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Research Article

Oxidative stress and toxicological impacts of monomehypo exposure on bone marrow and erythrocytes in male Japanese Quail

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ABSTRACT

Pesticide use in agriculture, particularly in developing countries, continues to pose a significant threat to avian populations. To investigate the potential harm caused by monomehypo pesticide, an experiment was conducted focusing on its effects on bone marrow and erythrocyte morphology in male Japanese quail. Forty-eight sexually mature quail weighing 120g were selected and divided into four groups (A, B, C and D), each comprising 12 quail. After a two-week adaptation period in their natural environment, the trial commenced. Group A served as the control, while groups B, C and D were exposed to monomehypo @ doses of 120ug/kg BW, 160ug/kg BW, and 200ug/kg BW, respectively. The study evaluated the concentrations of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and reduced glutathione (GSH). The results demonstrated a significant ($P < 0.05$) decrease in POD, SOD, CAT, and GSH levels with an increase in dosage. Conversely, oxidative stress markers, thiobarbituric acid reactive substances (TBARS), and reactive oxygen species (ROS) exhibited a significant increase ($P < 0.05$) in all exposed groups. This study also analyzed morphological changes in erythrocytes, revealing various abnormalities such as blebbed nuclei, micronucleated cells, notched nucleated cells, condensed nucleated cells, lobed nuclei cells, nucleus remnants, and cells lacking cytoplasm. These alterations were observed across all experimental groups and intensified with both higher doses and longer exposure. The results indicated that exposure to monomehypo showed a toxic effect and caused minor to major alterations in male Japanese quail. These results emphasize the urgent need to address the detrimental impacts of pesticides on avian populations, particularly in developing countries where birds are disproportionately affected.

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Introduction

Living organisms are commonly subjected to an extensive range of synthetic and naturally derived substances. The Industrial Revolution and the subsequent advancements in synthetic chemistry have led to the creation of numerous novel chemicals, including fertilizers and pesticides (Osman et al. 2023). Approximately two million tons
pesticides are manufactured and utilized annually worldwide. The United States consumes 24% of this total, while European countries account for 45% of pesticide usage. The remaining 25% is distributed among other regions globally. The most

employed pesticides, namely Lindane, dichlorodiphenyltrichloroethane (DDT), and malathion, make up 70% of the overall pesticide use (Rajak et al. 2023). Toxic pesticides are increasingly targeting a wide range of organisms. Fruit and vegetable crops alone consume 27% of the total pesticide consumption (Umapathi et al. 2022). Insecticides comprise 80% of pesticide use, herbicides make up 15%, plant fungal diseases account for 1.46%, and the remaining 3% consists of other forms of pesticides. However, on a global scale, herbicide consumption stands at 47.5%, insecticides at 29.5%, fungicides at 17.5%, and the remaining 5.5% represents other types of pesticides. India is responsible for 76% of the worldwide pesticide consumption, compared to the global usage rate of 44% (Cestonaro et al. 2022). The presence of pesticides in ecosystems has detrimental effects, which vary depending on the concentration, quantity, and duration of exposure to the contaminants (Voltz et al. 2022). Pesticides exhibit rapid lethality when orally exposed. Despite their widespread popularity and use, mixing, applying, and treating plants and animals with pesticides pose serious health risks (Rashid et al. 2022). Monomehypo, a systemic insecticide with contact and stomach action, disrupts nerve transmission in insects and binds to receptors in the nervous system. It belongs to the Carbamate group, specifically the 3rd age group, and is available in G Formulation (Granules) with a concentration of 5% w/w. Monomehypo is particularly effective against *Chilo suppressalis* W. and *Tryporyza incertulas* W. Dithiocarbamate pesticides and carbamate compounds induce oxidative stress by increasing the production of reactive oxygen species (ROS) (Suliman et al. 2020), leading to endoplasmic reticulum interference and mitochondrial dysfunction (Li et al. 2022). These chemicals also impair leukocyte development and interfere with the function of immune cells (Li et al. 2021). Experimental evidence has shown that carbamate pesticides increase the risk of liver, kidney, thyroid, bladder, uterus, bone, brain, and adrenal gland tumors in humans (Mdeni et al. 2022). Epidemiological studies have confirmed the damaging impacts of pesticide exposure on various organs, including the liver, brain, colon, and lungs. Previous investigations have also revealed the fatal consequences of insecticides, such as cancer, in different individuals (Sharma et al. 2020). All pesticides possess a certain level of toxicity. Even products with low toxicity can cause health problems if animals are exposed to sufficient quantities. The risk of health issues depends not only on the toxicity of the ingredients but also on the level of exposure to the product. Avian species are highly sensitive to industrial chemicals and pesticides, making them valuable model organisms for assessing chemical toxicity in ecotoxicology. Birds can be exposed to toxic substances directly through food or dermal contact during preening and grooming. Oral intake is considered the most significant route of exposure for avian species. Therefore, conducting oral toxicity tests is crucial in determining the toxicological effects of any compound under investigation on avian species (Djekkoun et al. 2021).

Materials and Methods

Experimental design

The experiment was conducted in the research laboratory of the Department of Zoology at the Islamia University of Bahawalpur, Pakistan. All the birds were reared in wire cages for 45 days. In this present study, 48 sexually mature Japanese quail weighing 120g were utilized. The quails were divided into four groups, namely A, B, C, and D, with 12 quails in each group. Throughout the experimental duration, the birds were provided with a corn-soybean meal-based feed containing 22% protein and water twice a day. Monomehypo was administered orally to groups B, C, and D at doses of 120 µg/kg BW, 160µg/kg BW, and 200µg/kg BW, respectively, for 45 days. Group A served as the control group. The birds were monitored daily, and samples were collected on the day 15th, 30th, and 45th day. The birds were housed at room temperature with a humidity level of 60-65%. The birds were subjected to a light/dark cycle of 12 hours and had continuous easy access to food and water throughout the experiment.

Blood Sampling and Morphological Changes in Erythrocytes

Blood sample (5 ml) was collected from jugular vein of each quail at day 15th, 30th, and 45th of the trail. For morphological and nuclear changes in red blood cells of exposed quail, fine thin blood films were prepared from fresh blood of each quail without any anticoagulants. All the blood films were immediately dried, fixed with absolute alcohol and stained with Giemsa solution for 5 to 10 secs. 3000 erythrocytes from each quail were observed for scoring nuclear changes and micronucleus frequencies under oil immersion (100X) lens. All the measurements were carried out with computer-assisted light microscope (Cimrin et al. 2023).

Oxidative and Antioxidant Study in Bone Marrow

On the 15th, 30th, and 45th day of the trial, bone marrow samples were collected from each quail in every group. The collected samples were preserved in serum cups for subsequent procedures. Various oxidative enzymes were determined according to previous protocols including ROS (Miladinovic et al. 2021), TBARS (Tarladgis et al. 1960), and GSH (Owens and Belcher 1965). The status of various antioxidant enzymes was measured according to previous protocols including POD (Owens and Belcher 1965), SOD (Al-Matubsi et al. 2011), and CAT (Goth 1991) in the bone marrow of exposed quail.

Statistical analysis

Obtained data were statistically subjected to one-way analysis of variance (ANOVA) using IBM, SPSS statistics version 20. Post hoc Tukey's test was employed to compare the means of the treatments at a significance level of $P < 0.05$.

Results

Morphological and nuclear alterations in erythrocytes

The formation of micronuclei in erythrocytes was observed as the most prominent abnormality in the monome hypo-exposed quail in groups B to D (120-200 µg/kg BW) at experimental days 15, 30, and 45. There was a significant ($P < 0.05$) increase in erythrocytes with lobed nuclei, blebbed nuclei, and notched erythrocytes in group D compared to group C. The presence of micronuclei significantly increased ($P < 0.05$) in all treatment groups (B, C, and D) compared to the control group A throughout the 45 days.

The occurrence of lobed nucleated cells showed a significant increase ($P < 0.05$) in treatment groups B and D compared with the control group A at different time points. Treatment groups B and C exhibited a significant increase ($P < 0.05$) in nucleus ruminants in red blood cells compared with control group A at various time intervals. The presence of blebbed nucleated cells significantly increased ($P < 0.05$) in treatment group B at all-time points compared with the control group A. Additionally, treatment group D also showed a significant increase in blebbed nucleated cells as compared to the control group. Furthermore, treatment groups B, C, and D demonstrated a significant increase in condensed nucleated cells compared with control group A throughout the experimental period. The absence of cytoplasm in cells significantly increased in treatment group D compared to control group A ($P < 0.05$) at different time intervals. Treatment groups B, C and D displayed a significant increase in notched nucleated cells compared to control group A over the 45 days. Fig. 1 represents all morphological and nuclear changes in erythrocytes of exposed quail.

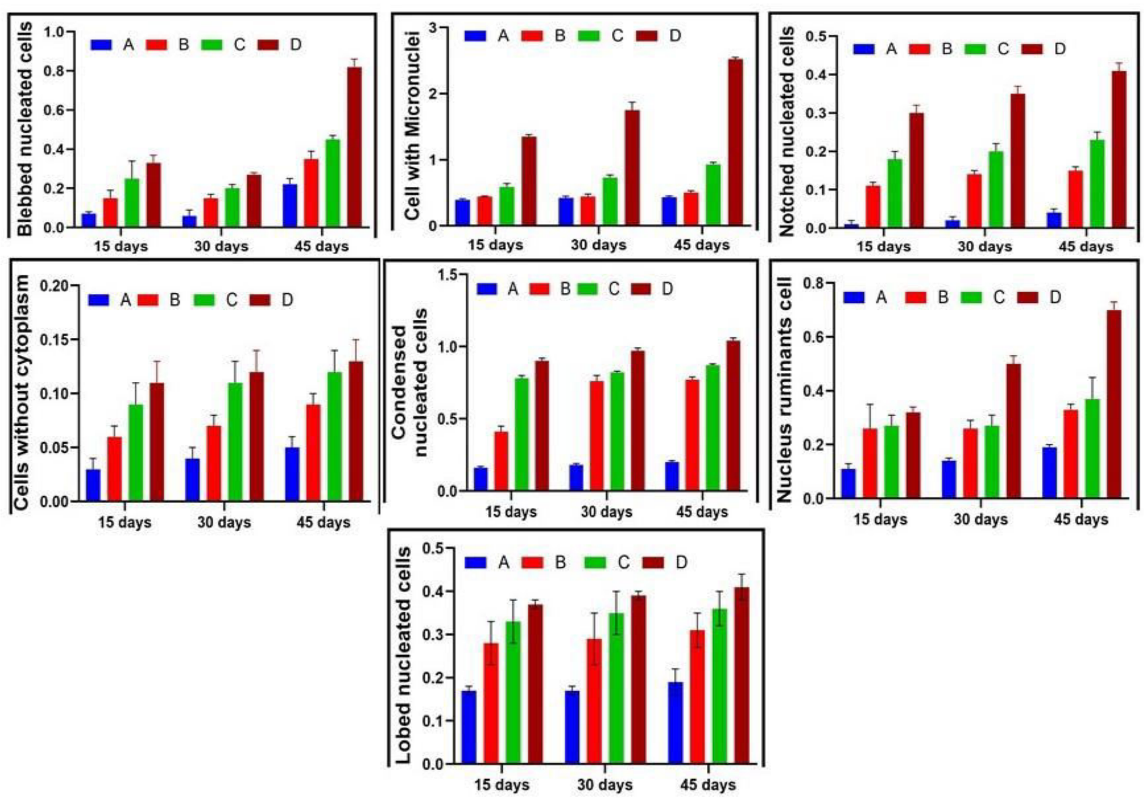

Fig. 1: Effects of Monomehypo exposure on erythrocyte (morphological and nuclear ailments) in male Japanese quail

Oxidative and Antioxidant Status in Bone Marrow

In terms of oxidative stress markers and antioxidant status, this study investigated the activity of ROS, TBARS, GSH, POD, SOD, and CAT (Fig. 2). The results revealed that monomehypo exposure had a slightly significant effect on ROS activity after 30 days of treatment, with group D exhibiting a significant increase ($P < 0.05$) compared to the control group. This suggests that monomehypo may contribute to the generation of ROS, leading to oxidative damage in the quail. Additionally, the activity of TBARS, an indicator of lipid peroxidation, showed a slightly significant effect after 30 days of monomehypo exposure, with group D displaying a significant increase compared to the control group. This indicates that monomehypo may induce lipid peroxidation and cause oxidative damage to cell membranes. Furthermore, the activity of GSH, an important antioxidant defense molecule, demonstrated a slightly significant effect after 45 days of monomehypo exposure, with group D exhibiting a significant decrease compared to the control group. These findings suggest that monomehypo might interfere with the antioxidant defense system, resulting in a reduction in GSH levels. In terms of the antioxidant enzymes evaluated (POD, SOD, and CAT), no significant changes in activity were observed after 15, 30, and 45 days of monomehypo exposure in the control groups. However, a significant decrease ($P < 0.05$) in activity was observed in all the groups exposed to monomehypo, indicating that monomehypo may impact the activity of antioxidant enzymes in male Japanese quail.

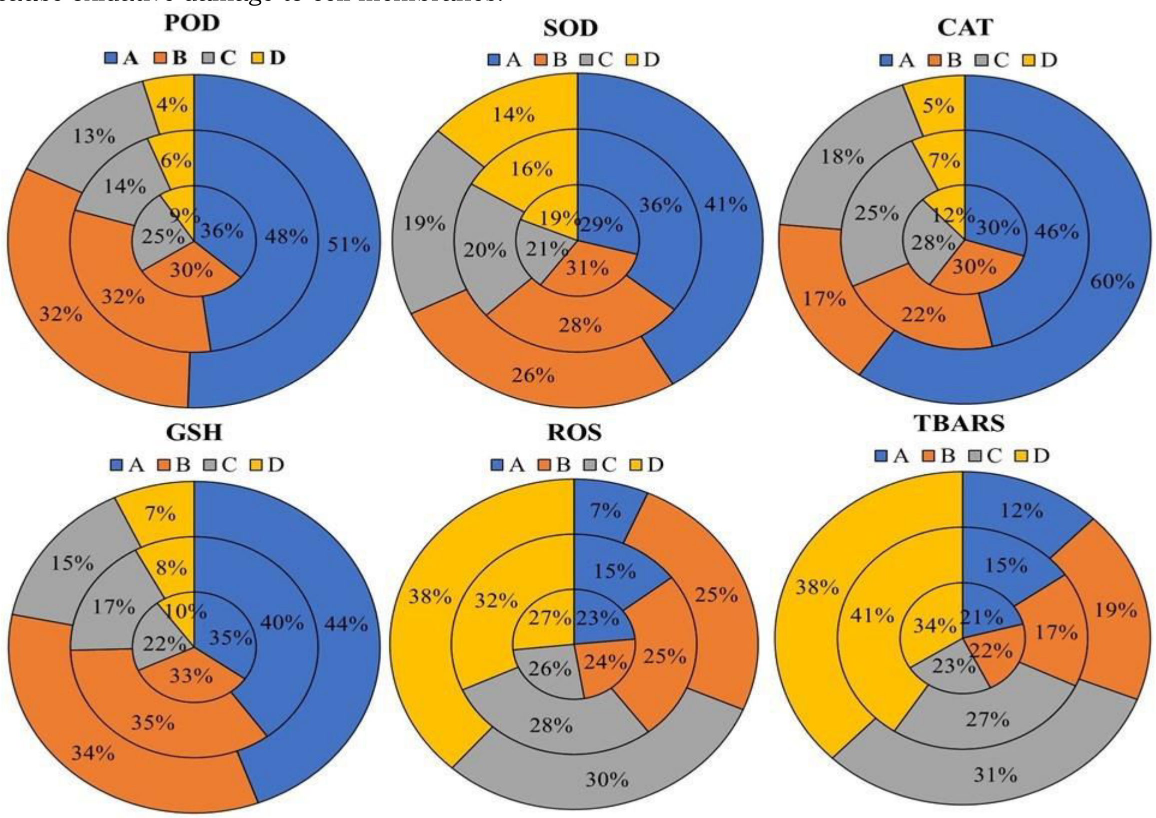

Fig. 2: Oxidative and antioxidant status in bone marrow of male Japanese quail exposed to various concentration of monomehypo

Discussion

Monomehypo is a neurotoxin pesticide that causes toxicity in birds, mammals and aquatic species. According to reports, insecticides and pesticides can cause DNA damage that manifests as the production of micronuclei, chromosomal aberrations, and microtubule abnormalities (Kocaman and Topaktaş 2010; Hussain et al. 2015; Suljevic et al. 2019). The presence of micronuclei in the cytoplasm is thought to be a biomarker of DNA damage (Saleh and Sarhan 2007). Micronuclei are tiny, distinct nuclei that are 1/3rd the diameter and 1/10th of the main nucleus. They have the same color, refraction, and texture as the nucleus. When the whole or chromosomal segments are not integrated in the primary nucleus following cell division, monomehypo treatment may lead to micronuclei (Hussain et al. 2012). Red blood cells develop micronuclei as a result of genetic damage, such as damage to the chromosomes, which causes fragments to lag during anaphase or lagging acentric chromosomes or cytoplasmic chromatin-containing bodies to fail to be incorporated into daughter nuclei (Ghaffar et al. 2015).

In our study, small satellite nuclei were observed that are independent from the cell's primary nucleus seen in the cytoplasm. The creation of cytoplasmic chromatin-containing entities that cannot be integrated into daughter nuclei (clastogenesis) because of genetic damage is frequently connected with micronuclei and following acentric chromosomes during anaphase (Khosrow et al. 2019). The micronuclei observed in our study exhibited consistent color and texture. Our findings are in aligned with (Quero et al. 2016), who also reported a positive correlation between the frequencies of micronuclei and notched nuclei in parrots. These abnormalities were simultaneously observed, affecting the same nucleus in three different cells.

In our present study, inconsistent but significant ($P < 0.05$) morphological changes were observed in the erythrocytes in a time and dose-dependent manner. These alterations included condensed nucleated erythrocytes, nuclear ruminant erythrocytes, blebbed membrane nuclei along with lobed nuclei, notched nuclei, and micronuclei. These Changes in the structure of red blood cells may be attributed to oxidative stress on the mitochondria, leading to the potential release of cytochrome c from the voltage-dependent anion channels in the outer mitochondrial membrane. This triggers apoptotic changes such as the cleavage of cytoskeletal proteins like gelsolin and fodrin, as well as increased production of caspase-activated DNase (CAD) in the nucleus, responsible for the deterioration and fragmentation of nuclear lamins proteins. Similar findings were reported by Hussain et al. (2012), who determined that exposure to ATZ (atrazine) lowered intracellular ATP levels and mitochondrial membrane potential in a variety of cells, leading damaging to both morphology and respiratory function. The frequency of formation of micronuclei increased in erythrocytes of male Japanese quail that were subjected to higher doses of monomehypo. As well as the micronuclei formation increased with the increase in exposure time. These findings align with previously published research. Our study also revealed decreased levels of antioxidant enzymes including reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and increased oxidative stress, increased production of reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS), in the bone marrow of male Japanese quail. These findings are consistent with previous studies that have demonstrated the toxic effects of pesticides on quail (Arslan et al. 2022; Taha et al. 2013; Taha et al. 2020). These results suggest that exposure to monomehypo can induce oxidative stress by altering the antioxidant enzyme status in the bone marrow and causing morphological changes in erythrocytes in male Japanese quail.

Conclusion

This current study demonstrates that monomehypo pesticide exposure has detrimental effects on male Japanese quail, particularly in terms of genotoxicity, cytotoxicity, and oxidative stress. The observed morphological alterations in erythrocytes, such as micronuclei formation and changes in nucleus shape, highlight the potential damage caused by monomehypo. The increased levels of ROS and lipid peroxidation, coupled with decreased antioxidant enzyme activity, further support the induction of oxidative stress by monomehypo. Overall, this study contributes to our understanding of the harmful effects of monomehypo pesticides on avian health and underscores the importance of implementing sustainable and eco-friendly agricultural practices to mitigate the potential risks to bird populations.

Ethical Statement

All the experiments were conducted according to animal ethical principles. The University ethical committee approved the experimental layout.

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This current study was conducted without any external funding.

Competing Interest

The authors declare they have no relevant financial or non-financial interests to disclose.

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Data Availability

The data can be obtained from the corresponding author on a reasonable request.

Consent for publication

All the authors consented to publication.

Author Contribution

AK and AJ designed the study. HA, GA, and QUN experimented. SA, AA, and MIS wrote the manuscript.

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