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BIOCHEMICAL ANALYSIS FOR EXPLORATION OF TOXICITY OF ARSENIC

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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 important organs of body such as Liver, kidney, muscle and brain 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.Conclusions: Clinical signs including diarrhea, appetite loss and decrease in body weight was observed in all treated groups and control group birds gain weight. Histoptahologoical examination of different tissues, In Liver, revealed the congestions, pyknosis and cytoplasmic vacuolation were main histopathological changes observed in liver. In kidney histopathological changes observed were swelling of tubular epithelium and destruction of blood cells. In brain changes observed was reduction of granular layer and purkinji cell layer not present. In Muscles Absence of nucleus in few mayo fibers occurred. The current study that arsenic causes the severe toxicity in body organs (Liver, kidney, muscle, brain) of birds.

INTRODUCTION

Quail possess advantages such as rapid growth, compact size, enhanced laying capabilities, a brief life cycle, flavorful meat, and quicker hatch times compared to other avian species. (Siyadati *et al.*, 2011).

Arsenic is a chemical element its atomic number is 33 and AS is symbol. This study investigates the

detrimental impact of arsenic on ATP production, specifically targeting the citric acid cycle by inhibiting lipoic acid, a crucial cofactor for pyruvate dehydrogenase. These metabolic disruptions lead to multi-system organ failure and subsequent death (Hughes et al., 2002). Severe arsenic poisoning manifests as initial symptoms of vomiting, diarrhea, vascular injuries, and muscle cramps, affecting organs like the kidneys, liver, and skin in chronic cases (Chowdhury et al., 2001).

Liver and kidneys are identified as primary organs for arsenic-induced toxicopathological effects, with pathological variations directly linked to arsenic accumulation in various organs (Nandi et al., 2005; Radi, 2019). Creatinine, a breakdown product of muscle creatinine phosphate, becomes elevated in the blood due to insufficient kidney filtration, highlighting its role as a marker for renal dysfunction (Gross et al., 2005).Additionally, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, along with the AST/ALT ratio, are commonly utilized as biomarkers for liver health. Elevated AST levels also serve as biochemical markers for liver, kidney, and brain conditions (Gaze, 2007). So this study was conduct to estimate the adverse effects of heavy metal such as arsenic on Histopathological aspects of the quail birds.

Material and Methods:

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, plastic glass jars, Embedding molds, microtome disposable blades, water bath, incubator, water bath, glass slides, and cover slip.

Chemicals

Arsenic, formalin, glacial acetic acid, alcohol, benzoyl, wax, xylene, hematoxylin, eosin, Canada balsam.

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

Tissues samples were collected from high and low dose treated birds by slaughtering and immediately preserved in 10% formalin solution.

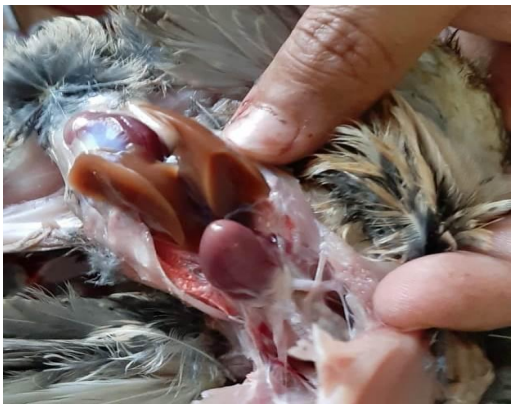


Figure 01: collection of sample.

Preparation of slides for histopathology

1. **Procedure of microtomy**

Tissues of all important organs of 6 micrometer width were cut and fixed in saline solution for 5 hours at room temperature.

2. **Dehydration process**

To get rid of additional liquid from tissues drying up procedure occurs. By sequence of frequently aggregating alcohol fractions and by other drying

agents additional water contents are detached from tissues which are secure in aqueous.

3. Clearing of tissues

Tissues then transferred to clove oil until they become transparent. After that tissues were put in benzoyl for 10-12 minutes twice for clearing purpose. After that tissues were shifted in 1:1 mixture of benzoyl and paraffin wax for 20-25 minutes at 60^0c twice.

4. Embedding

After this process tissues were prepared to make blocks. The blocks mounted on wooden blocks. The segments were cut out of paraffin block at the width of 6 micrometer by using Richert microtome.

5. Deparaffinization

Pieces were attached to pre washed albumenized glass slides and prolonged at 60^0c on fisher slide warmer and then shifted to oven at temperature 60^0C for whole deparaffinization.

6. Staining

For removal of any remaining wax slides were shifted to xylene for half an hour. Dehydration of slides occurred by using of descending grades of alcohol. After that slides washed with the help of tap water for 5 minutes and for staining hematoxylin is used. Slides dehydrated again in rising grades of alcohol and at the end stained with eosin for 1/2-2 minutes. After completion of staining process slides were mounted in Canada balsam. The microphotographs of preserved slides were taken by microscope furnished by camera.

Results

Histological assessment of Liver kidney, muscles and brain in control and treated

We did not detect any precise variations throughout the course of trials in control group. While in treated birds specific changes were occurred in liver, kidney, muscles and brain. There were degenerative and necrotic changes in liver. We also observed vacuolation of cytoplasm due to disintegration of hepatocytes.

Necrotic changes in tubular epithelium observed in kidney. In muscle we observed the mayo fibers are damaged. While in brain we detected the molecular layer intact but showed vacuolated appearance. Purkinje cell layer was not present. Demarcation between molecular layer and granular layer was lost. Granular layer showed the vacuolation.

Comparative examination of histological valuation of Liver tissue samples in standard and treated bird group

Winter trial

Control group: control bird groups did not show any particular variations throughout their trials (Figure.02). While changes were observed in low dose and high dose treated birds(Figure.03),(Figure.04) respectively.

Figure 02: A Photomicrograph of liver of control group

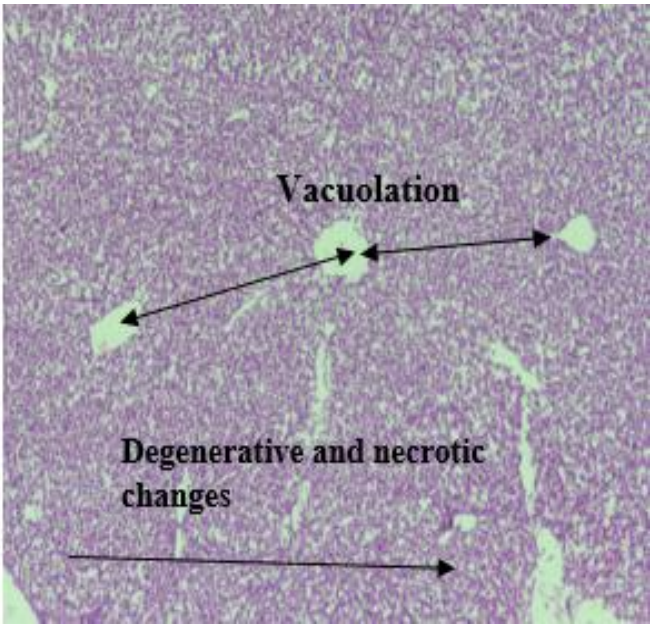


Figure 03: A Photomicrograph of liver showing Excessive vacuolation along with degenerative and necrotic changes in the hepatic parenchyma.

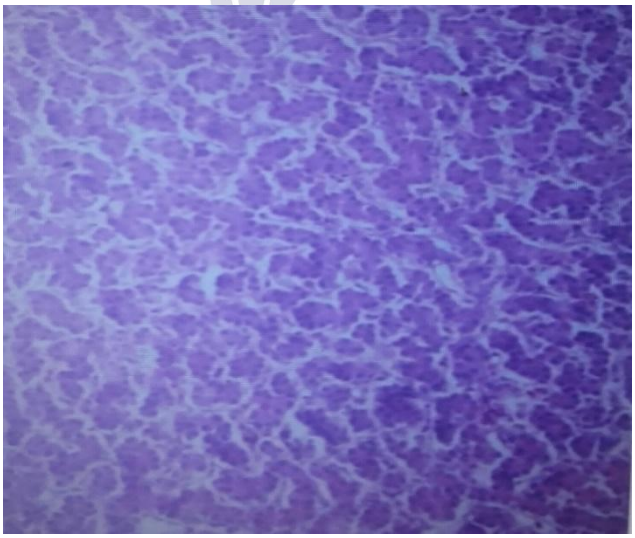
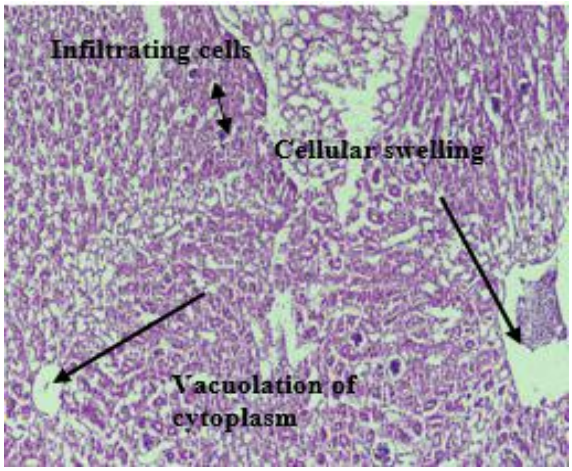


Fig.04: A Photomicrograph of liver showing vacuolation of cytoplasm infiltrating cells and cellular swelling



Spring trial

Control group: No changes were detected throughout the course of trials in control group (Figure.05). While changes were observed in low and high dose treated birds.(Figure.06), (Figure.07) respectively.

Figure 05: Control group showing normal liver tissue sample.

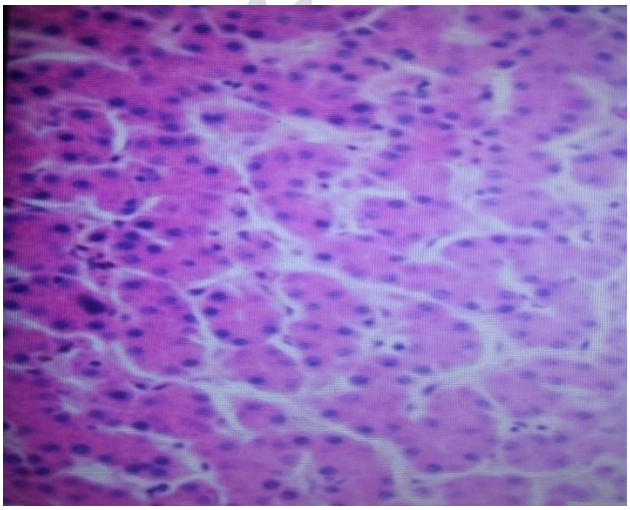


Figure 06: A photomicrograph of liver showing sinusoidal space present along with coagulated necrosis

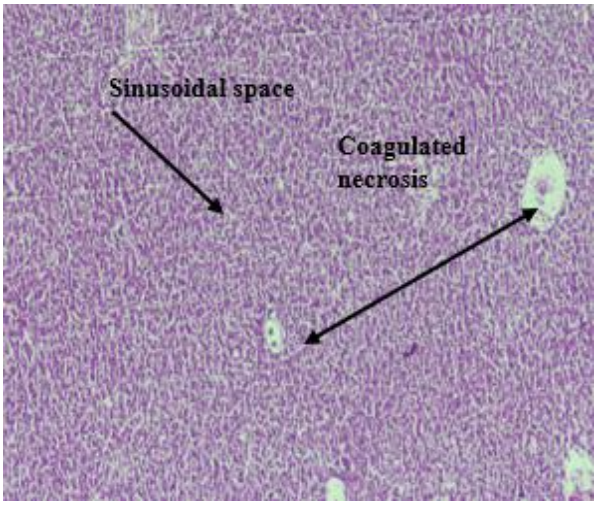
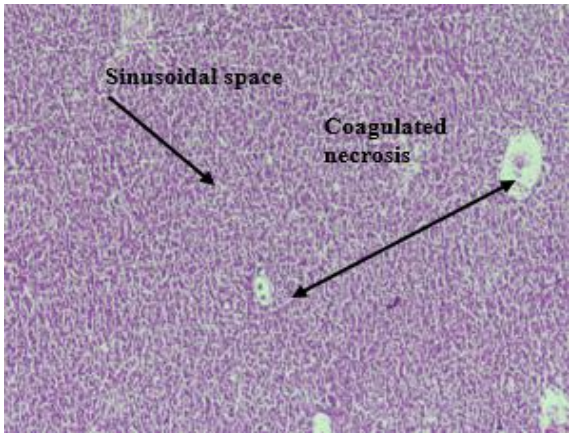


Figure 07: A photomicrograph of liver showing vacuolation and sinusoidal space present.



Relative investigation of histological valuation of Kidney tissue samples in standard and treated group

Winter trial

Control group: No changes were observed throughout the course of trials in controls groups (Figure.08). While changes were observed in low and high dose treated quails (Figure.09),(Figure.10) respectively.

Figure 08: Control group showing normal kidney tissue sample.

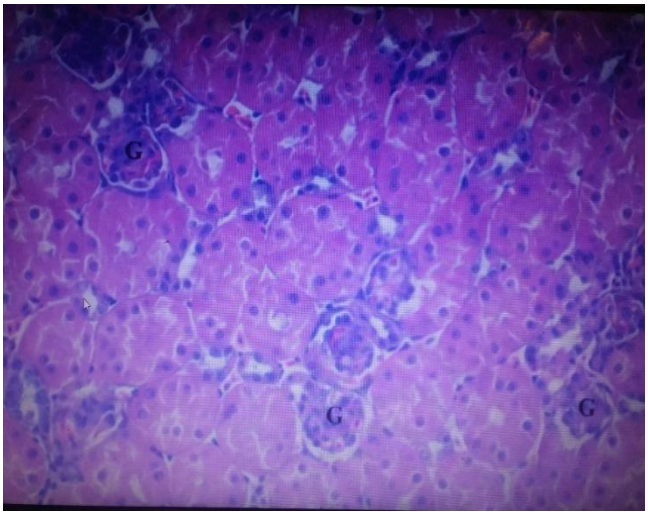


Figure 09: A Photomicrograph of kidney showing congestion along with degenerative and necrotic changes in the renal parenchyma and glomerulus space also increased.

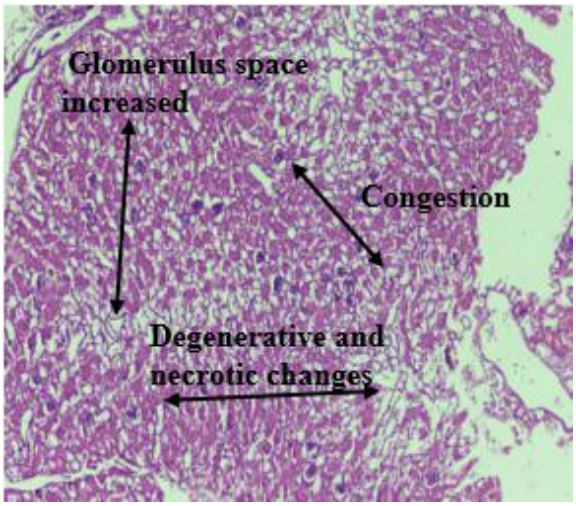
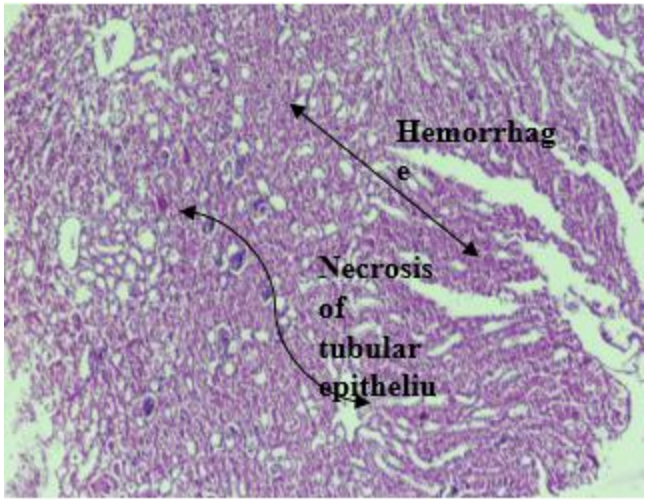


Figure 10: A Photomicrograph of kidney showing severe necrosis of tubular epithelium in the renal parenchyma and hemorrhage.



Spring trial

Control group: No changes were detected throughout the course of trials (Figure.11) Changes Were detected in low and high dose treated quails(Figure.12) and (Figure.13) respectively.

Figure 11: Control group showing normal kidney tissue sample.

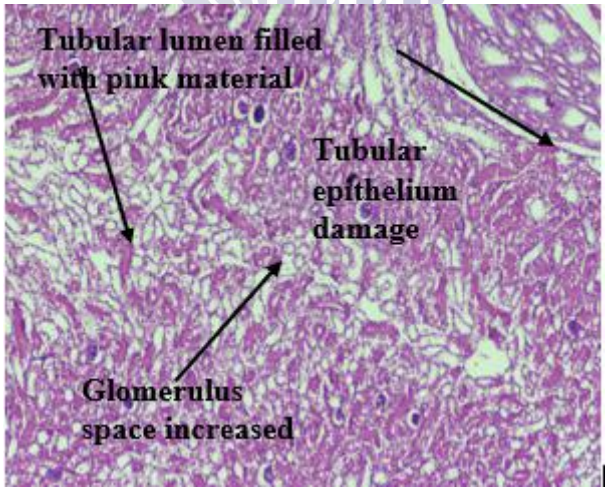


Figure 12: A Photomicrograph of kidney showing tubular lumen is filled with pink material, glomerulus space increased and tubular epithelium damage.

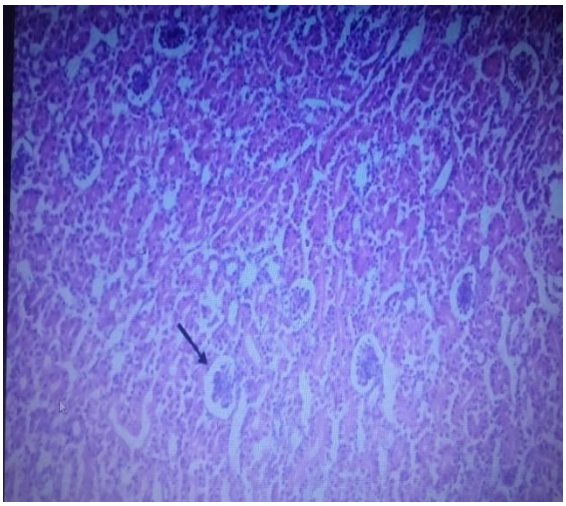
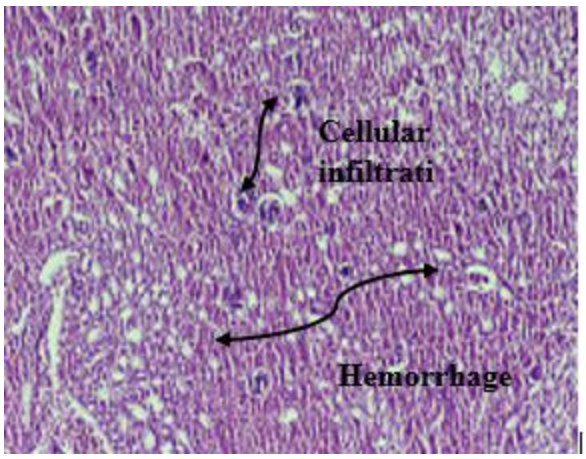


Figure 13: A photomicrograph of Kidney showing hemorrhage



Comparative analysis of histological assessment of Muscle tissue samples in control and treated group

Control group: In birds of control group no variations were detected during the course of trials (Figure.14).While changes were showed in low and high dose treated birds (Figure.15) and (Figure.16) respectively.

Figure 14: Control group showing normal muscle tissue sample.

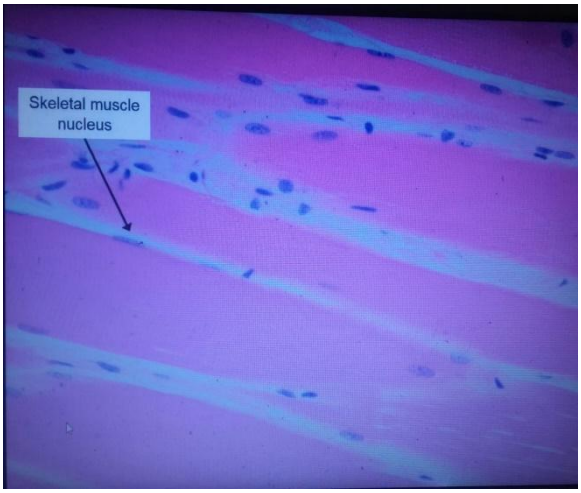


Figure 15: A Photomicrograph of myofibers showing destruction of muscle fibers.

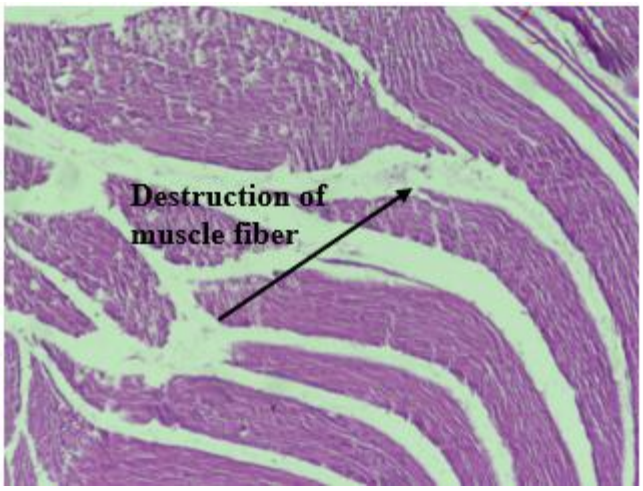
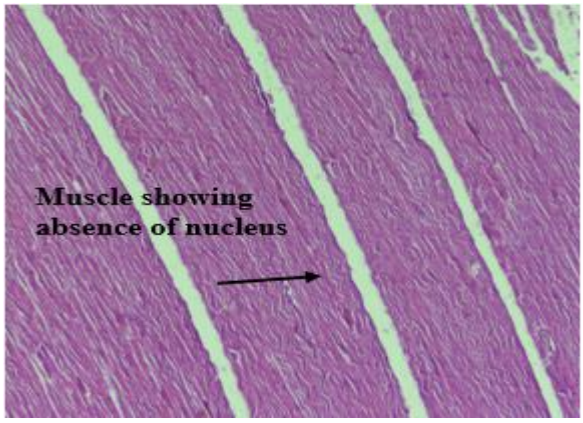


Figure 16: A Photomicrograph of muscle showing absence of nucleus.



Relative examination of histological valuation of Brain tissue samples in standard and treated bird group

Control group: There was no variations observed in birds of control group (Figure.17).Treated birds showing changes ,shown in (Figure.18 & 19).

Figure 17: A photomicrograph of brain showing normal brain tissue sample.

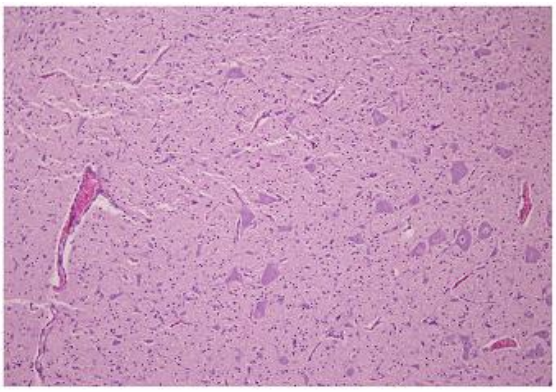


Figure 18: A Photomicrograph of cerebral cortex showing intracytoplasmic vacuolation in along with darkly stained neuronal cells.

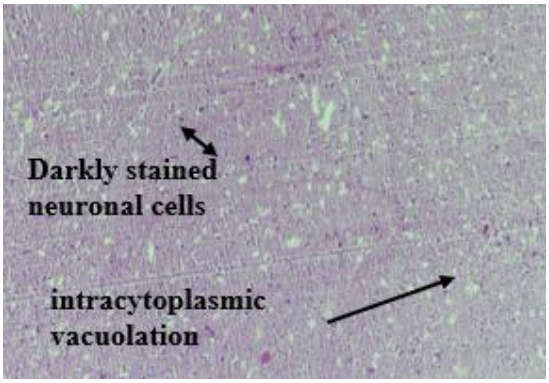
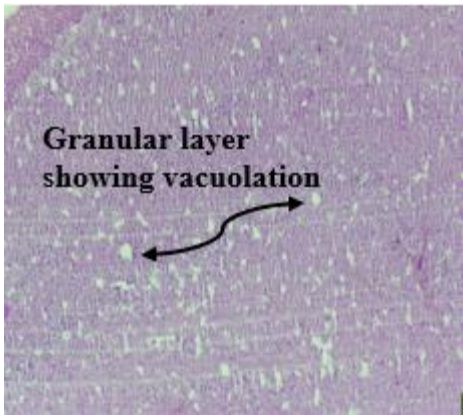


Figure 19: A Photomicrograph of brain tissue showing depletion of granular layer and granular layer showing vacuolation.



Comparative analysis of induced arsenic doses on body weights and temperature in 20 days long winter trial experiment.

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.

Body mass of control bird's increase in winter and spring trial. While body mass of treated birds decreases 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.20). In 20 day long trial experiment body temperature of bird showed at 1st, 5th, 10th,15th and 20th day(Figure.21).

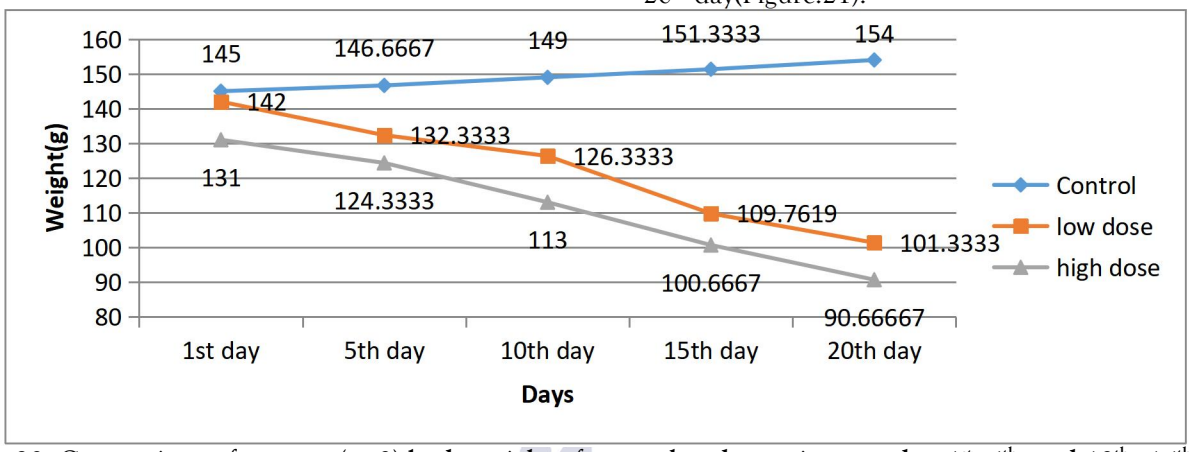


Figure 20: Comparison of average (n=3) body weight of control and arsenic treated at 1st, 5th, and 10th, 15th , and 20th day of winter experiment.

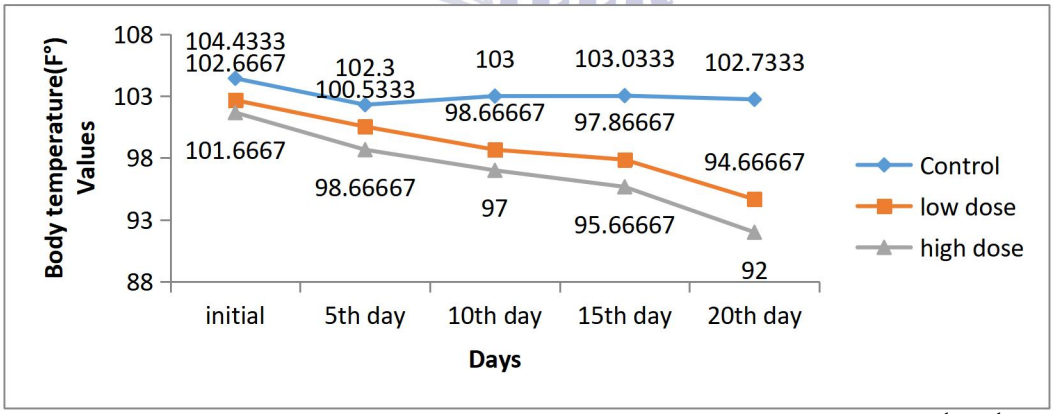


Figure 21: Comparison of average (n=3) temperature of control and arsenic treated at 1st, 5th 10th, 15th, and 20th day of winter experiment.

Comparative analysis of induced arsenic doses on body weights and temperature in 20 days long spring trial experiment

Comparison of body weight and temperature of control and treated groups by one way ANOVA showed significant results that is p=0.000(Table.3 &4). In 20 day long trial group body mass of standard and treated groups at 1st,5th,10th,15th and 20th day indicated in (Figure.22). In 20 day long trial experiment body temperature of bird showed at 1st, 5th, 10th,15th and 20th day(Figure.23).

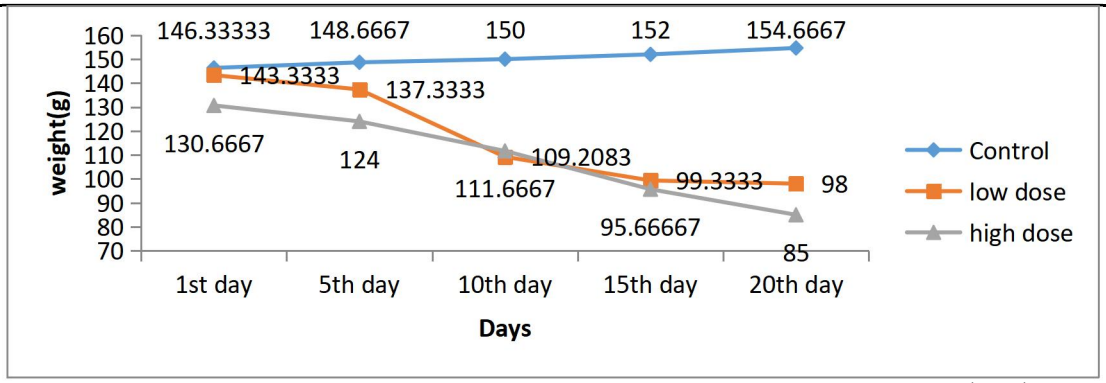


Figure 22: Comparison of average (n=3) body weight of control and arsenic treated at 1st, 5th 10th, 15th, and 20th day of spring experiment.

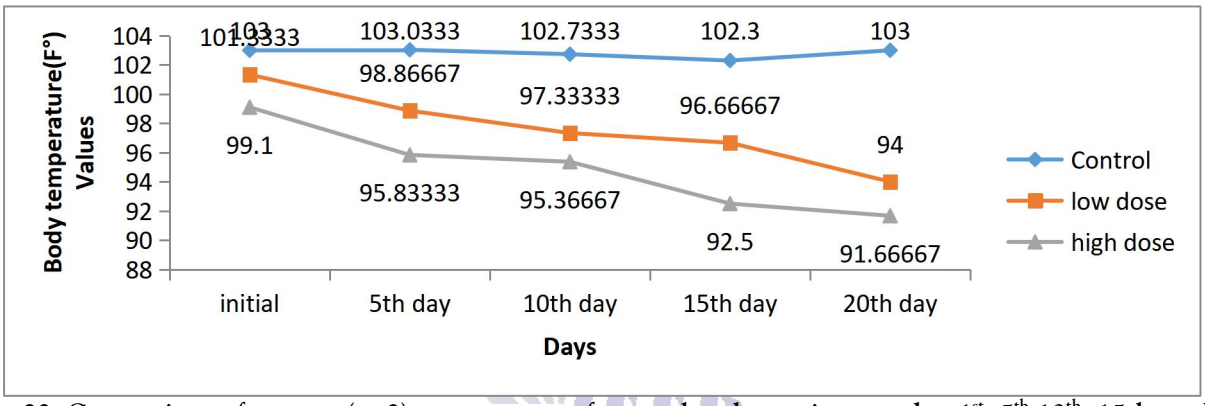


Figure 23: Comparison of average (n=3) temperature of control and arsenic treated at 1st, 5th 10th, 15th, and 20th day of spring experiment.

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).In present study exposure of quail to two concentration of arsenic are assessed at 10 and 20 days. Kidney biomarkers are creatinine and urea. Serum creatinine in this report has been significantly increased. This result was in line with former document in rats (Sener *et al.*, 2016)in chicks. Higher level of creatinine caused the renal failure.Liver is considered as the major organs for histological changes due to accumulation of arsenic and it also produced toxic metabolites. Metabolism of arsenic involved only in liver because arsenic induced hepatotoxicity. In current study, in control bird hepatic parenchyma presenting usual appearance of hepatic cords while arsenic treated bird showing vacuolation, congestion, condensed nuclei, necrotic and degenerative variations such as change in nucleus alike, pyknosis cells without nucleus and intracytoplasmic vacuolation. All these present findings supported by the earlier outcomes of (Mashkoor *et al.*, 2013; Sharaf *et al.*, 2013) in birds, (Ghosh *et al.*, 2014) in goats.Muscle regeneration ability inhibit due to exposure of arsenic and composition of muscle protein change. Spontaneous locomotors activity increased and muscle contractile activity also changed. This result of present study was supported by the documents of former workers (Yen *et al.*, 2010) in fish and mice and (Li *et al.*, 2009) in rodents.In current study oxidative damage of brain also occurred due to arsenic exposure. This result of present study was in line with the former study of (Noman *et al.*, 2015) in rats. High oxygen consumption rate and high level of poly unsaturated fatty acid cause the brain to oxidative stress.

Conclusion:

It may be determined from the current study that arsenic induced clinical symptoms in birds such as

decayed in body weight, declined feed intake and tediousness. Histopathological changes were noticed in liver, kidney brain and muscles of birds treated with arsenic. Main changes in liver noticed was damaged of hepatocytes. Hemorrhage was observed in kidney. Absence of nucleus occurred in muscle fibers. Demarcation between molecular layer and granular layer was lost it was observed in brain.

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