Toxic effect of induced arsenic doses on the complete blood count and serum biochemistry in Quails

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Abstract:

Arsenic is a natural metalloid which is present in the universe everywhere. Poisoning of arsenic is major issue that affects different species. Current study was performed to investigate the toxic effects of feeding inorganic heavy metals such as arsenic on complete blood count and blood chemistry of common quail. Total 120 birds were purchased from the market.60 birds were used in winter and 60 in spring trial. These divided into three groups one kept as control, second group was given (low concentration @ 1.5mg) and third group received are (high concentration @ 2.5mg) of arsenic. Clinical signs including diarrhea and appetite loss were exhibited in all treated birds. A decrease in body weight was observed in all treated groups, while control group birds gain weight. Impact of arsenic on blood parameters was that number of red blood cells, level of hemoglobin and MCH decreased. Number of white blood cells, value of MCV, alanine amino transferase, aspartate amino transferase and cholesterol increased. Analysis of data revealed that the reduction in number of red blood cells and level of hemoglobin were significant ($p<0.05$), while number of white blood cells increased. MCV, the significant value of Cholesterol was also observed in birds affected by high dose of arsenic as compared to control and low dose treated bird. In creatinine and globulins significantly changed was also observed.

Key Words: Mean corpuscular hemoglobin (MCH), Mean cell volume (MCV).

Introduction

The quail also known as batter is a slight moderate size game bird associated with pheasant family. Quail have the benefit of rapid growth rate, small size, better lying ability, short life cycle, good meat taste and shorter time of hatching than other birds (Siyadati *et al.,* 2011). Blood is special type of connective tissue, composed of erythrocytes, leukocytes and platelets in a fluid matrix. Analysis of blood is an vital tool to study the health condition of the animal and to recognize the pathological process (Christopher *et al.,* 1999). PH of the blood range from 7.35 to 7.45(Waugh and Grant, 2007).

Arsenic

Arsenic is a chemical element its atomic number is 33 and AS is symbol. Arsenic pollution of water has directed to extensive arsenic poisoning in many countries (Meharg, 2005; Kozul *et al.,* 2009). After entry in body in severe cases, the first toxic symptom are illustrated by severe vomiting, diarrhea, injuries in blood vessels and cramps in muscles (Chowdhury *et al.*,2001).

Hematological parameters

Hematology is the study of blood and blood cells. Hematology deals with the physiology, diagnosis and prevention of blood related diseases. There are many factors that affect the values of hematological parameters such as; age, sex, ethnic background and social, environmental and nutritional factors. Hemoglobin is oxygen carrying transport metallic protein in the red blood cells. It is also involved in the transport of other gases (Maton *et al.*,1993).

Red blood cells also recognized as erythrocytes are involved in transporting oxygen to the body tissues. (Erich, 1995).The size of red blood cells change extensively among vertebrate species. It has been considered that this improve the oxygen transfer from red blood cells to tissue (Snyder and Sheafor, 1999). White blood cells are also recognized as leukocytes and act as immunological line infectious diseases (Maton, 1997).There are five main types of leukocytes are present including ,eosinophil, basophils, lymphocytes, monocytes and neutrophils, last two are phagocytic(LaFleur, 2005).

Mean corpuscular volume is the quantity of normal capacity of red blood cells. Standard value of MCV is 80-100fl.MCV below and above normal range cause microcytic anemia and macrocytic anemia (Schrier and Auerbach, 2013). Mean corpuscular hemoglobin or mean cell hemoglobin is the usual

bulk of hemoglobin each red cells of blood in a test of blood. It is stated as typical whole blood count. Standard value of MCH is 27-31 picograms/cells.

MATERIALS AND METHODS

Experimental Birds

For experiment 120 common quails (90-140gm) were brought from the local market of quails in Multan. Female quails were selected for the experiment to reduce the interbreeding. Common quail or European quail having good health, 30-40 days age. These quails were kept in the animal house of bio park of Bahauddin Zakariya University, Multan.

Instruments

Instruments used in this experiment were cages, dissection kit, dissection board, blood test tubes, test tube rack, plastic glass jars, Embedding molds, microtome disposable blades, water bath, incubator, water bath, glass slides, and cover slip.

Birds' conditioning

Common quails were reserved in coops in fine lighted, washed and ventilated room. Millet used as feed for quails and provided two times per day. All quails were trained and used to animal house for two weeks earlier starting of experiment. For, identification of each bird, a combination of different colored markers were used to mark on their both right and left foot. Before the start of each experimental trial early mass and rectal temperature of all birds were noted into account.

Quails were categorized in following three sets.

1. **Group 1** control
2. **Group 2** Low concentration(@1.5mg/kg body weight) of arsenic
3. **Group 3** High concentration(@2.5mg/kg body weight) of arsenic

The doses were selected according to the LD_{50} value of metal for quail which was experimentally determined before the start of experiment. The heavy metal was persuaded in quail by oral management of Low concentration (@1.5mg/kg body weight) and High concentration (@2.5mg/kg body weight) of heavy metals-packed in gelatin capsules and given daily for 20 days. Experiment was repeated two times in winter and spring season.

Determination of hematological parameters

Autopsy of bird was done after 5th, 10th, 15th and 20 days of each trial. Hemoglobin level, complete red and white cells of blood, Mean corpuscular volume, mean corpuscular hemoglobin, creatinine, cholesterol, ALAT, ASAT, Uric acid, Total Bilirubin, Albumin, were determined in all groups

Figure 01: Blood sampling

Haemoglobin

Serum hemoglobin was determined by using Drabkin's reagent standard reported method.

Total Red Blood Cell Count (TRBCs): Haemocytometer was used for total red blood cell count. RBC number was considered by using following formula

$$\text{RBC (1012/L)} = \frac{\text{50(volume factor)*200(dilution factor)*the number of cells counted}}{\text{100}}$$

Total white Blood Cell count (TWBCs): Neubaur Ruling Haemocytometer was used to total white cells of blood. Blood was diluted (1:20) with diluting solution in a normal white cells of blood micropipette. Following formula was used to compute number of white blood cells.

$$\text{WBC (109/L)} = \frac{\text{Cells counted*20(dilution factor)}}{\text{Volume (0.4mm)}}$$

Mean corpuscular volume (MCV)

Following formula was used to estimate the mean corpuscular volume

$$\text{MCV (femptoliters)} = \frac{\text{Hematocrit\%*10}}{\text{RBC count (millions/µl)}}$$

Mean corpuscular hemoglobin (MCH)

Computable determination of mean corpuscular hemoglobin level per model was resolute by using following formula

$$\text{MCH (pg.)} = \frac{\text{Hb (gdl-1) *10}}{\text{RBC } (10^6 \mu L^{-1})}$$

Determination of serological parameters

Samples of blood were collected in blood collection tube containing EDTA as anticoagulant and centrifuged at speed (1300 RPM) for 10 minutes to separate the serum from the cells of blood. The level of following serum enzymes were checked in serum samples using commercial kits and by adopting following standard protocols.

Serum creatinine

Serum creatinine was resolved by using commercial kits which contained two reagents R1 and R2. Monoreagent was prepared by mixing 4 parts of R1 and 1 part of R2.10 µL of serum sample was diverse with 1000 µL of monoreagent. Sample was reared at 37^0C for approximately 10 minutes .Absorbance was observed by using micro lab 200 at AL-khidmet medical center laboratory .wavelength of micro lab was adjusted at Hg 546 nm.

Cholesterol

Cholesterol in serum sample was determined by using commercially available kits which contained a ready reagent(R) and a normal solution.

Total protein

It was determined by using commercial kits which contained two reagents R1 and R2.Monoreagent was prepared by mixing 4 parts of R1 and 1 part of R2. 20µL of serum sample was mixed with 1000 µL of monoreagent. Sample was reared at 37^0C for approximately 5 minutes. Absorbance was observed

by using micro lab 200 at AL-khidmet medical center laboratory. Wavelength of micro lab was adjusted at Hg 546 nm.

Albumin

Assessment of serum albumin was done by two reagents in micro lab. Serum sample of 10µL was combined with thousand µL of monoreagent and placed in incubator for half hour. Wavelength of micro lab was adjusted at Hg 546nm.

Serum urea

Serum was determined by using commercial kits which contained two reagents.R1 and R2. Monoreagent was arranged by mixing 4 parts of R1 and 1 part of R2.20 µL of serum sample was diverse with 1000 µL of monoreagent. Mixture was incubated at 25^0C for approximately 60 seconds. Absorbance was observed by using micro lab 200 at AL-khidmet medical center laboratory. Wavelength of micro lab was adjusted at Hg 546 nm.

Serum ASAT/GOT

Commercial kits was using for the determination of ASAT which contained two reagents R1 and R2.Monoreagent was prepared by mixing 4 parts of R1 and 1 part of R2. 100 µL of serum sample was mixed 1000 µL of monoreagent. Absorbance was observed by using micro lab 200 at AL-Khidmet medical center laboratory. Wavelength of micro lab was adjusted at Hg 546 nm.

Serum ALAT/GPT

Serum ALAT was determined by using commercial kits which contained two reagents R1 and R2.Monoreagent was prepared by mixing 4 parts of R1 and 1 part of R2. 100 µL of serum sample was mixed with 1000 µL of monoreagent. Absorbance was observed by using micro lab 200 at AL-Khidmet medical center laboratory. Wavelength of micro lab was adjusted at Hg 546 nm.

Serum uric Acid

Commercial kits were used for determination of serum uric acid which contained two reagents R1 and R2. Monoreagent was prepared by mixing 4 parts of R1 and 1 part of R2. 20 µL of serum sample was mixed with 1000 µL of monoreagent. Mixture was in incubated at 37^0C for approximately 10 minutes.

Absorbance was observed by using micro lab 200 at al khidmet medical Centre. Wavelength of micro lab was adjusted at Hg 546 nm.

Total Bilirubin

Serum Total bilirubin was determined by using commercial kit which contained two reagents R1 (Sulfanilic acid, Hydrochloric acid, Dimethysulphoxide) and R3 (Sodium nitrite).

Statistical Analysis

For comparison of different hematological factors between treated and un- treated birds in 10 and 20 days long trial group two sample T test was performed. Data were expressed as mean ± standard error. One way ANOVA was performed to check the medicinal effect with control group by using **SPSS software.**

Hematological valuation: In one way ANOVA test of **Red blood cells** indicated meaningful difference between standard and treated group that is p=0.000 throughout winter trial experiment(Table.2).In spring trial p=0.000(Table.4).

Hemoglobin test throughout winter trial experiment among control and treated group by one way ANOVA presented the significance difference that is p=0.019 for low dose and 0.001 for high dose. While in spring trial noteworthy modification between standard and high dose treated group is 0.003(Table.4).

Test of **white blood cells** in one way ANOVA presented significant difference between control and high dose treated group that is 0.002(Table.2).In spring trial white blood test by one way ANOVA indicated noteworthy difference between standard and high dose treated birds p=0.001(Table.4).

In one way ANOVA **Mean corpuscular hemoglobin** test showed notable variations among standard and high dose treated birds that is p=0.047(Table.2).While in spring trial it is p= 0.044(Table.4).

Total protein and albumin test in both trial experiment through one way ANOVA showed strong noteworthy modification between control and arsenic treated birds that is p=0.000(Table.2) and (Table.4).

In one way ANOVA **cholesterol and uric acid test** showed important changes between high dose treated birds and control group that is p=0.010 and p=0.001 respectively(Table.2).In spring trial this test also showed significant difference that is p=0.000 for both(Table.4).

Creatinine test by one way ANOVA presented vital changes in standard, low and high dose treated group p=0.000(Table.2).Same result is obtained in spring trial that is p=0.000(Table.4).

In one way ANOVA **Globulins test** exposed important deviations between control, low dose treated birds p=0.016 and high dose treated birds p=0.000(Table.2).In spring trial experiment significant variations among standard and low dose treated bird is p=0.003(Table.4).

Table 1: Comparison of various hematological parameters in 10 and 20 days of winter trial experiment after applying 2-sample t-test

Parameters	Days	Mean	St. Deviation	St. Error	P-value
RBC	10	2.2539	0.49117	0.1157	0.569
	20	2.3611	0.61969	0.14606	0.569
HB	10	9.9561	1.09868	0.25896	0.007
	20	8.8417	1.20770	0.28466	0.007
WBC	10	263.9444	2.48459	0.58562	0.000
	20	269.5000	2.83362	0.66789	0.000
MCV	10	70.3461	2.45505	0.57866	0.000
	20	82.0828	5.87899	1.38569	0.000
MCH	10	37.0978	1.81916	0.42878	0.000
	20	31.6911	2.40436	0.56671	0.000
ASAT	10	30.4217	3.10008	0.73070	0.000
	20	43.8383	4.80118	1.13165	0.000
ALAT	10	21.2389	2.85765	0.67356	0.000
	20	32.2500	3.91371	0.92247	0.000
Total protein	10	5.8011	0.47669	0.11236	0.002
	20	5.0794	0.80060	0.18870	0.002

Albumin	10	3.8133	0.45264	0.10669	0.863
	20	3.8411	0.50487	0.11900	0.863
Cholesterol	10	113.1667	3.43426	0.80946	0.000
	20	123.8889	4.19928	0.98978	0.000
Uric Acid	10	14.4172	2.03010	0.47850	0.000
	20	18.3722	2.15727	0.50487	0.000
Creatinine	10	0.3839	0.20018	0.04718	0.002
	20	0.6839	0.30946	0.07294	0.002
Globulins	10	2.5539	0.24727	0.05828	0.000
	20	2.0033	0.33705	0.07944	0.000

RBC's (Red blood cells), HB (Hemoglobin), WBC's (white blood cells), MCV (mean cell volume), MCH (Mean corpuscular hemoglobin), ASAT (Aspartate amino transferase), ALAT (Alanine amino transferase)

Table 2: Comparison of various hematological parameters between control and arsenic (low and High Dose) treated quail by one way ANOVA

Parameters	**Group**	**Dose**	**P-value**
RBCs	Control	Low	0.000*
		High	0.000*
Hb	control	Low	0.019*
		High	0.001*
WBCs	control	Low	0.177
		High	0.002*
MCV	control	Low	0.543
		High	0.210
MCH	control	Low	0.369

		High	0.047*
ASAT	control	Low	0.413
		High	0.175
ALAT	control	Low	0.463
		High	0.185
Total Protein	control	Low	0.000*
		High	0.000*
Albumin	control	Low	0.000*
		High	0.000*
Cholesterol	control	Low	0.146
		High	0.010*
Uric Acid	control	Low	0.33
		High	0.001*
creatinine	control	Low	0.000*
		High	0.000*
Globulin	Control	Low	0.016*
		High	0.000*

RBC's (Red blood cells), HB (Hemoglobin), WBC's (white blood cells), MCV (mean cell volume), MCH (Mean corpuscular hemoglobin), ASAT (Aspartate amino transferase), ALAT (Alanine amino transferase). P< 0.05 significant*.p> 0.05 Non-significant.

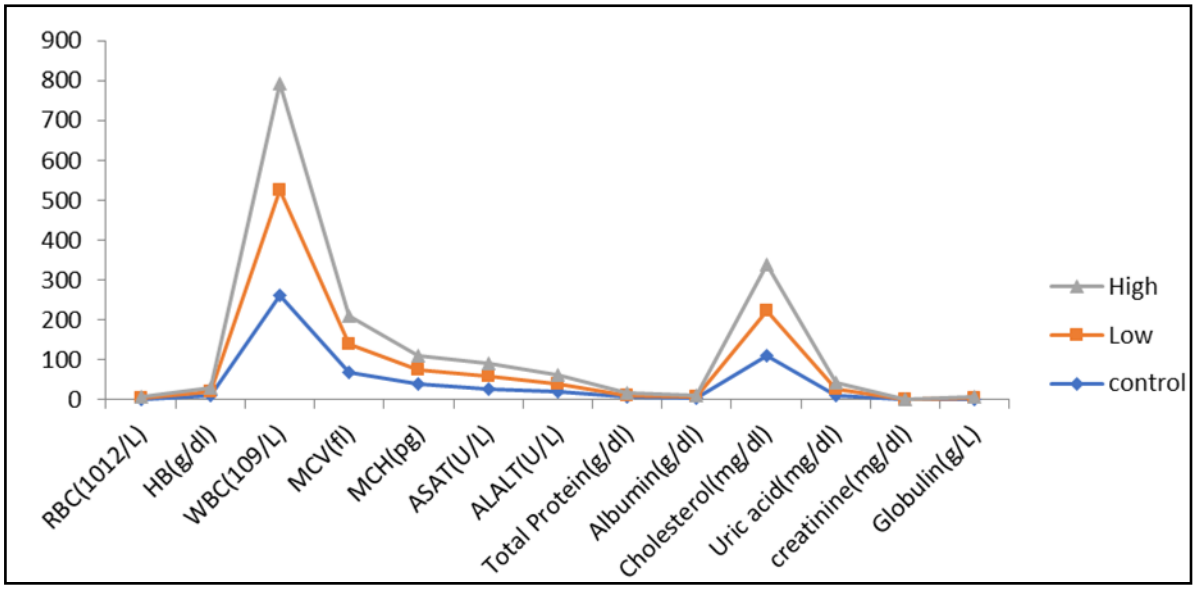

Figure 2: Comparison of average (n=6) blood parameters of control and arsenic treated (Low and high dose) bird in 10 day winter trial

Table 3: Comparison of various hematological parameters in 10 and 20 days long spring trial experiment after applying 2-sample t-test

Parameters	Days	Mean	St. Deviation	St. Error	P-value
RBC	10	2.3917	0.44015	0.10374	0.001
	20	1.8300	0.52123	0.12286	0.001
HB	10	12.2994	0.91810	0.21640	0.000
	20	10.0950	1.31672	0.31035	0.000
WBC	10	254.7222	2.82438	0.66571	0.000
	20	259.1667	2.47933	0.58438	0.000
MCV	10	85.0528	2.58855	0.61013	0.000
	20	92.2061	3.18123	0.74982	0.000
MCH	10	54.2384	1.70700	0.40234	0.000
	20	47.3772	3.17224	0.74770	0.000
ASAT	10	63.8867	2.36740	0.55800	0.000

	20	69.9589	2.43032	0.57283	0.000
ALAT	10	21.5367	1.77248	0.41778	0.000
	20	27.6283	2.59005	0.61048	0.000
Total protein	10	5.4083	0.66860	0.15759	0.251
	20	5.0928	0.93056	0.21934	0.252
Albumin	10	4.0706	0.50083	0.11805	0.057
	20	3.6928	0.64150	0.15120	0.058
Cholesterol	10	146.5000	3.07265	0.72423	0.000
	20	152.1667	2.81279	0.66298	0.000
Uric Acid	10	15.3644	1.27219	0.29986	0.000
	20	17.4800	1.47897	0.34860	0.000
Creatinine	10	0.5911	0.16712	0.03939	0.021
	20	0.7456	0.21200	0.04997	0.021
Globulins	10	2.4093	0.22631	0.05334	0.000
	20	1.9011	0.35793	0.08437	0.000

RBC's (Red blood cells), HB (Hemoglobin), WBC's (white blood cells), MCV (mean cell volume), MCH (Mean corpuscular hemoglobin), ASAT (Aspartate amino transferase), ALAT (Alanine amino transferase)

Table 4: Comparison of various hematological parameters between control and arsenic (low and High Dose) treated quail by one way ANOVA

Parameters	**Group**	**Dose**	**p-value**
RBC	Control	Low	0.000*
		High	0.000*
Hb	control	Low	0.057
		High	0.003*
WBC	control	Low	0.221
		High	0.001*

MCV	control	Low	0.076
		High	0.004*
MCH	control	Low	0.373
		High	0.044*
ASAT	control	Low	0.059
		High	0.004*
ALAT	control	Low	0.100
		High	0.013*
Total Protein	control	Low	0.000*
		High	0.000*
Albumin	control	Low	0.000*
		High	0.000*
Cholesterol	control	Low	0.075
		High	0.000*
Uric Acid	control	Low	0.027*
		High	0.000*
creatinine	control	Low	0.000*
		High	0.000*
Globulin	Control	Low	0.003*

RBC's (Red blood cells), HB (Hemoglobin), WBC's (white blood cells), MCV (mean cell volume), MCH (Mean corpuscular hemoglobin), ASAT (Aspartate amino transferase), ALAT (Alanine amino transferase). P< 0.05 significant*.p> 0.05 Non-significant

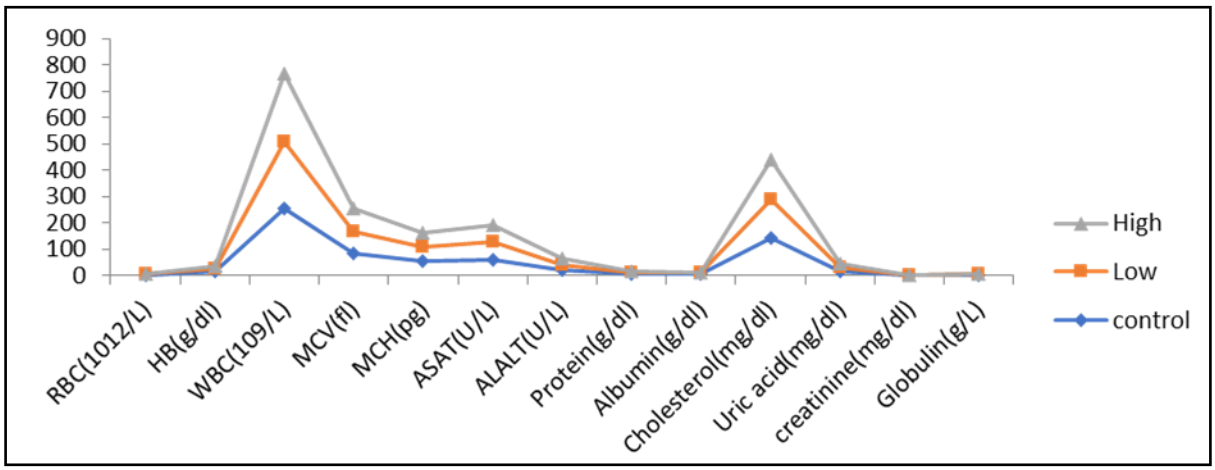

Figure 3: Comparison of average (n=6) blood parameters of control and arsenic treated (Low and high dose) bird in 10 day spring trial

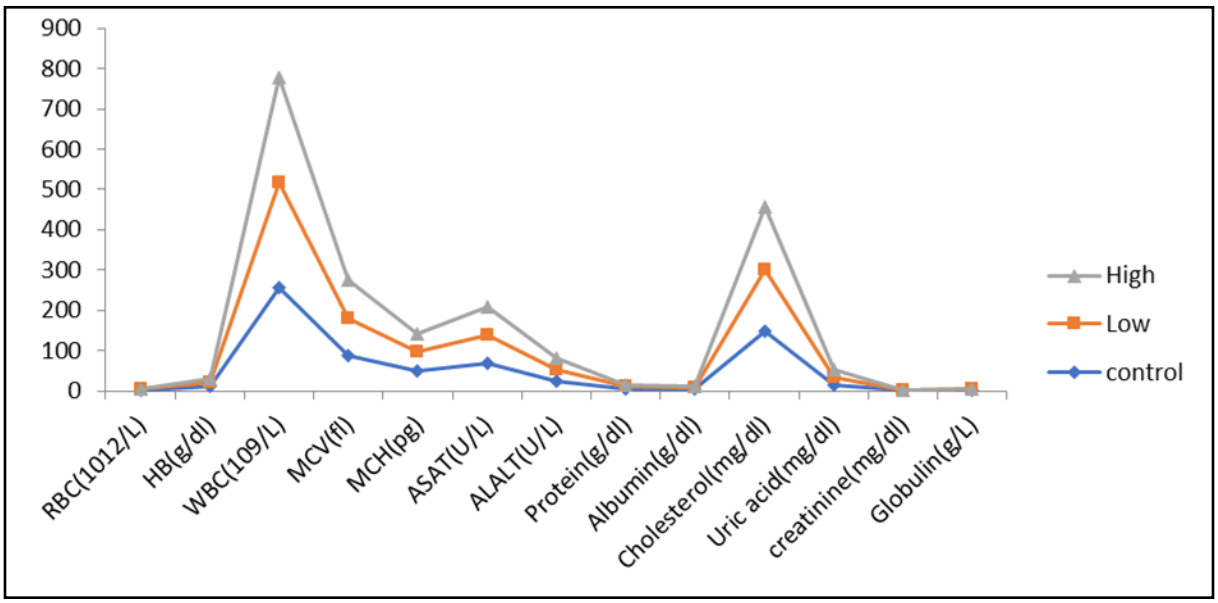

Figure 4: Comparison of average (n=6) blood parameters of control and arsenic treated (Low and high dose) bird in 20 day spring trial

In one way ANOVA when comparison is occurred in between control, low and high dose treated groups then highly significant results are obtained which is(p=0.000). Similarly low dose treated birds when associated with standard and high dose treated birds, it indicated significance (p=0.000).Highly significant result also obtained when high dose treated birds compared with standard and low dose treated birds (Table.6).

Body mass of control bird's increase in winter and spring trial. While body mass of treated birds decrease as compared to control group. In 20 day long trial group body mass of standard and treated

groups at 1st,5th,10th,15th and 20th day indicated in (Figure No.5). In one way ANOVA highly significant result is achieved when comparison is made between temperature of standard and treated groups which is p=0.000(Table.8). In 20 days long trial group body temperature of birds at 1st, 5th,10th ,15th and 20th day were shown in(Figure No.6).

Table 5: Comparison of body weight in control and arsenic (Low and High dose) treated quail. Comparison is made with one-way ANOVA

Group	Dose	p-value
Control	Low	0.000*
	High	0.000*
Low	Control	0.000*
	High	0.000*
High	Control	0.000*
	Low	0.000*

P<0.05 Significant*

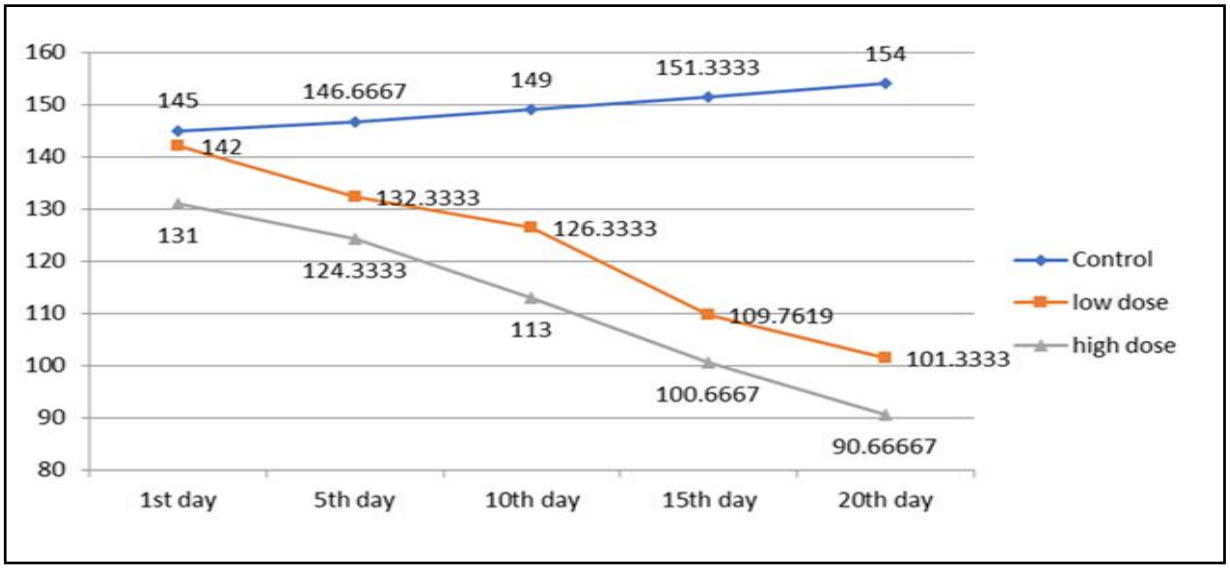

Figure 5: Comparison of average (n=3) body weight of control and arsenic treated

Table 6: Evaluation of body temperature in control and arsenic (Low and High dose) treated quail by one way ANOVA

Group	Dose	P-value
Control	Low	0.000*

	High	0.000*
Low	Control	0.000*
	High	0.000*
High	Control	0.000*
	Low	0.000*

P<0.05 Significant*

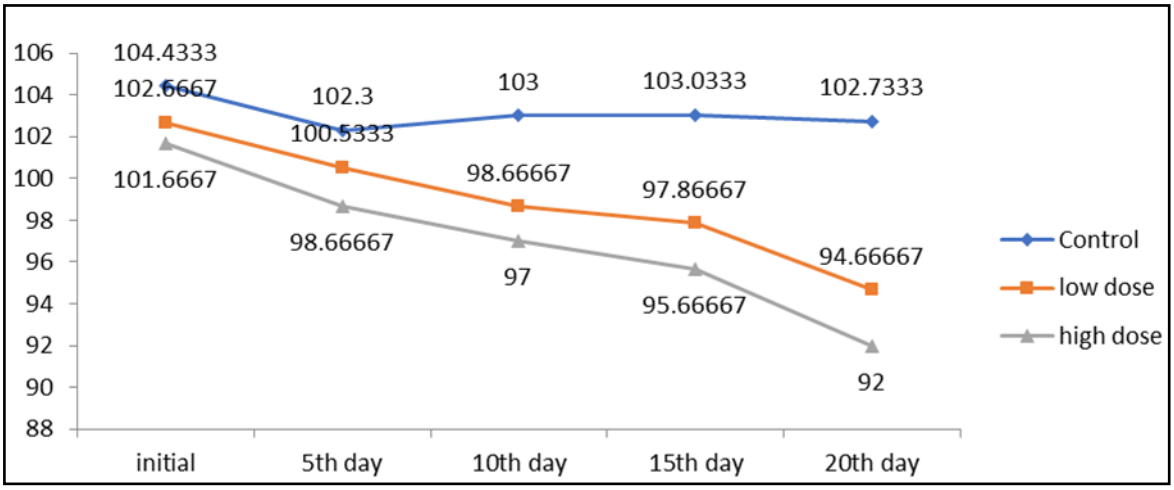

Figure 6: Comparison of average (n=3) temperature of control and arsenic treated birds at 1st, 5th 10th, 15th, and 20th day of winter experiment

Comparative analysis of induced arsenic doses on body weights and temperature of birds in 20 days long spring trial experiment: Comparison of control and treated groups in one way ANOVA showed significant results that is p=0.000(Table.7). Body weights of control bird group increase in 20 days long trial experiment while decrease in treated groups. .In 20 days experiment weight of bird showed at 1st, 5th, 10th, 15th and 20th day in (Figure No.7). In 20 day long trial experiment body temperature of bird showed at 1st, 5th, 10th,15th and 20th day(Figure No.7).

Table 7: Comparison of body weight in control and arsenic (Low and High dose) treated quail by one way ANOVA

Group	**Dose**	**P-value**
Control	Low	0.000*
	High	0.000*

Low	Control	0.000*
	High	0.000*
High	control	0.000*
	Low	0.000*

p>0.05 NS or non-significant, p<0.05 significant.*

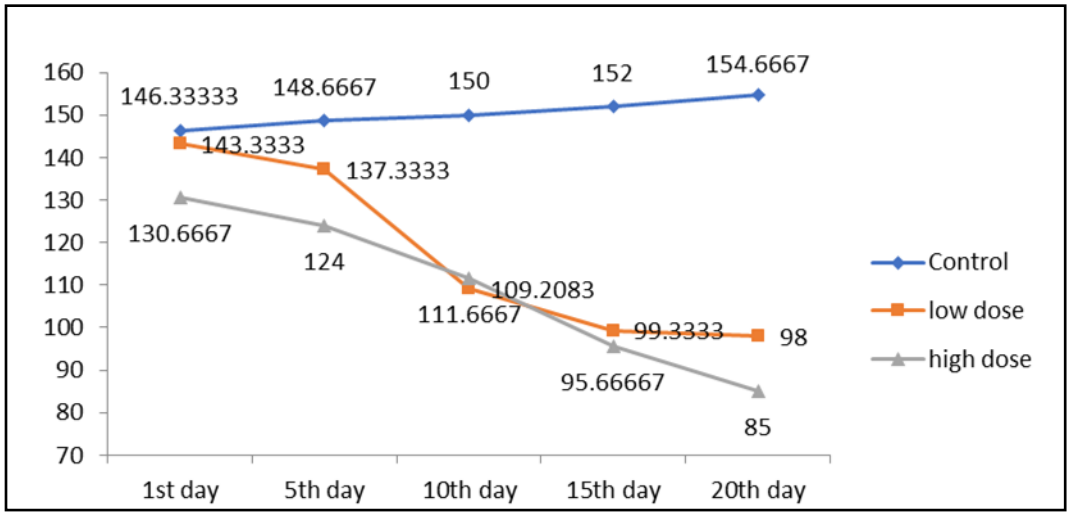

Figure 7: Comparison of average (n=3) body weight of control and arsenic treated birds at 1st, 5th 10th, 15th, and 20th day of spring experiment

Table 8: Comparison of body temperatures in control and arsenic (Low and High dose) treated quail by one way ANOVA

Group	**Dose**	**P-value**
Control	Low	0.000*
	High	0.000*
Low	Control	0.000*
	High	0.000*
High	Control	0.000*
	Low	0.000*

p>0.05 NS or non-significant, p<0.05 Significant*

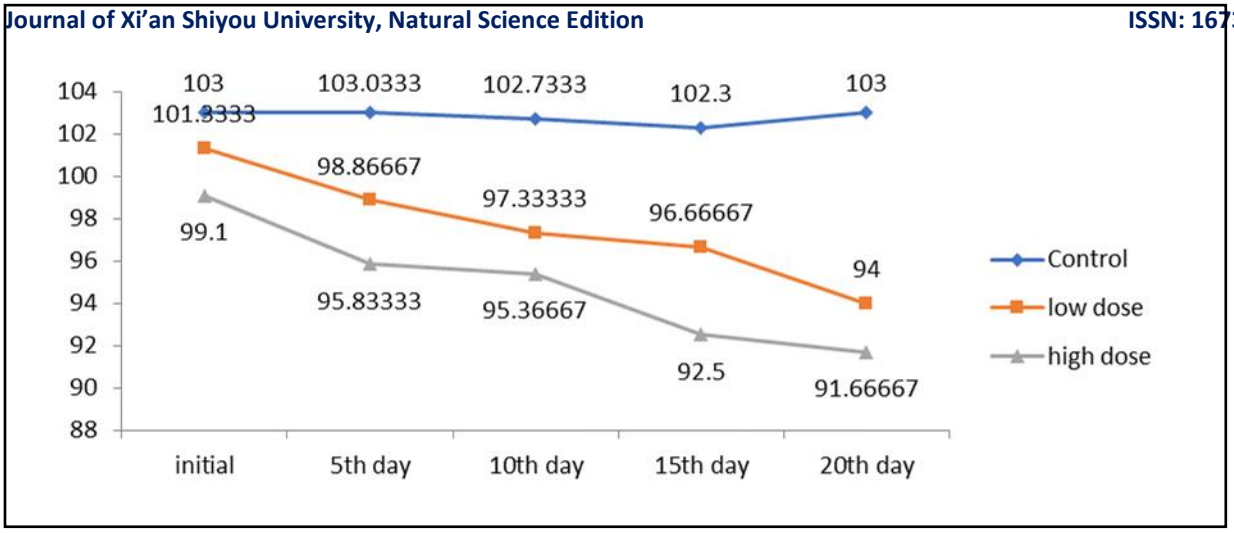

Figure 8: Comparison of average (n=3) temperature of control and arsenic treated birds at 1st, 5th 10th, 15th, and 20th day of spring experiment

Discussion:

In this investigation arsenic treated birds presented particular symbols such as reduction in body weight, Dullness, Feed intake decreased, increased thirst. These outcomes are related to former document such as in rats (Singh and Rana, 2007) and in mammals (Rahman *et al*., 2001).

Blood parameters are vital in identifying the functional condition of animals/Birds exposed to toxicants. RBC counts in this research declined significantly due to heavy metal introduction such as arsenic and this result is corroborated with the results of (Singh and Sarivastava., 2017) in fish and (Kumar *et al*., 2015) in albino mice. This reduction in red blood cells calculations is due to the destruction of undeveloped blood cells. This destruction of cells is due to the toxicity of heavy metals arsenic in quails. Lessening of both red blood cells and hemoglobin also show inhibition of hem-synthesis pathway. Hematological changes occurred in our study that values of MCV significantly increased in birds due to exposure of arsenic and this finding was in line with the report of (Ghaffar *et al*., 2017) in chicks (Ghaffar *et al*.,2016) in fish. MCH in this investigation decreased significantly and this finding was also reported former in fish (Singh and sarivastava., 2017).Lessened level of MCH are direct suggestion of hemoglobin damage due to exposure of arsenic. Declined level of MCH also indicate micro cystic anemia. In this investigation MCH significantly changes and this outcome support the result of previous report (Kumar *et al.*, 2015) in albino mice.

Conclusion

The study reveals that arsenic induces adverse effects in birds, leading to decreased body weight, reduced feed intake, and increased lethargy. Analysis of blood parameters demonstrates arsenic's toxicity, marked by significant declines in red blood cell count, hemoglobin, and mean corpuscular hemoglobin, while white blood cell count, mean corpuscular volume, alanine amino transferase, and aspartate amino transferase show notable increases.

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