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Research Article

Oxidative stress and toxicological impacts of Ethoxysulfuron exposure on bone marrow, and intestinal morphometry in male Japanese Quail

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ABSTRACT

Herbicides use in agriculture, particularly in developing countries, continues to pose a significant threat to avian populations. To investigate the potential harm caused by ethoxysulfuron herbicide, an experiment was conducted focusing on its effects on bone marrow, erythrocyte, and intestine 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 ethoxysulfuron @ doses of 07mg/kg BW, 09mg/kg BW, and 12.5mg/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 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 in all exposed groups. The results of CAT and SOD activity also exhibited a significant decrease in erythrocytes, whereas the content of LPO increased significantly in erythrocytes of all treated groups of Japanese quail. This study also analyzed morphological changes in the small intestine, revealing a decrease in villous height and crypt depth in the duodenum, jejunum, and ileum. These alterations were observed across all experimental groups and intensified with both higher doses and longer exposure. The results indicated that exposure to ethoxysulfuron 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 herbicides 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 herbicides (Osman et al. 2023). Approximately two million tons of 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, and malathion, makeup 70% of the overall pesticide use (Rajak et al. 2023). Toxic herbicides are increasingly targeting a wide range of organisms. Fruits and vegetable crops alone consume 27% of the total herbicide consumption (Umapathi et al. 2022). 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 herbicides in ecosystems has detrimental effects, which vary depending on the concentration, quantity, and duration of exposure to the contaminants (Voltz et al. 2022). Herbicides 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). Ethoxysulfuron, a systemic herbicide with contact and stomach action, disrupts nerve transmission in insects and binds to receptors in the nervous system. It belongs to the sulfonylurea group, a class of selective herbicides used to control broadleaf weeds and grasses in crops. These herbicides work by inhibiting the activity of acetolactate synthase (ALS) which is essential for the synthesis of certain amino acids. When ALS is inhibited, plants cannot produce amino acids, which are important for growth and protein synthesis. Herbicides induce oxidative stress by increasing the production of reactive oxygen species (ROS) (Suliman et al. 2020), leading to endoplasmic reticulum interference and mitochondrial dysfunction (Liu et al. 2022). These chemicals also impair leukocyte development and interfere with the function of immune cells (Hussain et al. 2012; Li et al. 2022). Experimental evidence has shown that sulfonylurea herbicides 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. Ethoxysulfuron was administered orally to groups B, C, and D at doses of 120 µg/kg BW, 160 µg/kg BW, and 200 µg/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 smear formation of bone marrow

Blood sample (5 ml) was collected from the jugular vein of each quail on day 15th, 30th, and 45th of the trail to determine the oxidative stress and antioxidative enzyme status in erythrocytes. All the bone marrow cells were immediately collected, formed thin smears, and stained with Giemsa solution for 5 to 10 secs. All the measurements were carried out with a computer-assisted light microscope (Cimrin et al. 2023).

Intestinal morphometry

Three birds from each group were decapitated on the 15th, 30^{th,} and 45th day of the experiment. Specimens from the duodenum, jejunum, and ileum were obtained for morphological measurements. Villus height and crypt depth were evaluated in all collected samples under blind conditions (Bancroft and Gamble 2008).

Oxidative and antioxidant study in bone marrow and erythrocytes

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 and erythrocytes 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

Photomicrograph of bone marrow cells

The photomicrograph of bone marrow cells of male Japanese quail is presented in (Fig. 1). The image revealed a representative section of bone marrow. The bone marrow cells appeared well preserved and exhibited characteristics consistent with normalcy. The bone marrow demonstrated a suitable density of cells, which indicates active hematopoiesis. Nuclei within the cells appeared intact and there was no evidence of nuclear fragmentation or other abnormalities. The cells exhibited uniform staining, which is an indication of good tissue processing. Cells were well dispersed and did not clump, that is an indication of proper thin smear formation. Notably, there were no signs of cellular damage, or degeneration in the observed bone marrow cells even when exposed to different concentrations of ethoxysulfuron. At the microscopic level, the herbicide exposure did not appear to cause evident structural and pathological changes in the bone marrow.

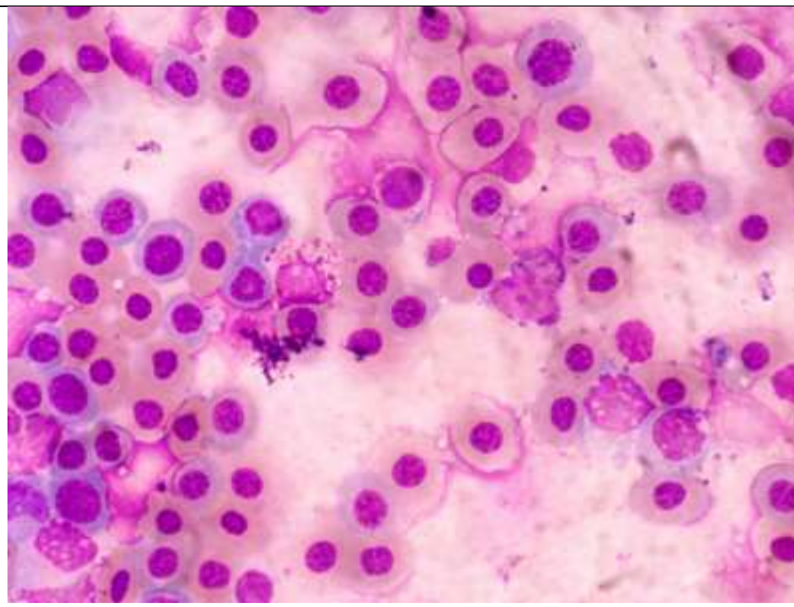

Fig. 1: Photomicrograph of thin smear showing normal bone marrow cells of male Japanese quail exposed to different concentrations of ethoxysulfuron

Intestinal morphometry

The morphological changes in the small intestine of Japanese quail are shown (Fig. 2). In the duodenum, a significant decrease in villous height was observed when quail were exposed to 9 mg/kg and 12.5 mg/kg of ethoxysulfuron as compared to the control group. A significant decrease in crypt depth of the duodenum was also noted on the 30th and 45th days of experimental work. Similarly, in the jejunum, a significant decrease in crypt depth and villous height was examined on 30th and 45th day after exposure of different concentrations of ethoxysulfuron.

Likewise, in the ileum section of the small intestine of Japanese quail, significant reductions in villous height and crypt depth were evident when the quail were exposed to 9 mg/kg and 12.5 mg/kg of ethoxysulfuron. These findings indicate that a higher concentration of ethoxysulfuron had a pronounced negative impact on the villous height and crypt depth of the small intestine in Japanese quail, implying potential adverse effects on their intestinal structure.

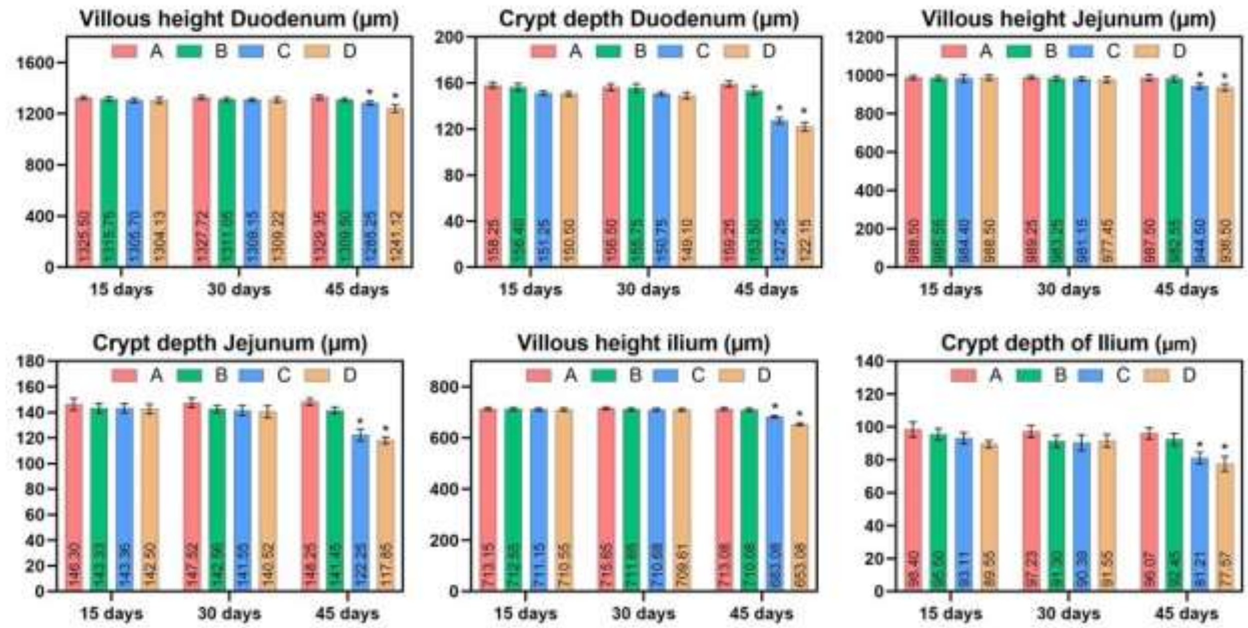

Fig. 2: Villous height and crypt depth of intestine (duodenum, jejunum and ileum) in male Japanese quail exposed to various concentrations of ethoxysulfuron

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. 3). The results revealed that ethoxysulfuron exposure had a slightly significant effect on ROS activity after 30 days of treatment, with group D exhibiting a significant increase compared to the control group. This suggests that ethoxysulfuron 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 ethoxysulfuron exposure, with group D displaying a significant increase compared to the control group. This indicates that ethoxysulfuron 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 ethoxysulfuron exposure, with group D exhibiting a significant decrease compared to the control group. These findings suggest that ethoxysulfuron 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 ethoxysulfuron exposure in the control groups. Similar results of a significant decrease in CAT and SOD activity in the erythrocytes were observed in male Japanese quail while there was a significant increase in the quantity of lipid peroxidation was observed after exposure to different doses of ethoxysulfuron at days 15th, 30th and 45th of the trial (Fig. 4). However, a significant decrease in activity was observed in all the groups exposed to ethoxysulfuron, indicating that ethoxysulfuron may impact the activity of antioxidant enzymes in male Japanese quail.

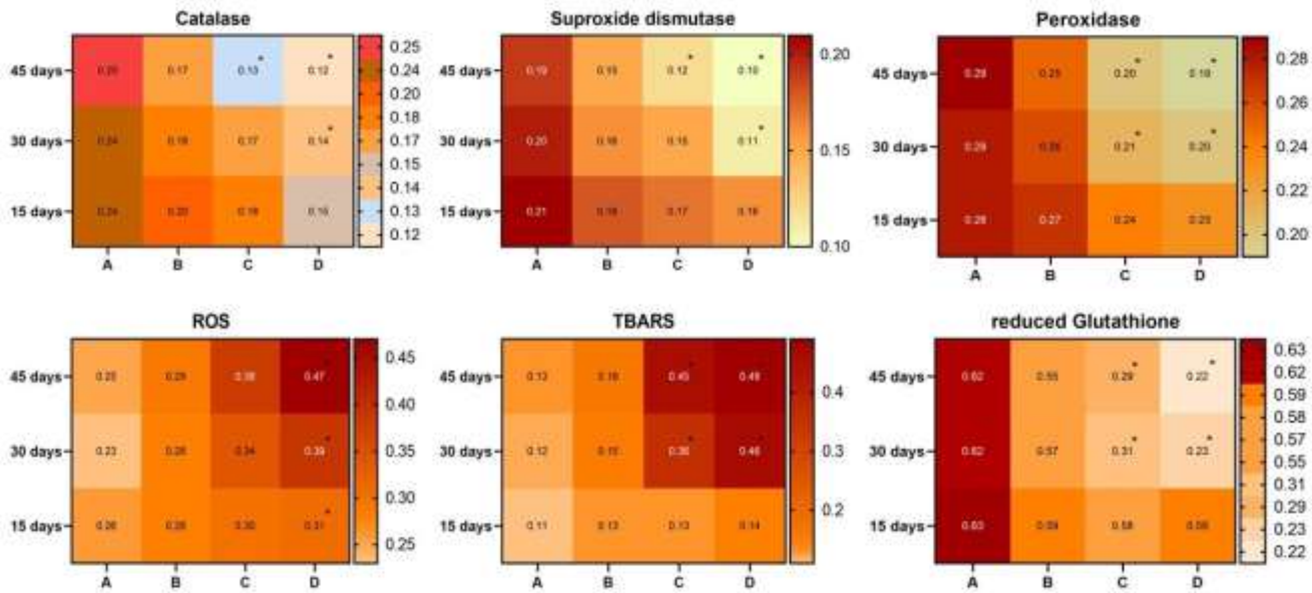

Fig. 3: Oxidative and antioxidant status in bone marrow of male Japanese quail exposed to various concentration on Ethoxysulfuron

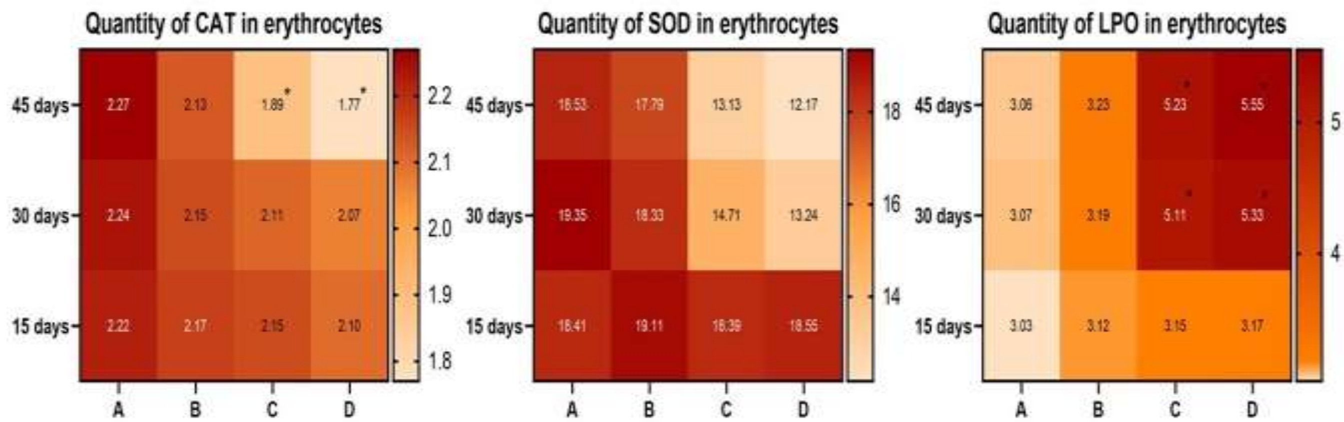

Fig. 4: Oxidative and antioxidant status in erythrocytes of male Japanese quail exposed to various concentration on Ethoxysulfuron

Discussion

The herbicide ethoxysulfuron, also known as 3-(4, 6-dimethoxyoyrimidin-2-yl)-1-(2ethoxyphenoxysulfonyl) urea, is a member of the sulfonylurea group and is employed as a selective herbicide**.** The phenyl ring of ethoxysulfuron has an ortho-ethoxy group, while the urea bridge has an additional oxygen atom. Ethoxysulfuron is used to manage the majority of annual and perennial wide vegetation and sedges in paddy and turf because it is very active at low application doses (Kocaman and Topaktaş 2010; Hussain et al. 2015; Suljevic et al. 2019).

In the current experimental work, ethoxysulfuron was proven to decrease the villous height and crypt depth of the small intestine thus impairing the absorption of nutrients. These findings are consistent with previous research that has demonstrated the adverse effects of pesticides in intestinal morphology (Crisol-Martínez et al. 2016; Qiu et al. 2020). In another study, it was reported that microorganisms were responsible for decrease in villous height and crypt depth in all parts (duodenum, jejunum, and ileum) of the small intestine in Japanese quail (Gesek and Lambert 2018). In contrast to our results, Li et al. (2023) reported that melittin (amphipathic peptide) increased the villous height for better absorption of nutrients in the intestine of quail. Villous height is an important indicator of absorptive surface area in the intestine, a decrease in villous height potentially leading to malnutrition (Rezaiei et al. 2018). Crypts are responsible for the formation of new epithelial cells in the intestine and a decrease in their depth may lead to disturbance in the repair process of the intestine, rendering the birds more susceptible to inflammation and intestinal injury (Manafi et al. 2016). It was noted that observed effects in the current study were dose-dependent, indicating that higher concentrations of ethoxysulfuron had more pronounced impacts on the morphology of the intestine in male Japanese quail. Our study also revealed decreased levels of antioxidant enzymes including reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and increased in 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). A significant decrease in antioxidant enzymes CAT and SOD and an increase in lipid peroxidation (LPO) was also observed in erythrocytes of quail exposed to various concentrations of ethoxysulfuron. It could be due to herbicide's potential to induce toxicity and damage to cellular components (Ruuskanen et al. 2020). Decreased catalase activity may result in reduced protection against hydrogen peroxide-induced oxidative stress. Ethoxysulfuron may disrupt the balance between oxidative and antioxidant defense systems.

These results suggest that exposure to ethoxysulfuron can induce oxidative stress by altering the antioxidant enzyme status in the bone marrow and erythrocytes and causing morphological changes in the small intestine in the male Japanese quail.

Conclusion

This current study demonstrates that ethoxysulfuron pesticide exposure has detrimental effects on male Japanese quail, particularly in terms of genotoxicity, cytotoxicity, and oxidative stress. The observed morphological alterations in the small intestine, such as decreased in villous height and decrease in crypt depth of duodenum, jejunum and ileum caused by ethoxysulfuron. The increased levels of ROS and lipid peroxidation, coupled with decreased antioxidant enzyme activity, further support the induction of oxidative stress by ethoxysulfuron. Overall, this study contributes to our understanding of the harmful effects of ethoxysulfuron pesticide 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

The current trial was executed according to the guidelines of bioethical committee of Islamia University of Bahawalpur regarding the welfare and use of laboratory animals.

Availability of data and material

The corresponding author is responsible for the availability of data for this experiment.

Acknowledgment

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Consent to participate

All the authors equally participated in this study.

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Competing Interest

Authors have no competing or financial interests.

Author Contribution

SS, JA, and AJ designed the study. SS, HA, and GA executed the research JA, GA, QUN and SA wrote the manuscript. AJ and JA revised the manuscript.

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