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SCIENCE AND SOCIETY INSIGHTS
Sci Soc Insights (2025), 1(4): 203-211 Research Article

Toxicological Impact of Lead and Arsenic Mixture on Hematological Parameters in Japanese Quail (Coturnix japonica)

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Article History: 25-290 Received: 08 Nov 2024 Revised: 08 Feb 2025 Accepted: 08 Feb 2025 Published Online: 2025

Citation: Shabbir G, Ramzan S, 2025. Toxicological impact of lead and arsenic mixture on hematological parameters in japanese quail (coturnix japonica). Sci Soc Insights 1(4): 203-211.

ABSTRACT

Heavy metals are affecting the surroundings through natural and anthropogenic sources. Lead (Pb) and arsenic (As) are extremely toxic metals with their potential effects on the health of living organisms around the globe. Birds may serve as valuable bio indicator of environmental health. Hematological and biochemical indices are among the most reliable markers for accessing animal and human health. In order to evaluate the toxicological effect of metals mixture (lead + arsenic) on the hematological profile in Japanese quail (Coutrnix japonica) twelve adult Japanese quails of body weight between 100-120g were bought from Jhang bazaar bird market Faisalabad. All birds were divided into three equal groups (n=4). Group I was named as control group and provided only with tap water and standard pellet feed; Group II: treated with the low dosage of lead (1 mg/kg) and arsenic (1 mg/kg); Group III: treated with the high dosage of lead (10 mg/kg) and arsenic (15 mg/kg body weight). Birds were slaughtered after 30 days of trial. Blood samples were collected from the animals of all groups in EDTA tubes. Different hematological parameters were estimated. Body weight gain of Low and high treated groups was decreased significantly ($p<0.05$) than control group birds. Results showed a significant ($p<0.05$) increase in the values of WBCs count (Monocyte, Lymphocytes, Basophils, Neutrophils and Eosinophil's, Erythrocyte sedimentation rate (ESR) and Mean corpuscular volume (MCV) in the treated groups as compared to control group. A significant ($p<0.05$) decrease in the values of Hematocrit, Hemoglobin and RBCs were observed in treated groups as compared to control group. Mean corpuscular Hemoglobin concentration (MCHC) showed a non-significant decrease and MCH showed a non- significant ($p>0.05$) increase in treated groups as compared to control group. Therefore, lead (Pb) and arsenic (As) exerted detrimental effects on hematology of birds.

***Keywords*:** Heavy metals, Lead (Pb) toxicity, Arsenic (As) exposure, Animal health and Japanese quail (Coturnix japonica)

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 (Xu et al 2022). Heavy metals with comparably high density (i.e. 5 g/cm3) 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 (Singh et al 2011).

The bio toxic 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 sub chronic), 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 (Duruibe et al 2007: Collin et al 2022).

Heavy metals are classified as the metals with an atomic number of more than 20 and a specific density of more than 5 g cm-3(Ali and Khan, 2018). On the basis of their role in physiology of living organisms, they can be divided into two types; essential and non-essential elements. Essential elements are those needed for various biochemical and physiological functions in the body by living organisms. Manganese, Iron, Cobalt, Copper and Zinc are some examples of essential elements. On the other hand, certain elements that have no specific physiological role in the body are non-essential elements. These non-essential heavy metals can cause potential effects in the organisms (Sauliute and Svecevicius G, 2015).

Lead, Mercury, Arsenic and cadmium are considered most harmful non-essential heavy metals (Fraga, 2005). A number of heavy metals are affecting the surroundings from natural and anthropogenic sources. Emissions of heavy metals from the anthropogenic resources are increasing day by day in many parts of the world because of the urbanization and industrialization. Birds consume heavy metals through their food, water, and surrounding environment (through preening). The relationship between exposure and toxic kinetics in birds must be understood in terms of ingestion of pollutants and their possible age and concentration dependent outcomes (Bond and Lavers, 2011).

Haematological and biochemical indices are among the most reliable markers for assessment of animal and human health (Ohaeri and Eluwa 2011The presence of As, Cd, B, and Pb metals over the recommended limits in birds indicates that their habitat is heavily polluted with heavy metals. According to previous research, elevated levels of heavy metals in exposed birds that are insufficient to cause direct death can result in behavioural abnormalities, reproductive impairment, and an increased risk of disease. Reproductive impairment may occur as a direct result of exposure, resulting in decreased clutch size, delayed hatching, nestling mortality, and a thin egg shell in birds (Das et al, 2004).

The heavy metals can be involved in different bodily changes such as neurological disorders, depression and learning difficulties. Heavy metals can also induce impairment of enzyme action, cellular damage, variations in genetic material (DNA) which may result in cancer and birth defects. Structural anomalies in erythrocytes such as abnormal nuclei, poikilocytosis, RBCs swelling, cell membrane degradation in fish exposure to toxic metal contamination were observed (Banarjee et al., 2008; Strunjak et al., 2009). Lead poisoning is primarily caused by its connection with organic components such as enzymes, signalling molecules, regulatory proteins, and the bone matrix (Cangelosi et al., 2017).

Arsenic contamination for wildlife and humans is a great concern and these environmental pollutants are increasing because of anthropological practices. Arsenic exists in both organic and inorganic forms. Inorganic arsenate (Arsenic (V)) and arsenite (arsenic (III)) are the main forms of arsenic which are presents in the soil and water. Four different oxidation states (arsenate, arsenite, arsenic and arsine) are important in regards to the toxicological effects of arsenic (Sharma and Sohn, 2009). Organo-arsenicals are formed when inorganic arsenic compounds are methylated by biological processes.

Arsenic may participate in intracellular oxidation-reduction reactions which can create Nausea, vomiting, diarrhea and muscle cramping are all symptoms of arsenic poisoning (Rahman et al., 2001). Metal toxicity induces the formation of reactive oxygen species which oxidize lipids, proteins, RNA, and DNA. Furthermore, it also disrupts the cell's structure which may lead to cell death (Cao et al., 2010). Arsenic can cause poising in different parts of body. Blood is the major target of arsenic toxicity. Chronic arsenic toxicity results in iron deficiency, leukopenia, hypoplasia of the bone marrow, aplastic anemia, thrombocytopenia, and thrombocytopenia and abnormalities in liver, Karyorrhexis, and late metabolism (Das et al., 2012).

Furthermore, it can cause reduced red blood cell, lymphocyte, and monocyte counts could possibly be a result of an increased rate of cell death, decreased metabolic activity in birds, impaired hemosynthesis, and decreased oxygen carrying capacity in blood forming organs (Ghaffar et al., 2015). 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

MATERIALS AND METHODS

Experimental Design

For this experiment adult Japanese quail (Coutrnix japonica) having weight between 100-120g were used. All the animal trials were conducted in animal house facility, University of Agriculture, Faisalabad for 30 days. Animals were acclimatized prior to the experimentation for 7 days. Animals were divided into three groups, 4 in each group captives in the stainless-steel cages at 20-22ºC temperature. Animals were given access to the tap water and standard food chew

with 12 h light and dark cycles. All animal ethics were followed during whole experimentation as set by ethical committee of University of Agriculture, Faisalabad.Lead and Arsenic were purchased from Sigma-Aldrich (Germany). Twelve adult and healthy Japanese quails of (100-120g) body weight were selected for the experiment. Animals were divided in three groups (4 birds/ group) First group served as control group. Control group were provided by standard pellet feed and tap water. Second group served as experimental group and treated with low doses of lead (1 mg/kg body weight) and arsenic (1 mg/kg body weight). Second group was also provided with standard pellet feed and tap water. Third group was experimental group and treated with high doses of Lead (Pb) (10 mg/kg) and arsenic (15 mg/kg) respectively. All animals in control and treated groups were fed orally through gavagesThe stock solution of lead was prepared by homogenizing 100 mg of lead chloride into 100 ml of distilled water. Same method is used for preparing the stock solution of Arsenic. Low and high dose of Lead were administered to Japanese quails once a day in different time intervals at 0.105 ml and 1.05 ml concentrations, respectively. Similarly, the low and high doses of Arsenic were administered once a day to Japanese quails in different time intervals at 0.6ml and 1.8ml concentrations respectively. Both lead and arsenic dosage were injected with the help of small tubes. Initial and final body weights of all the birds were measured with the help of digital weight balance in order to detect the changes in body weight after experimentation. All the birds were slaughtered with the help of the sharp surgical blade after completion of trial. All the safety and hygiene measures during slaughtering of animals were followed. Blood was collected in the EDTA tubes. Prior to hematological testing, a drop of blood was taken on a clean glass slide to examine the presence of any pathogen. Infection free blood was used for testing to avoid from the variations. Samples were collected during particular time in early hours of the day. All the blood samples were kept in freezer abruptly to prevent them from contamination and disintegration.

Hematological Analysis:

RBCs and WBCs counts were estimated by using the Neubauer Crystalline counting chamber. To calculate the leukocytes and other types of WBCs, the Hunter and Bonford (Hunter 1963) 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 (1975) was employed

Statistical analysis:

After the careful testation and investigation, all the derived data were analyzed statistically and expressed as mean ± SEM. It was further analyzed by using a basic statistical technique known as one way analysis of variance or ANOVA followed by Tukey's test. Both the analyses were performed by using SPSS statistical Software. In order to compare the control verses experimental groups, graph pad prism 5 software were used. $P<0.05$ were kept as significance level (Inkielewicz-Stepniak et al., 2012).

RESULTS

Body Weight

Current results suggested significant ($p<0.05$) differences in treated groups as compared to control. Body weight gain in high dosage group (63± 1.77) is close to the control (69.5 ± 1.93). But there is significant ($p<0.05$) decline in the body weight gain in low dosage group (33 ±5.87) as compared to control group.

Table 1: Show the body weight with different dosage of Pb and Ac

Groups	Initial BW (g) Mean ± SEM	Final BW (g) Mean ± SEM	Weight gain (g) Mean ± SEM	p
Control	104.5 ± 5.59	174± 2.03	69.5 ± 1.93^{c}	<0.05
Pb + Ar (Low dose)	104.75 ± 1.08	137.75 ± 4.17	33 ±5.87 ^{b}	<0.05
Pb + Ar (High dose)	103.75 ± 9.60	166.75± 1.88	63± 1.77^{a}	<0.05

Values are expressed as mean ± SEM

Means that do not share a letter are significantly different at $p<0.05$

Hematological variations

Hematological variations are shown in Table 2. significant ($p<0.05$) differences in treated groups as compared to control in hematocrit percentage. Lead and arsenic at low (41.58 ± 1.10) and high (34.25 ± 1.51) doses significantly ($p<0.05$) decreased the hematocrit percentage in the blood of the experimented groups as compared to the control group (51.25 ± 1.10). After 30 days of the experiment, a significant ($p<0.05$) difference in hemoglobin content was observed in treated groups as compared to control group. Hemoglobin levels were significantly ($p<0.05$) declined in the high (8.25 ± 0.32) and low (12.25 ± 0.91) dose groups than in the control group (15.35 ± 0.37) significant ($p<0.05$) differences were observed in Erythrocyte Sedimentation Rate (ESR) in treated groups as compared to control group. Erythrocyte Sedimentation Rate (ESR) levels were significantly ($p<0.05$) lowered in the high (5.45 ± 0.13) and low (3.52 ± 0.25) dose groups than in the control group (1.82 ± 0.12). Between the control and experimental groups, a significant ($p<0.05$) difference in RBCs count was observed. Lead and arsenic at low (3.52 ± 0.15) and high (2.7 ± 0.08) doses significantly ($p<0.05$) decreased the RBCs count than the control group (4.5 ± 0.12).

After 30 days of lead and arsenic intoxication, a significant ($p<0.05$) difference in total leukocyte count was observed between control and treated Japanese quails. The number of WBCs was significantly increased ($p<0.05$) in low (0.31 ± 0.01) and high (0.55 ± 0.01) groups compared to the control group (0.19 ± 0.04). There was no significant difference in the number of basophils and eosinophils between the low and high dose group However, when compared to the control group, a significant ($p<0.05$) increase was observed in significant ($p<0.05$) differences in platelet count in treated groups as compared to control. Lead and arsenic at low (210 ± 8.63) and high (254 ± 8.49) doses increased platelets significantly ($p<0.05$) more than the control group (176.5 ± 5.75). The mean corpuscular volume (MCV) was identical in the low dose group and control group, a significant ($p<0.05$) increase was observed in the low (116 ± 6.46) and high (134 ± 3.85) dose treated group when compared to the control (116 ± 2.02).

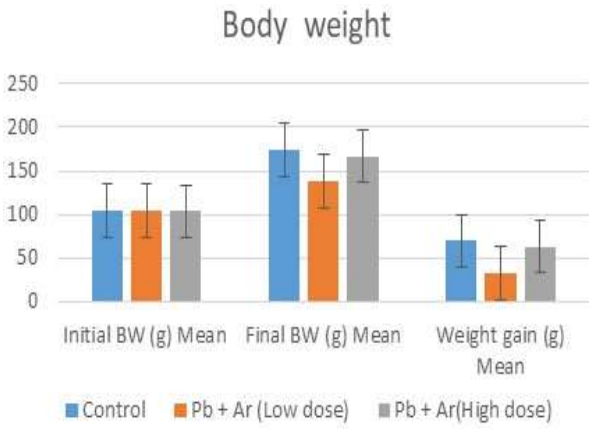

Fig 1: Graph 1 show the body weight with different dosage of Pb and Ac.

Table 2: Show the Hematological variations with different dosage of Pb and Ac

Measurements	Control Mean ± SEM	Pb + Cr (Low dose) Mean ± SEM	Pb + Cr (High dose) Mean ± SEM	p
Hematocrit (%)	51.25 ± 1.10c	41.58 ± 1.10b	34.25 ± 1.51a	<0.05
Hemoglobin (g/dL)	15.35 ± 0.37c	12.25 ± 0.91b	8.25 ± 0.32a	<0.05
Erythrocyte sedimentation rate (ESR)	1.82 ± 0.12c	3.52 ± 0.25b	5.45 ± 0.13a	<0.05
RBCs (1012/L)	4.5 ± 0.12a	3.52 ±0.15b	2.7 ± 0.08c	<0.05
WBCs (10^9/L)	13.12 ± 0.42c	20.25 ± 0.85b	15.55 ± 0.15a	<0.05
Monocytes (10^9/L)	0.19 ± 0.04c	0.31 ± 0.01b	0.55 ± 0.01a	<0.05
Lymphocytes (10^9/L)	2.05 ± 0.10c	3.58 ± 0.06b	4.1 ± 0.09a	<0.05
Basophils (10^9/L)	0.05 ± 0.02c	0.18 ± 0.02b	0.32 ± 0.01a	<0.05
Neutrophils (10^9/L)	2.12 ± 0.45c	4.2 ± 0.10b	9.2 ± 0.09a	<0.05
Eosinophils (10^9/L)	0.3 ± 0.04c	0.58 ± 0.06b	1.32 ± 0.108a	<0.05
Platelets (10^9/L)	176.5 ± 5.75c	210 ± 8.63b	254 ± 8.49a	<0.05
MCV (fL)	116 ± 2.06b	116 ± 6.46b	134 ± 3.85a	<0.05
MCH (pg)	33.5 ± 0.64a	34.25 ± 4.62a	38 ± 1.73a	<0.05
MCHC (g/dL)	29 ± 0.40a	29 ± 2.61a	28 ± 0.57a	<0.05

Non-Significance increase in mean cell hemoglobin (MCH) were showed by both low (34.25 ± 6.62) and high (38± 1.73) dose experimented groups when compared with that of control group (33.5 ± 0.64). Lead and arsenic at high doses (28 ± 0.57) resulted in a non-significant decrease in MCHC, whereas low doses (29 ± 2.61) of lead and arsenic had no significant effect on MCHC when compared to the control group (29 ± 0.40) as shown in above Table 2.

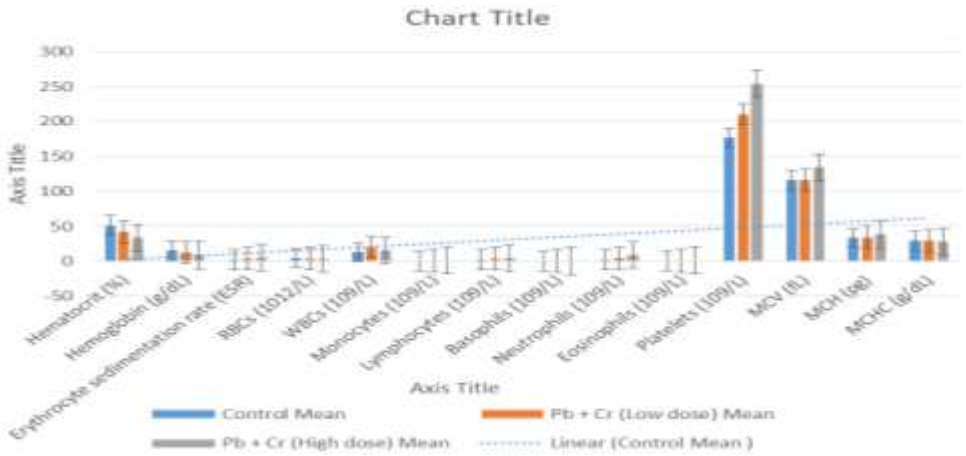

Fig 2: Graph 2 show the Hematological variations with different dosage of Pb and Ac.

Behavioural changes

Potential behavioural variations were recorded in experimented groups in the form of grades (0-4) for the period of 30 days. Weekly behavioural variations (Alertness, feeding, drinking, crowing, foamy pop, mating) in control and experimented groups were observed.

Changes in 1st Week:

After first week, lead and arsenic resulted in the increase in the behavioural variations in low dosage group (10) and high dosage group (09) as compared to control group (08).

Table 3: Show the Behavioral changes in 1st week

Behavioral Parameters Dosage	Range	Control	Low Dosage	High
Alertness	0-4	1	2	2
Feeding & drinking	0-4	4	4	3
Crowning	0-4	1	2	2
Foamy Poop	0-4	2	2	1
Mating	0-4	0	0	0
Total		8	10	9

Changes in 2nd Week:

Lead and arsenic showed a slightly decrease in behavioural variations in high dosage group (08) with respect to that of low dosage (09) and control group (09) at the end of second week. There was no difference in behavioural variations in low dosage group and control group.

Table 4: Show the Behavioral changes in 2nd week

Behavioral Parameters Dosage	Range	Control	Low Dosage	High
Alertness	0-4	2	2	3
Feeding & drinking	0-4	3	3	2
Crowning	0-4	1	1	1
Foamy Poop	0-4	3	3	2
Mating	0-4	0	0	0
Total		9	9	8

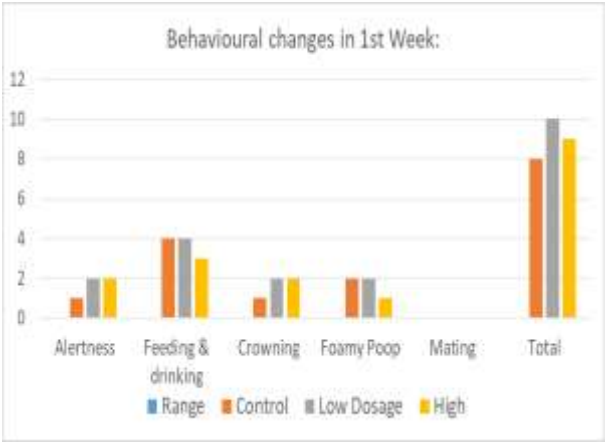

Fig 3: Graph 3 show the Behavioral changes in 1st week.

Fig 4: Graph 4 show the Behavioral changes in 2rd week.

Changes in 3rd Week:

A slightly decrease in behaviour of high dosage group (12) and low dosage group (11) was observed. However, an assessable reduction in behaviour of low dose group (11) was detected with respect to control group (13).

Table 5: Show the Behavioral changes in 3rd week

Behavioral Parameters Dosage	Range	Control	Low Dosage	High
Alertness	0-4	2	4	4
Feeding & drinking	0-4	4	2	2
Crowning	0-4	2	2	3
Foamy Poop	0-4	3	2	0
Mating	0-4	2	1	3
Total		13	11	12

Changes in 4th Week:

After fourth week of experiment, potential behavioural variations took place in both the low dosage (08) and high dosage group (11) as compared to control group (15).

Changes after 30 Days:

Overall behavioural variations decreased in both the low (38) and high dosage (40) experimented groups as compared to control group (45). These variations can be easily assessed in Table 7.

Table 6: Show the Behavioral changes in 4th week

Behavioral Parameters Dosage	Range	Control	Low Dosage	High
Alertness	0-4	2	4	4
Feeding & drinking	0-4	4	2	1
Crowning	0-4	3	0	2
Foamy Poop	0-4	2	0	0
Mating	0-4	4	2	4
Total		15	8	11

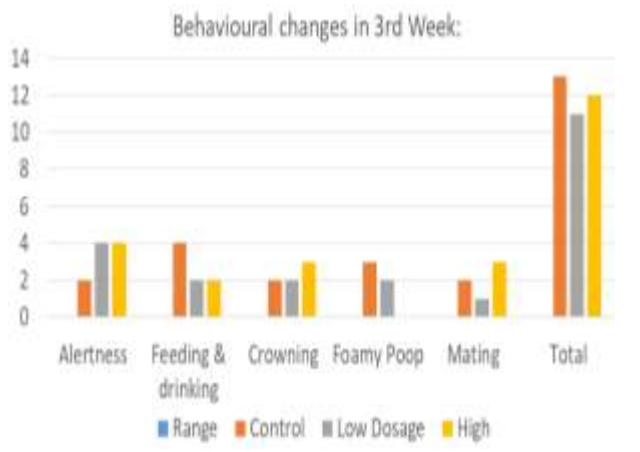

Fig 5: Graph 5 show the Behavioral changes in 3th week.

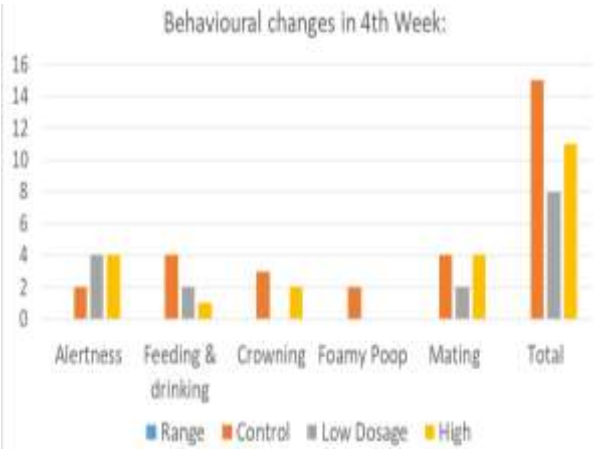

Fig 6: Graph 6 show the Behavioral changes in 4th week.

Table 7: Show the Behavioral changes after 30 days

Weeks	Range	Control	Low Dosage	High Dosage
1st week	20	8	10	9
2nd Week	20	9	9	8
3rd Week	20	13	11	12
4th Week	20	15	8	11
Grand Total	80	45	38	40

DISCUSSION

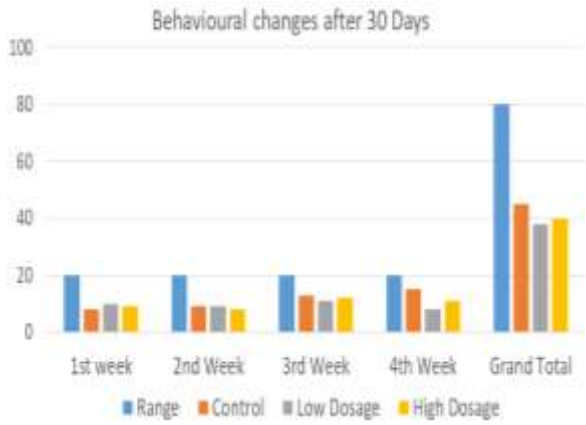

Fig 7: Graph 7 show the Behavioral changes after 30 days.

Absorption, distribution and accumulation of heavy metals in tissues and organs are determined by a variety of factors. These factors are chemical nature of heavy metals. Blood cells and other hematological parameters are well-established as the best biomarkers for assessing the toxic potential of various toxic substances, even at low concentration. Additionally, hematological parameters reflect the pathophysiological state of various animals which are exposed to various toxic substances (Hussain *et al*., 2012; Khan *et al.,* 2013). The haemopoietic process is among the most susceptible target site to assess the heavy metal-based toxicity. Arsenic and lead are absorbed into the intestine and then transported via the bloodstream. They can be distributed in the bloodstream through red blood cells and plasma proteins primarily albumin.

Significant ($p<0.05$) differences in treated groups as compared to control. Body weight gain in high dosage group (63± 1.77) is close to the control (69.5 ± 1.93). Ibrahim *et al.* (2012) also reported similar results of decreased body weight in experimented groups. Ghazanfarpour *et al*. (2019) also reported a significant decrease in the rats exposed to lead acetate. The loss of body weight can be due to the anorexia caused by the ingestion of lead. It may also be due to muscle massthat can cause cachexia. The loss of muscle mass can be caused by oxidative stress caused by lead in body.

In the present study, level of Hematocrit reduced significantly ($p<0.05$) in high (34.25 ± 1.51) and low (41.58 ± 1.10) dose groups than in control group (51.25 ± 1.10). Reduced hematocrit percentages can be caused by heavy metal toxicity (Ghaffar *et al*., 2015). Significant decrease in Hematocrit values in albino mice exposed to lead for 120 days. Ssignificant reduction in arsenic-exposed rats. The reduced percentage of hematocrit may be due to the effect of lead on heme synthesis because it inhibits the major functions of enzymes (ferrochelatase, delta-

aminolevulinic acid dehydratase and delta-aminolevulinic acid synthase) (Piomelli, 2002).

In the current study, the value of Hemoglobin (Hb) also showed a significant decrease ($p<0.05$) in high (8.25 ± 0.32) and low (12.25 ± 0.91) treated groups than control group (15.35 ± 0.37). Bollini *et al*. (2010) also reported that haemoglobin (Hb) was significantly ($p<0.05$) reduced in the women exposed to arsenic. Reduced hemoglobin levels lead to RBC deformity and degeneration. Reduced haemoglobin level due to metals mixture toxicity may be due to inhibition of heme synthesis caused by the activity of lead.

Our findings indicated a significant increase ($p.> 0.05$) in the ESR value in the high (5.45 ± 0.13) and low dose groups (3.52 ± 0.25) as compared to the control group (1.82 ± 0.12). Ghaffar *et al.* (2015) obtained comparable results in adult male poultry exposed to arsenic. Furthermore, a significant decrease ($p<0.05$) in the Value of RBCs count in high (2.7 ± 0.08) and low dose groups (3.52 ±0.15) as compared to control groups (4.5 ± 0.12) was observed. Erythrocytes are a prime target of Lead (Pb) poisoning and it plays a critical role in innate immunity. Grandjean *et al.* (1989) demonstrated a lead-dependent delay in regeneration of human RBCS. Previously, similar findings were made in rats rabbits and children. When lead enters the systemic circulation, it accumulates at a rate of 95% in erythrocytes. The decrease in red blood cell count could also be a result of an increased rate of cell destruction, decreased metabolic activity in birds, impaired hemosynthesis, and decreased oxygen carrying capacity in blood producing tissues (Ghaffar *et al.,* 2015).

In current study a significant increase ($p<0.05$) in the Value of WBCs was observed in the high (20.25 ± 0.85) and low (15.55 ± 0.15) dosage groups as compared to control group (13.12 ± 0.42). Escalation in total white blood cells and neutrophil populations may be due to hypersensitivity reactions and underlying tissue damage. WBCs, lymphocytes and neutrophils were all increased in rats exposed to arsenic. Additionally, lead acetate-exposed rats had significantly higher levels of white blood cells (WBCs) which suggest that the metal is likely to trigger an immune response by stimulating the body's immune system to produce more WBCs. It also increases cell death due to arsenic lipophilic nature. The increase in immune parameters may be a due to oxidative stress.

The overall differential count of leukocytes was significantly ($p<0.05$) increased. The differential count of leukocytes was calculated as the total number of all leukocyte types, including monocytes, lymophocytes, basophils, eosinophils, and neutrophils. WBCs, lymphocytes and neutrophils were all increased in rats exposed to arsenic. Gupta *et al.* (2003) also observed an increase in the values of monocytes, lymophocytes, basophils, eosinophils, and neutrophils in rats exposed to lead. Arsenic-intoxicated birds have also been shown to produce fewer monocytes, lymphocytes and various immune parameters (Khan *et al*., 2014; Sattar *et al.,* 2016). The decrease in differential count of leukocytes count could also be a result of an increased rate of cell destruction, impaired hemosynthesis, decreased metabolic activity in birds and decreased oxygen carrying capacity in blood producing tissues (Ananth *et al.,* 2014).

In the current study, a significant increase in platelet value was observed in the high (254 ± 8.49) and low (210 ± 8.63) dosage groups compared to the control group (176.5 ± 5.75). An increase in Platelet counts of *Cyprinus carpio*. *Cyprinus carpio* is exposed to lethal levels of lead. This finding is consistent with our results of exposure to metals mixtures (Lead+Arsenic) at low and high doses. In comparison to the control group (116 ± 2.06), mean corpuscular volume (MCV) was higher in Japanese quails exposed to low (116 ± 6.46) and high (134 ± 3.85) doses of metals mixture (Lead+Arsenic). Jacob *et al.* (2000) also reported a negative correlation between blood lead levels and MCV and MCH (Jacob *et al*., 2000). The MCV value in rohu was increased when came into contact with lead. The results were in accordance with our research. Non-Significant increase in mean cell hemoglobin (MCH) was showed by both low (34.25 ± 4.62) and high (38 ± 1.73) dose experimented groups when compared with that of control group (33.5 ± 0.64). Mean corpuscular haemoglobin concentration (MCHC) showed a non- significant difference between treated and control groups. Low doses of lead and arsenic had no significant effect on MCHC when compared to the control group. Kim *et al.* (2017) also observed a non-significant decrease in the value of MCHC in juvenile rockfish exposed to dietary lead (Kim *et al.,* 2017). Lead and arsenic intoxication resulted in behavioral changes such as increased alertness or restlessness, feeding and foamy stools. The behavioral changes induced by these toxicants have been ascribed to the development of neurotoxicity in birds. Birds exposed to these metals may develop impaired neuronal ion channels (particularly sodium ions) and basic enzymes involved in neuronal function. The neuronic disorder can be caused by the dysfunction of enzymes such as acetylcholinesterase, monoamine oxidase, and Na+ / K+ ATPase. This may result in increased acetylcholine activity and, consequently, over stimulation of the cholinergic nerve. As a result, the birds may exhibit behavioural instabilities and imbalances. The same behavioural changes were observed in quails exposed to various pesticides (Hussain *et al.,* 2012; Hussain *et al.,* 2014).

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.

CONCLUSION

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.

DECLARATIONS

Funding: This study didn't receive any funding from any agencies in the public, commercial, or non-profit sector.

Conflicts of Interest: Authors have no conflicts of interest.

Data Availability: Data will be available from the corresponding author upon request.

Ethics Statement: Not Applicable

Authors' Contribution: Ghulam Shabbir; Conceptualization, Data Curation, Methodology, Wiring Original draft, Formal Data Analysis, Sania Ramzan; Writing, Review and Editing, Data Analysis and Data Collection

Generative AI Statements: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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