Asian J Agric & Biol. 2024(4).
DOI: 10.35495/ajab.2023.357

AJAB

Original Article

Bifenthrin induced toxic effects on haematological, reproductive and histo-morphological profile in adult male quail (*Coturnix japonica)*

Rabia Kalsoom[1], Hani Z. Asfour[2], Hafiz Muhammad Ali[3], Abdul Qayyum[3*], Shazia Anjum[4], Faisal Maqbool[1], Nuzhat Sial[1], Riaz Hussain[3], Sultan H. Alamri[5], Nadeem Ali[6], Nisreen Rajeh[7], Irfan Irshad[8], Asif Idrees[9]**

[1]Department of Zoology, The Islamia University of Bahawalpur, 63100, Pakistan
[2]Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah-21589, Saudi Arabia
[3]Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, 63100, Pakistan
[4]Faculty of Chemical and Biological Sciences, The Islamia University of Bahawalpur, 63100, Pakistan
[5]Department of Family Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah-21589, Saudi Arabia
[6]Center of Excellence in Environmental Studies, King Abdulaziz University, Jeddah-21589, Saudi Arabia
[7]Department of Clinical Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah-21589, Saudi Arabia
[8]Institute of Continuing Education and Extension, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan
[9]KBCMA, College of Veterinary and Animal Sciences, Narowal, Pakistan

Received:
December 17, 2023
Accepted:
May 03, 2024
Published Online:
May 26, 2024

Abstract

Currently terrestrial ecologies are polluted by numerous chemical compounds unceasingly leading to high risk of exposure to variety of life. Among these, bifenthrin is widely used for the control of different sucking and chewing insects and other leaf miner insects around the globe. The current study elaborated the toxic effects of oral administration of bifenthrin in male Japanese quails at sub-lethal (less than 1% mortality) concentrations (10mg/kg, 20mg/kg and 30mg/kg b.w.) during a period of 30 days. During the course of the study, the quails did not show any behavioural or clinical signs. However, a significantly ($p<0.05$) decreased RBCs and platelets counts and haemoglobin concentration while a significantly ($p<0.05$) increased MCV, MCHC, total leucocytes and neutrophils were observed in the birds administered with higher concentrations of bifenthrin. Moreover, the incidence of lobed nuclei, blebbed nuclei, condensed nuclei, notched nuclei, binuclear, pear shaped and micro-nuclei were significantly ($p<0.05$) increased in the erythrocytes of the groups C and D during the experiment. The diameter of seminiferous tubules, height of germinal epithelium and the number of seminiferous tubules containing normal spermatozoa were significantly ($p<0.01$) decreased while the number of pyknotic cells and degenerated seminiferous tubules were increased significantly ($p<0.05$) towards the end of the experiment (day-30) in the quails of groups C and D compared to the control group. Hence, the alterations in the hematological indices and histopathological changes in heart, spleen and testes indicate potential toxicity of bifenthrin and its adverse effects in the Japanese quail even at sub-lethal concentrations.

Keywords: Bifenthrin, Blood profile, Nuclear alterations, Seminiferous tubules, Histo-pathology, Testes

How to cite this:

Kalsoom R, Asfour HZ, Ali HM, Qayyum A, Anjum S, Maqbool F, Sial N, Hussain R, Alamri SH, Ali N, Rajeh N, Irshad I and Idrees A. Bifenthrin induced toxic effects on haematological, reproductive and histo-morphological profile in adult male quail (*Coturnix japonica)*. Asian J. Agric. Biol. 2024(4): 2023357. DOI: https://doi.org/10.35495/ajab.2023.357

**Corresponding author email:*
abdul.qayyum@iub.edu.pk
shazia.anjum@iub.edu.pk

This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License. (https://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Recently the attention towards the potential deleterious effects of herbicides, pesticides and industrial effluents has extremely increased all over the world (Ghaffar et al., 2020; Hussain et al., 2021). The persistent use of these compounds at crops and animal feed industries is harmful for target and non-target animals and food web (Arslan et al., 2017; Xiong et al., 2017; Rubin et al., 2019; Jayakumar et al., 2020; Safdar et al., 2022). These synthetic chemicals are causing significant endocrine abnormalities like alterations in the development and hormone secretary potential of pituitary gland, brain and gonads (Xu et al., 2018; Zhou et al., 2019; Scarano et al., 2019) and dysfunctions of hypothalamus, pituitary gland, thyroid, pancreas, adipose tissue, cardiovascular system, liver, kidney and reproductive system (Gaston et al., 2020; Gripp et al., 2017; Razali et al., 2019). Currently, synthetic pyrethroids are widely used class of insecticides, accounting for more than 25% of the global insecticide market (Yang et al., 2018). The increase in pyrethroid applications may be credited to perception of less toxic alternative to organophosphate pesticides (Fong et al., 2016). In fact, the pyrethroids are considered as less detrimental to mammals and birds although detrimental effects are reported in fish (Farag et al., 2021; Ullah et al., 2019). These organic compounds are found to cause the formation of reactive oxygen species (ROS) that induces the oxidative stress (Shafqat et al., 2023; Sule et al., 2022; Ullah et al., 2019). Avian species occupy a remarkable position in the ecosystem, are good indicator of a healthy ecosystem and are a sign of early warning to environmental issues (Sheikh et al., 2021; Luqman et al., 2021). Avian species die due to ingestion of pesticides and their remains may decompose rapidly or might be eaten by the scavengers (Arya et al., 2019). For several years, birds were used as bio-monitor species of environmental contaminations (González-Gómez et al., 2020). Moreover, sub-lethal toxicity effects of synthetic pesticides cypermetherin have been studied on spermatogenesis and male reproductive organs in rat that are significant to elaborate the toxicological effects caused by low quantities of pesticides used in the field (Dahamna et al., 2010). Likewise, the erythrocytic abnormalities are a good indicator to determine the oxidative stress due to cypermethrin in broiler chicks (Sharaf et al., 2010), chlorpyrifos in wistar rats (Uchendu et al., 2018) and toxicity of dietary lead (Abd et al., 2023) or monomehypo (Khan et al., 2023) in Japanese quails.

Bifenthrin (BIF) is a synthesized 3rd generation pyrethroid insecticide that is extensively used these days to eliminate the harmful insects (Farag et al., 2021; Fong et al., 2016). BIF disturbs the sodium ion channel and induces neuro- and immuno-toxicity in the insects (Wang et al., 2017). Additionally, to these toxic effects, the drug also causes serious consequences like hormone dysregulation, hepato- and immuno-toxicity in the Zebrafish (Park et al., 2020; Eghan et al., 2023). It was found to be an endocrine disrupter with anti-estrogenic activity via disrupting the hypothalamic-pituitary-gonadal axis cause a declined reproduction and morphological abnormalities in the fish embryos (Pan et al., 2024; Patisaul, 2021). Nevertheless, the data are not available regarding the hematological and histo-pathological effects of BIF in the Japanese quails. Hence, the study was conducted to investigate the clinically non-apparent sub-lethal effects of bifenthrin on various haematological parameters, absolute and relative weights of brain, lungs, trachea, liver, proventriculus, gizzard, intestine, kidneys, testes, spleen and heart and to analyse the nuclear abnormalities in red blood cells in different birds treated.

Material and Methods

Experimental design and treatment

The present study was executed at The Department of Zoology, the Islamia University of Bahawalpur. A total of 48 sexually grown-up male Japanese quail, weighing between 80 to 100gm were bought from the commercial bird market of Bahawalpur. The quails were kept in the natural environment for about two weeks and fed with standard commercial feed according to 10% of body weight. Clean pure drinking water was supplied ad-libitum. After adaptation, the birds were divided into four groups (A, B, C and D) each containing 12 quails.

Bifenthrin (10% w/v Emulsifiable concentration) was purchased from Jaffar Group of Companies, Pakistan, 2.5% w/w solution was prepared and was fed orally to the birds in drinking water, accordingly for different concentrations of BIF i.e. 10mg/kg, 20mg/kg and 30mg/kg body weight to the birds of groups B, C and D, respectively for a period of 30 days. While the birds of group A were served as control (0 mg/kg body weight). All the experimental procedures used in the study were executed according to the guidelines and prior permission of the office of Directorate of Research and Bioethics Committee, The Islamia University of Bahawalpur for the use and welfare of laboratory animals.

Haematological and biochemical analysis

All the birds were monitored twice daily for behavioural and clinical signs. At the time of sampling (on the experimental days-10, 20 and 30), 04 birds from each group were randomly slaughtered for collection of blood in the EDTA coated vacutainers for hematological analyses, as previously described (Ayub et al., 2018; Malik et al., 2018).

Histo-pathological analysis

After collection of the blood, the visceral organs (brain, lungs, trachea, liver, proventriculus, gizzard, intestine, kidneys, testes, spleen and heart) were collected to determine the absolute and relative weights of the organs and the microscopic histo-pathological abnormalities. For this purpose, the tissue pieces of about 01cm were preserved in 10% formalin solution and processed by paraffin sectioning technique (Ali et al., 2024; Akram et al., 2021). For microscopic observations, about 4-5µm thick histological sections were prepared and stained with hematoxylin and eosin staining technique, for histo-morphological studies, as previously described (Ali et al., 2017; Sikandar et al., 2020).

Statistical analysis

The recorded data were subject to statistical analysis by using one way ANOVA (Analysis of Variance). Mean ± SE values were determined and Multiple Mean Comparison was implied through Tukey's test. $p<0.05$ was accepted as statistically significant difference.

Results

Hematologic analysis

The total erythrocytes / RBCs count and hemoglobin (HGB) concentration decreased significantly ($p<0.05$) at the day-20 in the groups C and D and the values were further decreased at the day-30 in the quails of group D (Fig 1a-c). With the increased exposure of bifenthrin in the Japanese quail, the leukocyte / WBCs count was increased significantly ($p<0.05$) in each group B, C and D. Similarly, the hematocrit (HCT) values were goes on decreasing after 10 day to days-20 and 30 at the exposure of bifenthrin from groups B to D (Fig 1a-c). The values of mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentrations (MCHC) were also increased significantly ($p<0.05$) in the groups C and group D up-to the end of the experiment at days-30 (Fig 1 d-f).

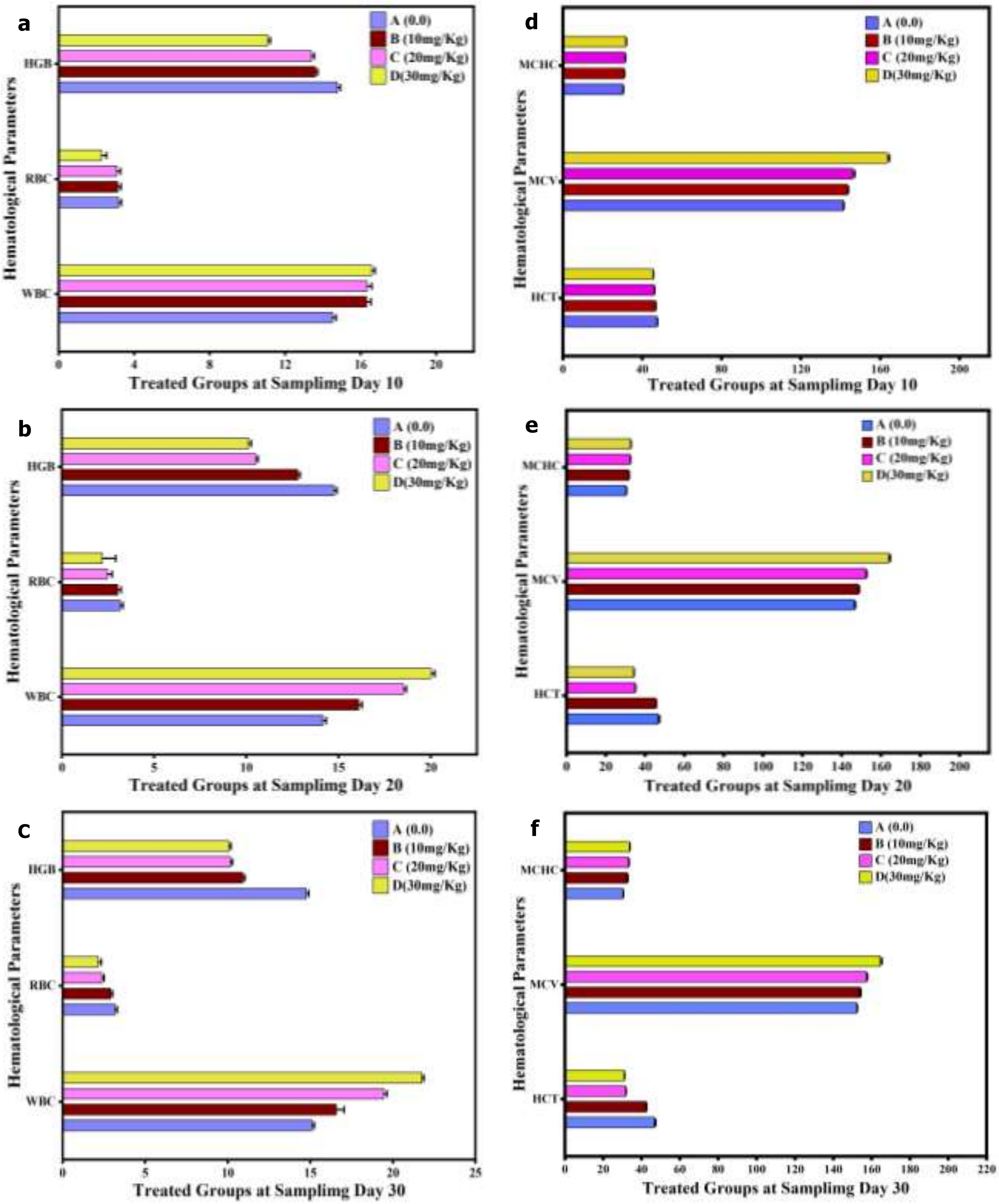

Figure-1. **Haematological parameters of birds of different treatment groups: B (10mg/kg b.w.), C (20mg/kg b.w.) and D (30mg/kg b.w.) of bifenthrin compared to the control group A, at days-10 (a, d), 20(b, e) and 30 (c, f).**

Nuclear abnormalities

The results revealed that various nuclear abnormalities in RBCs significantly increased in the groups exposed to different concentrations of bifenthrin as compared to control group. It was observed that the percentile rate of erythrocyte with hetero-pycnotic nuclei, lobed nuclei, blebbed nuclei, cells with micro- and broken nuclei were significantly ($p<0.05$) increased in the groups D than the groups B, C and control group A, during the experiment (Fig 2). There was significant increase in the occurrence of binuclear and pear shapes in the erythrocytes of the birds of group D at day-30 exposed to higher bifenthrin concentration of 30mg/kg (Fig 2). The condensed and notched nuclei were significantly ($p<0.05$) increased in the groups C and D at the days-20 and 30 in the groups exposed to higher concentrations of the drug (Fig 2-3).

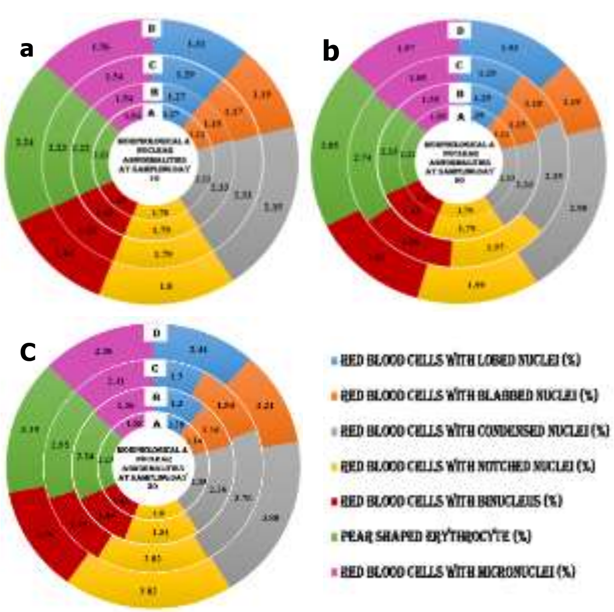

Figure-2. Nuclear abnormalities in RBCs of the birds of different treatment groups: B (10mg/kg b.w.), C (20mg/kg b.w.) and D (30mg/kg b.w.) of bifenthrin compared to the control group A, at days-10 (a), 20 (b) and 30 (c) of sampling.

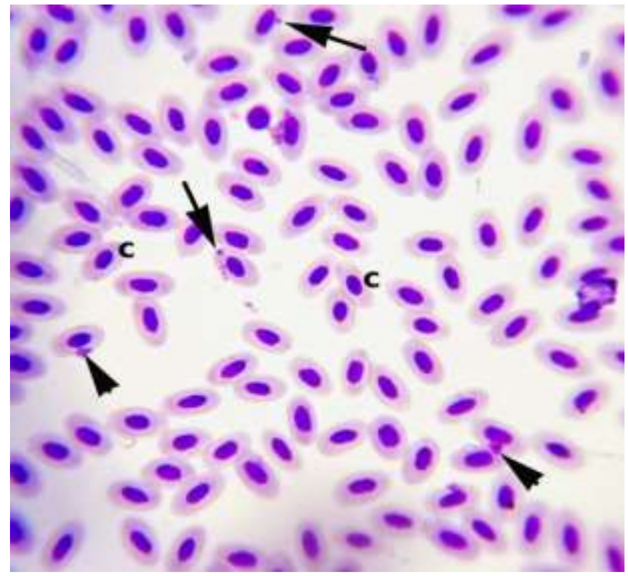

Figure-3. **Photomicrograph of erythrocyte of mature birds given higher concentrations of bifenthrin showing different nuclear and morphological ailments like micronucleus (arrow), lobed nuclei (arrow heads) and condensation of nuclei (c).**

Morphology and histological observations of visceral organs

Body weight of *C. japonica* exposed to different concentrations of bifenthrin significantly ($p<0.05$) decreased in the groups exposed different concentrations (10mg/kg b.w., 20mg/kg b.w. and 30mg/kg b.w.) of bifenthrin, as compared to control group A. The absolute (Figure 4) and relative (Figure 5) weights of liver and intestine increased significantly ($p<0.05$) in the groups C and group D at 20 and 30-days of exposure of bifenthrin. The weight of kidneys increased from group A to group D with an increase in the concentration of bifenthrin in a time dependant manner. Similarly, the absolute and relative weights of brain, spleen, lungs, proventriculus, gizzard and heart increased significantly ($p<0.05$) in the groups C and D compared to group A (Figure 4-5). Histo-pathologically, there observed necrosis of cardiac myocytes, edema and myofibrillosis, presence of inflammatory exudates, necrosis of cardiac myocytes and breakdown of cardiac myofibers (Fig 6). While the spleen sections revealed dis-organisation of splenic cords and sinuses, presence of inflammatory materials and depletion of splenic cells (Fig 7).

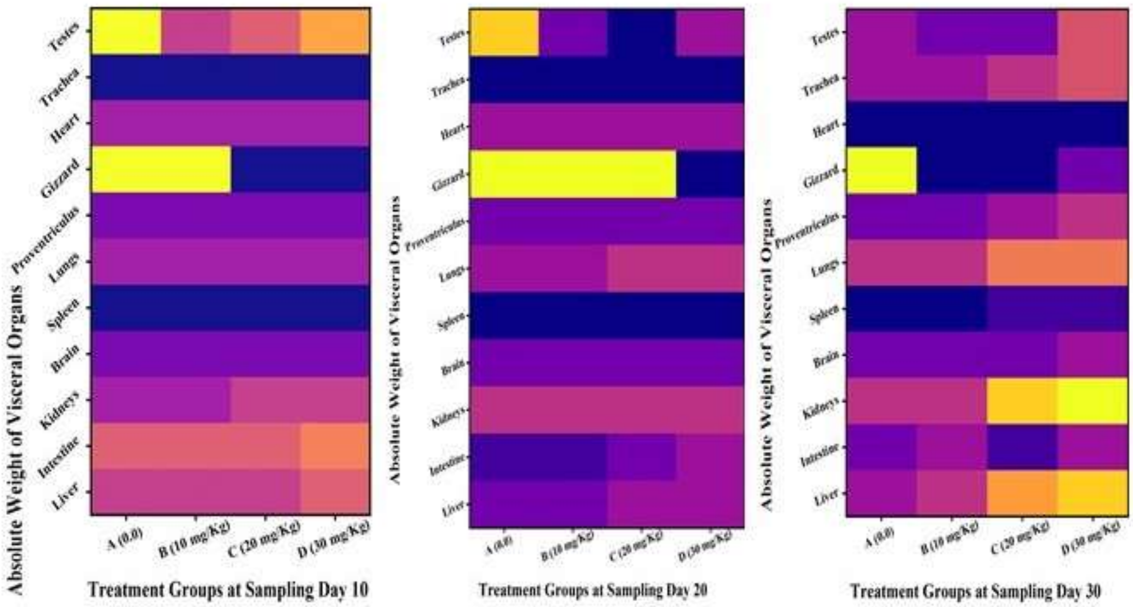

Figure-4. Absolute weights of visceral organs of quail of different treatment groups B (10mg/kg b.w.), C (20mg/kg b.w.) and D (30mg/kg b.w.) of bifenthrin compared to the control group A, at sampling days-10, 20 and 30.

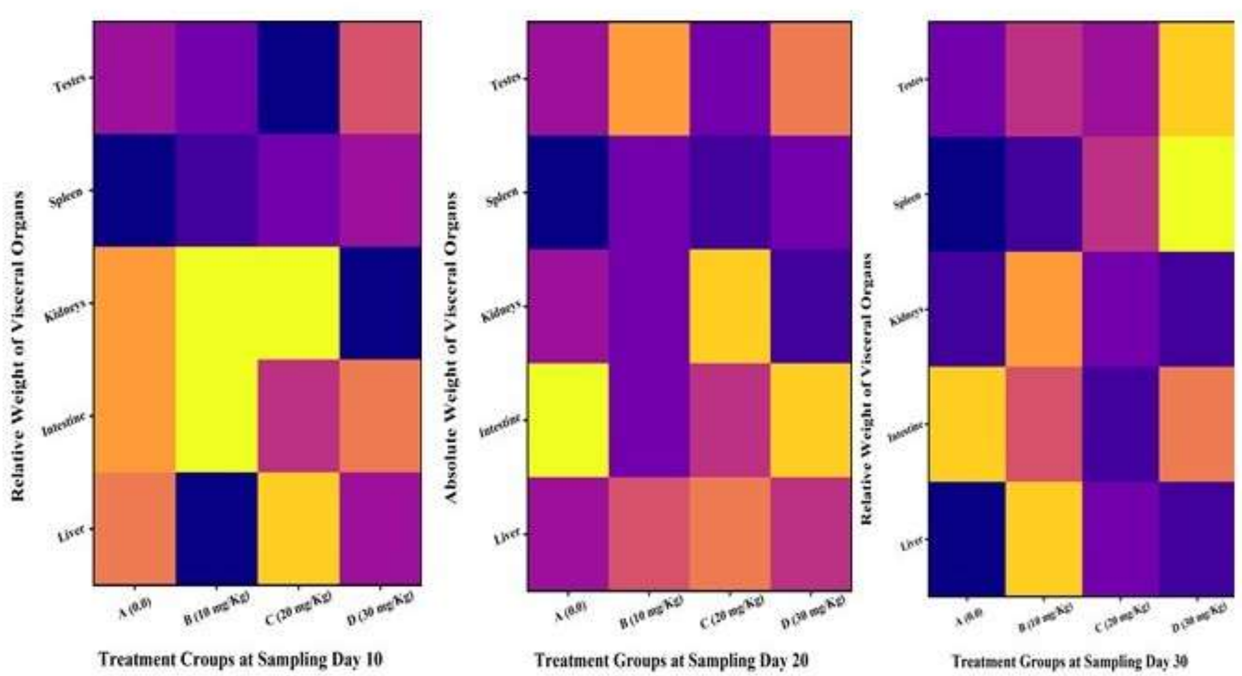

Figure-5. Relative weights of visceral organs of quail of different treatment groups B (10mg/kg b.w.), C (20mg/kg b.w.) and D (30mg/kg b.w.) of bifenthrin compared to the control group A, at sampling days-10, 20 and 30.

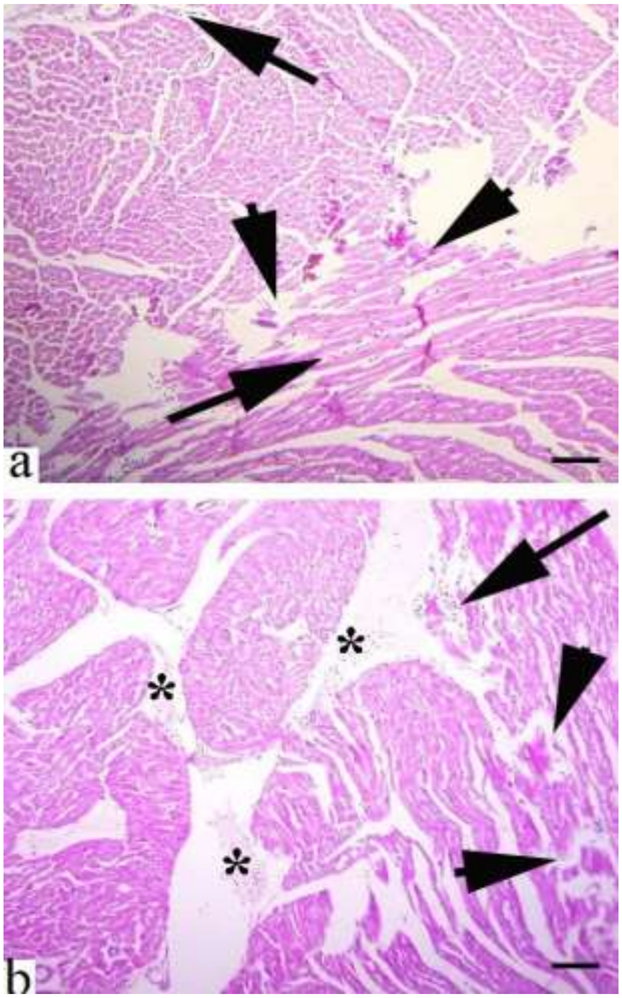

Figure-6. **Histo-pathological sections of heart of adult Japanese quails depicting necrosis of cardiac myocytes (arrow head) and presence of edema (arrow) in myofibrillosis (a). Moreover, necrosis of cardiac cells (arrow head) and presence of inflammatory exudate (arrow) and edema fluid (*) were observed along with the breakdown of cardiac myofibers (b). Scale bar = 50µm.**

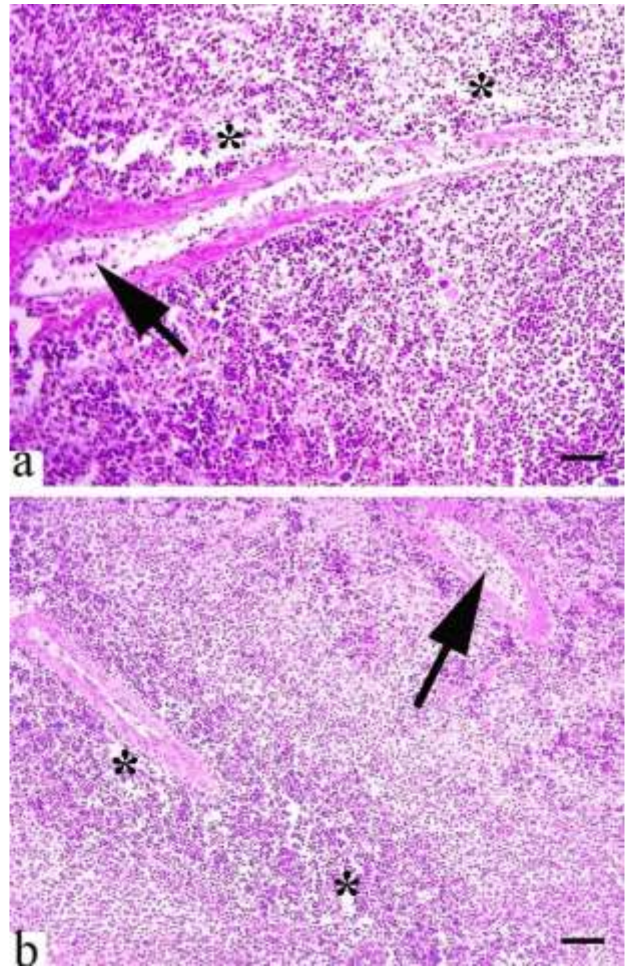

Figure-7. **Histological sections of spleen of adult quails exposed to different doses of bifenthrin showing different histo-pathological alterations like disorganisation (a) and depletion (b) of splenic parenchyma showing inflammatory exudate (arrow) and depletion of splenic cells (*). Scale bar = 50µm.**

Morphology and histological observations of testes

The testes of the quails in the group A (control group) were found to be regular in size, while the testes of quails subjected to different doses of bifenthrin had been significantly ($p<0.05$) decreased in size at days-30 in all groups as compared to control group. In the group D, the testicular length and width of quails has been extensively smaller in comparison to the control group (Figure 8).

Histo-pathological observations of the testes of the birds in the control group found to have all the successive tiers of spermatogenesis including spermatogonia, primary and secondary spermatocytes and spermatids.

Figure-8. The dimensions and size of testes of Japanese quail exposed to different concentrations of Bifenthrin at the end of the experiment (day-30). A = Control, B = 10mg/kg b.w., C = 20mg/kg b.w., D = 30mg/kg b.w.

While, the testes of quails subjected with different doses of bifenthrin found out admixture of necrotic cells inside the lumen of seminiferous tubules with arrest in spermatogenesis. The diameter of seminiferous tubules, height of germinal epithelium and the number of seminiferous tubules containing normal spermatozoa were significantly ($p<0.01$)

decreased in the quails of groups from C to D compared to the control group (Fig 9-10). The diameter of seminiferous tubules was decreased while the tubules having unusual sperms were additionally significantly increased from the day-10 ($p<0.05$) of the experiment in the treated groups up-to the end of the experiment (day-30; $p<0.01$) (Fig 9, Table 1). At the histo-pathological level, the number of pyknotic cells and degenerated seminiferous tubules were found to be increased significantly ($p<0.05$) along with an admixture of necrotic cells in the lumen of seminiferous tubules. Moreover, there observed an arrest of spermatogenesis, necrosis of spermatids and sloughing of germinal epithelium of seminiferous tubules, pyknosis, admixture of necrotic cells in the lumen of seminiferous tubules and degeneration of seminiferous tubules. The tubules were found to be congested having an increased number of abnormal cells (Fig 10, Table 2).

Discussion

Bifenthrin is extensively used insecticide in agriculture and at storage houses of crops and grains at worldwide. Despite, its beneficial effects against the harmful insects and pests, the compound causes serious deleterious impacts of different non-target organisms. However, the data were not available regarding the effects of bifenthrin exposed to Japanese quail. The dietary LC_{50} of the drug has been reported to be 1280mg/kg and 4450mg/kg for bobwhite quail and mallard ducks, respectively (Tomlin, 2000) and we have selected quite low concentrations (below than 1% mortality) of the drug to elaborate the sub-lethal effects in Japanese quail that the selected doses of the drug might not cause the death of the bird but impart non-apparent sub-clinical but severe physiological health effects. Hence, the current study revealed the alterations in the hematological parameters and tissue morphology. It has been previously shown that RBCs, HGB, hematocrit and lymphocytes were significantly ($p<0.05$) decreased while leukocyte counts were significantly increased in fresh water fish on exposure to Emamectin benzoate (Kumar et al., 2022) or Butachlor (Ghaffar et al., 2015).

There found a significant increased incidence of different morphological and nuclear abnormalities like pear shape RBCs, microcytes, pear shape RBCs, erythrocytes with micro-, lobed, blebbed or notched nuclei and cells with nuclear remnants were observed, as like quinalphos insecticide caused genetic alterations and nuclear changes in the peripheral RBCs of silver barb (Sadiqul et al., 2016). Our results also showed significant increase in the WBCs count and increased values of hemoglobin, erythrocyte, hematocrit and lymphocyte in the Japanese quail exposed to different concentrations of bifenthrin, as also previously reported (Ghaffar et al., 2018; Qureshi et al., 2016) wherein the authors exposed the common carp (*Cyprinus carpio*) to different concentrations of fipronil and found that erythrocyte count, hemoglobin and hematocrit were decreased significantly ($p<0.05$) and MCV, WBCs, neutrophils, monocytes and lymphocytes were significantly increased (Ghaffar et al., 2015).

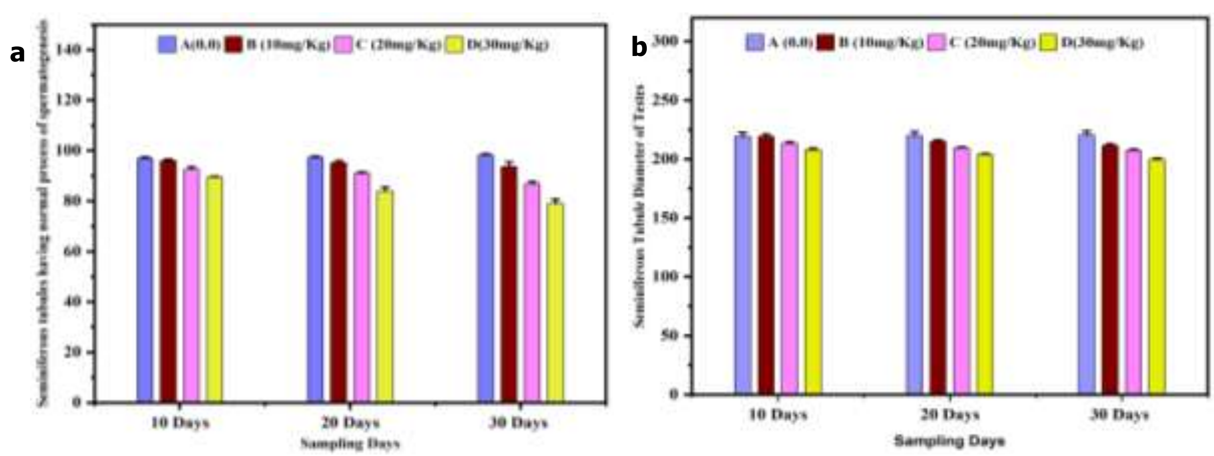

Figure-9. The seminiferous tubules with normal process of spermatogenesis (a) and diameter of seminiferous tubules (b) in the testes of Japanese quail exposed to different doses of Bifenthrin at different days of sampling. A = Control, B = 10mg/kg b.w., C = 20mg/kg b.w., D = 30mg/kg b.w.

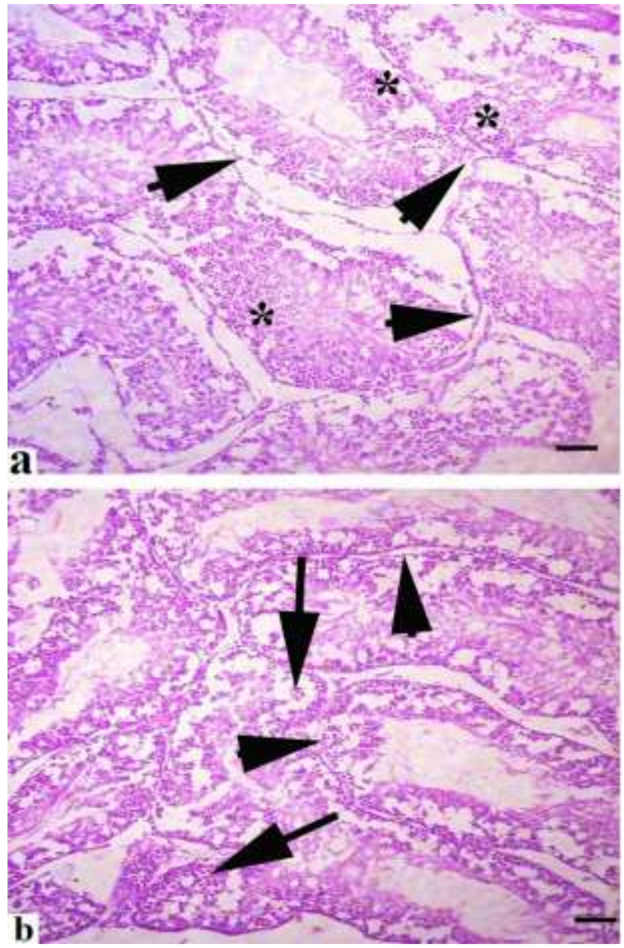

Figure-10. **Histo-micrograph of testicular tissues of Japanese quails exposed with bifenthrin showing various histo-pathological alterations including arrest of spermatogenesis (arrow heads), necrosis of spermatids (*) and sloughing of germinal epithelium of seminiferous tubules (a) along with pyknosis, admixture of necrotic cells in the lumen of seminiferous tubules (arrow) and degeneration (arrow head) of seminiferous tubules (b). Scale bar = 50µm.**

Moreover, total serum proteins, MCHC and MCV were significantly ($p<0.05$) decreased that indicate that fish were suffering from microcytic hypochromic anemia (Ghaffar et al., 2015). The reduction in the absolute and relative weights of different visceral organs may be attributed to systemic toxicity in the Japanese quail.

There observed reproductive modifications in the testes of quails in the form of significant decrease in the weight and testicular morphometry. Similar to our findings, it was previously found that the relative weight of testes was decreased in the quails exposed to triazophos in comparison to control group (Ghaffar et al., 2015). Moreover, at histologically level, the number and diameter of seminiferous tubules and height of spermatogenic epithelium was decreased in the quails exposed to triazophos compared to control group (Ghaffar et al., 2015). In the same context, many environmental toxicants produce variations of functions of reproductive and other body systems simultaneously with impact on the behavior and referred to as neuroendocrine disrupters function via hypothalamo-pituitary-organ axis (Hussain et al., 2011; Hussain et al., 2012; Hussain et al., 2014). The decrease in the relative weight of testes could be correlated with preceding findings displaying a decrease in the relative weight of testes at 90 days of treatment by various levels (62.5mg/kg and 125mg/kg b.w.) of pirimiphos-methyl (Ngoula et al., 2007) or of chlorpyriphos-ethyl at the dose of 7.5mg/kg, 12.5mg/kg and 17.5mg/kg b.w./day (Joshi et al., 2007) in rats or by 37.5mg, 56.25mg and 75mg of AS/kg b.w. in Japanese quail (Ferdinand et al., 2017). The substantive diminution of spermatogenic cellular population lead to widening of the intra-tubular area of the seminiferous tubules along with an admixture of necrotic cells, arrest of spermatogenesis, reduced seminiferous tubule diameter and epithelial height. The insecticides usually produce oxidative strains, free radicals and variations in the antioxidant levels, the scavenging enzyme machinary and lipid peroxidation (Sule et al., 2022). It has been observed that insecticides have the efficiency to pass the blood-testis barrier inducing oxidative pressure and lipid peroxidation that destroy the organic membranes in the testes (Tijani et al., 2024; Zuščíková et al., 2023) resulting in necrosis, infection and degeneration of the spermatogenic tissues (Babazadeh and Najafi, 2017).

Conclusion

These results signify the detrimental effects of bifenthrin on the health and physiological well-being of *C. japonica* suggesting the potential risks and harm to avian species when exposed to this pesticide. The comprehensive analysis and data presented in this study emphasize the impact of bifenthrin and other similar pesticides on avian species and ecosystems, urging for more stringent regulations and environmentally conscious practices to mitigate these harmful effects. The research conducted on the toxic impacts of bifenthrin in adult *C. japonica* revealed significant and serious alterations in the hematological indices and histopathological changes in different organ tissues. Bifenthrin exposure resulted in disruptions in the blood parameters and histological changes in the organs indicating potential toxicity and adverse effects on the health of these birds that warranted to comprehensively understand the mechanisms underlying these effects and to devise strategies to mitigate the impact of pesticides on wild avian health.

Acknowledgment

This research work was funded by Institutional Fund Projects under grant no. (IFPIP: 2003-140-1443). Therefore, the authors gratefully acknowledge the technical and financial support from the Ministry of Education and King Abdulaziz University (KAU), DSR, Jeddah, Saudi Arabia.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: This research work was funded by Institutional Fund Projects under grant no. (IFPIP: 2003-140-1443), Ministry of Education and King Abdulaziz University (KAU), DSR, Jeddah, Saudi Arabia.

Contribution of Authors

Kalsoom R: Performed the experiments & collected data, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Asfour HZ: Literature review & funding acquisition, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Ali HM: Performed the experiments & collected data, Project administration and article write up, Performed the data analysis, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Qayyum A: Performed the data analysis, Read and approved the final manuscript.
Anjum S: Conceived idea and designed research methodology, Data analysis and article write up, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Maqbool F: Performed the experiments & collected data, Performed the data analysis, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Sial N: Conceived idea and designed research methodology, Data analysis and article write up, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Hussain R: Conceived idea and designed research methodology, Literature review & funding acquisition, Project administration and article write up, Data analysis and article write up, Performed the data analysis, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Alamri SH: Editing, reviewing and drafting of article, Read and approved the final manuscript.
Ali N: Project administration and article write up, Performed the data analysis, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Rajeh N: Performed the data analysis, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Irshad I: Performed the data analysis, Editing, reviewing and drafting of article, Read and approved the final manuscript.
Idrees A: Editing, reviewing and drafting of article, Read and approved the final manuscript.

References

Abd AAR, Rasmi HAJ, Issa AR and Majeed FM, 2023. The histopathological and oxidative stress profiles in japanese quails (Coturnix japonica) induced by dietary lead. Arch Razi Inst. 78(2):523-530. https://doi.org/10.22092/ARI.2022.359352.2407.

Akram R, Ghaffar A, Hussain R, Khan I, de Assis Santana VL, Mehmood K, Naz S, Iqbal R, Imran HM, Qamar MR and Zhu H, 2021. Hematological, serum biochemistry, histopathological and mutagenic impacts of triclosan on fish (Bighead carp). Agrobiol. Rec. 7: 18-28.

Ali HM, Hussain S, Ahmad MZ, Siddique AB, Ali S, Mohiuddin M, Ehsan M, Nadeem M, Qayyum A, Hussain R, Khan I, Farraj DAA and Alzaidi E, 2024. Molecular identification of different toxinogenic strains of Clostridium perfringens and histo-pathological observations of camels died of per-acute entero-toxaemia. Heliyon – Microbiol. 10(2024): e27859. https://doi.org/10.1016/j.heliyon.2024.e27859.

Ali HM, Qureshi AS, Hussain R, Urbinati G, Mustafa MZ, Ali F, Manan A and Massaad-Massade L, 2017. Effects of natural environment on reproductive histo-morphometric dynamics of female dromedary camel. Anim. Reprod. Sci. 181: 30-40. https://doi.org/10.1016/j.anireprosci.2017.03.012.

Safdar U, Ahmed W, Ahmed M, Hussain S, Fatima M and Tahir N, 2022. A Review: Pesticide Application in Agriculture and its Environmental Consequences. International J. Agric. Biosci. 11: 125-130. https://doi.org/10.47278/journal.ijab/2022.017

Arslan H, Özdemir S and Altun S, 2017. Cypermethrin toxication leads to histopathological lesions and induces inflammation and apoptosis in common carp (Cyprinus carpio L.). Chemosphere 180: 491-499. https://doi.org/10.1016/j.chemosphere.2017.04.057

Arya AK, Singh A and Bhatt D, 2019. Ch 10: Pesticide applications in agriculture and their effects on birds: An overview. In: Contaminants in Agriculture and Environment: Health Risks and Remediation. pp: 129-137. https://doi.org/10.26832/AESA-2019-CAE-0163-010

Ayub I, Tanveer Z, Sadaf H, Ahmad I, Liaquat N, Talat R, Ali HM, Yousaf M and Asadullah, 2018. Sodium dodecyl sulfate polyacrylamide gel electrophoretic expression of serologic proteins from sera of patients with gastric cancer. Pak-Euro J. Med. Life Sci. 1(1): 1-4.

Babazadeh M and Najafi G, 2017. Effect of chlorpyrifos on sperm characteristics and testicular tissue changes in adult male rats. Vet. Res. Forum. 8(4): 319-326.

Dahamna S, Bencheikh F, Harzallah D, Boussahel S, Belgeit A, Merghem M and Bouriche H, 2010. Cypermetherin toxic effects on spermatogenesis and male mouse reproductive organs. Commun. Agric. Appl. Bio. Sci. 75 (2): 209-216.

Eghan K, Lee S, Yoo D, Kim CH and Kim WK, 2023. Adverse effects of bifenthrin exposure on neurobehavior and neurodevelopment in a zebrafish embryo/larvae model. Chemosphere 341:140099. https://doi.org/10.1016/j.chemosphere.2023.140099

Farag MR, Mahmoud HK, El-Sayed SAA, Ahmed SYA, Alagawany M and Abou-Zeid SM, 2021. Neurobehavioral, physiological and inflammatory impairments in response to bifenthrin intoxication in Oreochromis niloticus fish: Role of dietary supplementation with Petroselinum crispum essential oil. Aqua. Toxicol. 231: 105715. https://doi.org/10.1016/j.aquatox.2020.105715

Ferdinand N, Herman NV, Omer Bebe NK, Augustave K, Valence M, Ghislaine NT, Herve T, Dorice AK, Sorelle D and Yacouba M, 2017. Antouka Super® induced oxidative stress and reproductive toxicity in male Japanese quail (Coturnix coturnix japonica). Heliyon. 3(10): e00410. https://doi.org/10.1016/j.heliyon.2017.e00410.

Fong S, Louie S, Werner I, Davis J and Connon R, 2016. Contaminant effects on california bay–delta species and human health. San Francisco Estuary and Watershed Science, 14, 2016. https://doi.org/10.15447/sfews.2016v14iss4/art5.

Gaston SA, Birnbaum LS and Jackson CL, 2020. Synthetic chemicals and cardiometabolic health across the life course among vulnerable populations: a review of the literature from 2018 to 2019. Curr. Environ. Health Rep. 7(1): 30-47. https://doi.org/10.1007/s40572-020-00265-6.

Ghaffar A, Hussain R, Abbas G, Kalim M, Khan A, Ferrando S, Gallus L and Ahmed Z, 2018. Fipronil (Phenylpyrazole) induces hemato-biochemical, histological and genetic damage at low doses in common carp, Cyprinus carpio (Linnaeus, 1758). Ecotoxicol. 27(9): 1261-1271. https://doi.org/10.1007/s10646-018-1979-4.

Ghaffar A, Hussain R, Khan A, Abbas,RZ and Asad M, 2015. Butachlor induced clinico-hematological and cellular changes in fresh water fish Labeo rohita (Rohu). Pakistan Vet. J. 35(2): 201-206.

Ghaffar A, Hussain R, Noreen S, Abbas G, Chodhary I, Khan A, Ahmed Z, Khan M, Akram K, Ulhaq M, Ahmad N, Ali F, Niaz M and Mk K, 2020. Dose and Time-Related Pathological and Genotoxic Studies on Thiamethoxam in Fresh Water Fish (Labeo rohita) in Pakistan. Pakistan Vet. J. 40: 151-156.

González-Gómez X, Simal-Gándara J, Fidalgo Alvarez LE, López-Beceiro AM, Pérez-López M and Martínez-Carballo E, 2020. Non-invasive biomonitoring of organic pollutants using feather samples in feral pigeons (Columba livia domestica). Environ. Pollut. 267: 115672. https://doi.org/10.1016/j.envpol.2020.115672

Gripp HS, Freitas JS, Almeida EA, Bisinoti MC and Moreira AB, 2017. Biochemical effects of fipronil and its metabolites on lipid peroxidation and enzymatic antioxidant defense in tadpoles (Eupemphix nattereri: Leiuperidae). Ecotoxicol. Environ. Saf. 136: 173-179. https://doi.org/10.1016/j.ecoenv.2016.10.027

Hussain R, Ali F, Javed MT, Jabeen G, Ghaffar A, Khan I, Liaqat S, Hussain T, Abbas RZ, Riaz A, Gul ST and Ghori MT, 2021. Clinico-hematological, serum biochemical, genotoxic and histopathological effects of trichlorfon in adult cockerels. Toxin Reviews 40 (4): 1206-1214. https://doi.org/10.1080/15569543.2019.1673422

Hussain R, Mahmood F, Khan A, Javed MT, Rehan S, Mehdi T, 2012. Cellular and biochemical effects induced by atrazine on blood of male Japanese quail (Coturnix japonica). Pest. Biochem. Physiol. 103: 38–42

Hussain R, Mahmood F, Khan MZ, Khan A, Muhammad F, 2011. Pathological and genotoxic effects of atrazine in male Japanese quail (Coturnix japonica). Ecotoxicol 20:1-8

Hussaina R, Khan A, Mahmood F, Rehan S, Ali F, 2014. Clinico-hematological and tissue changes induced by butachlor in male Japanese quail (Coturnix japonica). Pest. Biochem. Physiol. 109:58-63

Jayakumar S, Muralidharan S and Dhananjayan V, 2020. Organochlorine Pesticide Residues Among Colonial Nesting Birds in Tamil Nadu, India: A Maiden Assessment from Their Breeding Grounds. Arch. Environ. Cont. Toxicol. 78(4): 555-567. https://doi.org/10.1007/s00244-020-00709-y

Joshi SC, Mathur R and Gulati N, 2007. Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat. Toxicol. Ind. Health 23(7): 439-44. https://doi.org/10.1177/0748233707080908.

Khan A, Afsheen H, Afzal G, Nisa QU, Alam S, Ali A, Shamsher MI and Jamal A, 2023. Oxidative stress and toxicological impacts of monomehypo exposure on bone marrow and erythrocytes in male Japanese Quail. Continental Vet J. 3(2):71-77.

Kumar V, Swain HS, Das BK, Roy S, Upadhyay A, Ramteke MH, Kole RK and Banerjee H, 2022. Assessment of the effect of sub-lethal acute toxicity of Emamectin benzoate in Labeo rohita using multiple biomarker approach. Toxicol Rep. 9:102-110. https://doi.org/10.1016/j.toxrep.2022.01.001.

Luqman Z, Masood S, Ali HM, Rehman TU, Altaf M, Iqbal N, Bilal RM, Din S, Hussain N and Aslam S, 2021. Effects of in-ovo administration of L-arginine on the histo-morphometry of thigh muscles, meat characteristics and growth performance in japanese quails (Coturnix coturnix japonica). Pakistan Vet Journal 41(3): 456-458. https://doi.org/10.29261/pakvetj/2021.037.

Malik S, Sial N, Shahzad MI, Hasanat A, Ali HM and Daniyal M, 2018. Prevalence and characterization of trypanosoma species from livestock of cholistan desert of Pakistan. Trop. Biomed. 35(1): 140–148.

Ngoula F, Watcho P, Dongmo MC, Kenfack A, Kamtchouing P and Tchoumboué J, 2007. Effects of pirimiphos-methyl (an organophosphate insecticide) on the fertility of adult male rats. Afr. Health Sci. 7(1): 3-9. https://doi.org/10.5555/afhs.2007.7.1.3.

Pan J, Liu P, Yu X, Zhang Z and Liu J, 2024. The adverse role of endocrine disrupting chemicals in the reproductive system. Front Endocrinol (Lausanne). 14:1324993. https://doi.org/10.3389/fendo.2023.1324993.

Park S, Lee JY, Park H, Song G and Lim W, 2020. Bifenthrin induces developmental immunotoxicity and vascular malformation during zebrafish embryogenesis. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 228: 108671. https://doi.org/10.1016/j.cbpc.2019.108671.

Patisaul HB, 2021. Endocrine disruption and reproductive disorders: impacts on sexually dimorphic neuroendocrine pathways. Reprod. 162(5):F111-F130. https://doi.org/10.1530/REP-20-0596.

Qureshi IZ, Bibi A, Shahid S and Ghazanfar M, 2016. Exposure to sub-acute doses of fipronil and buprofezin in combination or alone induces biochemical, hematological, histopathological and genotoxic damage in common carp (Cyprinus carpio L.). Aquat. Toxicol. 179: 103-14. https://doi.org/10.1016/j.aquatox.2016.08.012.

Razali NSM, Amin NM, Omar WBW, Ikhwanuddin M and Kadir NHA, 2019. Proteomic analysis and assessment of heavy metals in hepatopancreas of mud crabs from Setiu and Kuala Sepetang. Asian J. Agric. Biol. Special Issue: 17-24.

Rubin BS, Schaeberle CM and Soto AM, 2019. The Case for BPA as an Obesogen: Contributors to the Controversy. Front. Endocrinol. (Lausanne). 10:30. https://doi.org/10.3389/fendo.2019.00030.

Sadiqul IM, Ferdous Z, Nannu TA, Mostakim GM and Rahman MK, 2016. Acute exposure to a quinalphos containing insecticide (convoy) causes genetic damage and nuclear changes in peripheral erythrocytes of silver barb, Barbonymus gonionotus. Environ. Poll. 219: 949-956. https://doi.org/10.1016/j.envpol.2016.09.066.

Scarano W, Amina B, Alonso L, de Aquino A, Fantinatti B, Justulin Jr L, Barbisan LF, Freire P, Flaws J and Lemos B, 2019. Exposure to an Environmentally Relevant Phthalate Mixture During Prostate Development Induces MicroRNA Upregulation and Transcriptome Modulation in Rats. Toxicol. Sci. 171. https://doi.org/10.1093/toxsci/kfz141.

Shafqat S, Abbass J, Khan A, Afsheen H, Afzal G, Nisa QU, Alam S, Shamsher MI and Jamal A, 2023. Oxidative stress and toxicological impacts of Ethoxysulfuron exposure on bone marrow, and intestinal morphometry in male Japanese Quail. Continental Vet J. 3(2):78-85.

Sharaf S, Khan A, Khan MZ, Aslam F, Saleemi MK and Mahmood F, 2010. Clinico-hematological and micronuclear changes induced by cypermethrin in broiler chicks: Their attenuation with vitamin E and selenium. Exp. Toxicol. Pathol. 62(4): 333-341. https://doi.org/10.1016/j.etp.2009.05.002

Sheikh IS, Asmat TA, Bajwa MA, Mustafa MZ, Rashid N, Rafeeq M, Kiani MMT, Ali HM and Raziq A, 2021. Effects of immune modulators on growth and economic performance of broiler chickens. Journal of Animal and Plant Sciences: 31(4): 966-973. https://doi.org/10.36899/JAPS.2021.4.0292.

Sikandar A, Zaneb H, Nasir A, Adil M, Ali HM, Muhammad N, Rehman TU and Rahman HF, 2020. Effects of Bacillus subtilis on performance, immune system and gut in Salmonella-challenged broiler chickens. South Afr. J. Anim. Sci. 50(5): 654-662.

Sule RO, Condon L and Gomes AV, 2022. A Common Feature of Pesticides: Oxidative Stress-The Role of Oxidative Stress in Pesticide-Induced Toxicity. Oxid. Med. Cell. Longev. 2022: 5563759. https://doi.org/10.1155/2022/5563759.

Tijani AS, Daba TM, Ubong IA, Olufunke O, Ani EJ and Farombi EO, 2024. Rutin attenuated hexachlorobenzene-induced testicular injury via regulation of oxidative stress, steroidogenic enzymes and apoptotic process in male rats. Eur. J. Med. Chem. Rep. 10: 100121. https://doi.org/10.1016/j.ejmcr.2023.100121.

Tomlin CDS, 2000. The Pesticide Manual, A World Compendium. 12th Ed.; British Crop Protection Council: Surrey, England. Pp: 502-504.

Uchendu C, Ambali SF, Ayo JO and Esievo KAN, 2018. Chronic co-exposure to chlorpyrifos and deltamethrin pesticides induces alterations in serum lipids and oxidative stress in Wistar rats: mitigating role of alpha-lipoic acid. Environ. Sci. Pollut. Res. 25(20):19605–19611. https://doi.org/10.1007/s11356-018-2185-x.

Ullah S, Li Z, Zuberi A, Arifeen MZ and Baig MMFA, 2019. Biomarkers of pyrethroid toxicity in fish. Environ Chem Lett 17, 945–973 (2019). https://doi.org/10.1007/s10311-018-00852-y

Wang X, Gao X, He B, Jin Y and Fu Z, 2017. Cis-bifenthrin causes immunotoxicity in murine macrophages. Chemosphere 168: 1375-1382. https://doi:10.1016/j.chemosphere.2016.11.121

Xiong J, An T and Peng Pa, 2017. Accelerated biodegradation of BPA in water-sediment microcosms with Bacillus sp. GZB and the associated bacterial community structure. Chemosphere 184. https://doi:10.1016/j.chemosphere.2017.05.163

Xu J, Zhou L, Wang S, Zhu J, Liu T, Jia Y, Sun D, Chen H, Wang Q, Xu F, Zhang Y, Liu H, Zhang T and Ye L, 2018. Di-(2-ethylhexyl)-phthalate induces glucose metabolic disorder in adolescent rats. Environ. Sci. Pollut. Res. 25(4): 3596-3607. https://doi:10.1007/s11356-017-0738-z

Yang JS, Symington S, Clark JM and Park Y, 2018. Permethrin, a pyrethroid insecticide, regulates ERK1/2 activation through membrane depolarization-mediated pathway in HepG2 hepatocytes. Food Chem. Toxicol. 121: 387-395. https://doi:10.1016/j.fct.2018.09.009

Zhou L, Chen H, Xu Q, Han X, Zhao Y, Song X, Zhao T and Ye L, 2019. The effect of di-2-ethylhexyl phthalate on inflammation and lipid metabolic disorder in rats. Ecotoxicol. Environ. Saf. 170: 391-398. https://doi:10.1016/j.ecoenv.2018.12.009.

Zuščíková L, Bažány D, Greifová H, Knížatová N, Kováčik A, Lukáč N and Jambor T, 2023. Screening of toxic effects of neonicotinoid insecticides with a focus on acetamiprid: A review. Toxics 11(7): 598. https://doi:10.3390/toxics11070598.