative effect of vitamin C on the haematological changes induced by exposure of chlorpyriphos and lead acetate in Wistar rats. Comp Clin Pathol. 2014;23:829-834. doi:10.1007/s00580-013-1697-5
23. Alijagi A, Suljevi D, Focak M. Trivalent Cr(III) and hexavalent Cr(VI) chromium affect morphology and diversity of hematopoietic outcome in Japanese quail (Coturnix japonica). Hacettepe J Biol Chem. 2017;45(2):263-268. doi:10.15671/HJBC.2017.161
24. Vutukuru SS. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, Labeo rohita. Int J Environ Res Public Health. 2005;2(3-4):456-462. doi:10.3390/ijerph2005030010
25. Abedi Z, Khalesi MK, Eskandari SK. Biochemical and hematological profiles of common carp (Cyprinus carpio) under sublethal effects of trivalent chromium. Iran J Toxicol. 2013;7(20):782-792.
26. Jacob B, Ritz B, Heinrich J, Hoelscher B, Wichmann HE. The effect of low-level blood lead on hematologic parameters in children. Environ Res. 2000;82(2):150-159. doi:10.1006/enrs.1999.4011
27. Shah N, Khan A, Ali R, et al. Monitoring bioaccumulation (in gills and muscle tissues), hematology, and genotoxic alteration in Ctenopharyngodon idella exposed to selected heavy metals. Biomed Res Int. 2020;2020:6185231. doi:10.1155/2020/6185231
28. Mugahi MN, Heidari Z, Sagheb M, Barbarestani M. Effects of chronic lead acetate intoxication on blood indices of male adult rats. DARU J Pharm Sci. 2003;11(4):147-151.
29. Ghazanfarpour E, Fatemi M, Ghandehari F. Protective effect of Lactobacillus fermentum on lead-induced hematological and body weight alterations in rats. Iran J Toxicol. 2019;13(3):15-20. doi:10.32598/IJT.13.3.596.1
30. Dworzański W, Sembratowicz I, Cholewińska E, et al. Effects of different chromium compounds on hematology and inflammatory cytokines in rats fed high-fat diet. Front Immunol. 2021;12:614000. doi:10.3389/fimmu.2021.614000
31. Mitchell EB, Johns J. Avian hematology and related disorders. Vet Clin North Am Exot Anim Pract. 2008;11(3):501-vi. doi:10.1016/j.cvex.2008.03.004
32. El-Boshy ME, Refaat B, Qasem AH, et al. The remedial effect of Thymus vulgaris extract against lead toxicity-induced oxidative stress, hepatorenal damage, immunosuppression, and hematological disorders in rats. Environ Sci Pollut Res Int. 2019;26(22):22736-22746. doi:10.1007/s11356-019-05562-8
33. Hamidipour F, Pourkhabbaz H, Banaee M, Javanmardi S. Bioaccumulation of lead in the tissues of Japanese quails and its effects on blood biochemical factors. Iran J Toxicol. 2016;10(2):13-21. doi:10.32598/IJT.10.2.315.1
34. Hussain R, Mahmood F, Khan A, Javed MT, Rehan S, Mehdi T. Cellular and biochemical effects induced by atrazine on blood of male Japanese quail (Coturnix japonica). Pestic Biochem Physiol. 2012;103(1):38-42. doi:10.1016/j.pestbp.2012.03.001
35. Hussain R, Khan A, Mahmood F, Rehan S, Ali F. Clinico-hematological and tissue changes induced by butachlor in male Japanese quail (Coturnix japonica). Pestic Biochem Physiol. 2014;109:58-63. doi:10.1016/j.pestbp.2014.01.005

CONFLICTS OF INTEREST
The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none was reported.

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ETHICAL APPROVAL AND INFORMED CONSENT
The experiments were conducted in keeping with all the guidelines for the Prevention of Cruelty to Animals Act (1890), Punjab Wildlife Act (1974), and all the hygiene and safety measures. This study was approved by the Ethical Committee of the University of Agriculture Faisalabad (Approval number: ER3729; Date: 28 January 2021).

DATA AVAILABILITY
The data supporting this research are available from the authors on reasonable request.

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