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Toxicological Effects of Glyphosate on Hematological and Genotoxic Parameters in *Coturnix japonica* (Japanese Quail)

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ABSTRACT

Significant ecological and environmental issues have emerged due to the increased use of agrochemicals to meet the food demands of a growing population. In the agricultural industry, glyphosate is widely applied to various food crops. However, prolonged exposure to this chemical has been associated with serious endocrine-disrupting effects in animals, along with potential health implications for humans. This study aimed to evaluate the hematological and genotoxic effects of glyphosate in Japanese quail (*Coturnix japonica*). A total of 18 adult quails were obtained and randomly divided into four groups (A–D). Glyphosate was administered orally for 21 days. Group A served as the control, while groups B, C, and D received glyphosate at doses of 334.0 mg/kg, 33.40 mg/kg, and 3.340 mg/kg body weight, respectively. The results revealed significant hematological abnormalities, including anemia, thrombocytopenia, and oxidative stress. Red blood cell (RBC) count and hemoglobin (Hb) levels showed a marked decline over time in the higher exposure groups. These findings corroborate earlier studies indicating glyphosate's toxic effects across various species and underscore the potential ecological risks posed by its widespread use.

INTRODUCTION

The rising global population has necessitated an increase in food production, leading to the widespread use of agrochemicals in modern agriculture. Among these, pesticides have seen a particularly sharp rise in use, paralleling the expansion of conventional and industrial farming practices [1]. Over the past five decades, the influx of synthetic chemicals—especially pesticides into the environment has accelerated at a pace surpassing that of other major drivers of global environmental change, including greenhouse gas emissions [2]. This trend raises significant ecological and public health concerns, particularly regarding the long-term impact of these substances on both ecosystems and human well-being.

The global production of formulated pesticides increased significantly from 2.5 million metric tons (MT) in 2005 to 4.8 million MT in 2019. This total includes various categories such as insecticides, fungicides, bactericides, herbicides, and nematicides [3].

Birds can be exposed to pesticides through ingestion of contaminated food or water, dermal contact, or inhalation of airborne particles. Their foraging behavior often leads them into pesticide-treated areas, making them particularly susceptible to exposure [4]. As integral components of functional ecosystems, birds play a key ecological role and are widely regarded as effective bioindicators of environmental health and ecosystem integrity [5].

Pesticides can exert harmful effects on animals by inhibiting key enzymes and disrupting endocrine functions [6]. Many organic and inorganic pesticides persist in the environment for extended periods, posing long-term risks to human health, wildlife, and ecosystems, including avian species [7, 8].

Glyphosate, a widely used broad-spectrum herbicide, is commonly applied to control annual grasses and weeds across a variety of crop systems [9]. In mammals, including humans, glyphosate has been associated with cytotoxic and genotoxic effects [10].

The Japanese quail (*Coturnix japonica*) is a semi-migratory bird belonging to the Phasianidae family and Galliformes order [11]. Their diet is diverse, consisting primarily of weed seeds (90%), cereal grains (18%), and insects (8%), along with fruits, legumes, and grasses [12]. Quails play a

vital ecological role by contributing to pollination, seed dispersal, and environmental restoration[13].

Glyphosate, the active ingredient in glyphosate-based herbicides such as Roundup®, has been the most widely used herbicide since its commercial release in 1974 [14, 15]. It is a highly potent, broad-spectrum herbicide [16], with a variable half-life ranging from 12 days to several months depending on environmental conditions [17]. In aquatic environments, glyphosate disintegrates relatively quickly through adsorption to suspended and bottom particles. While certain glyphosate formulations like Rodeo® are approved for aquatic use, others contain surfactants that are highly toxic to aquatic organisms and are therefore restricted [18].

Historically, glyphosate has been considered relatively safe, with 99% of glyphosate residues in food falling below the maximum residue limits (MRLs) set by the European Union and the U.S. Environmental Protection Agency [19]. However, growing evidence now points to its potentially harmful effects on humans and other vertebrates [20] [21]. For example, Roundup® exposure (10 ppm) in laying hens has been shown to reduce hatchability and induce oxidative stress and lipid peroxidation in chicks [22]. Moreover, pesticides, including glyphosate, have been found to negatively affect immune function in poultry, leading to reduced white blood cell counts, lower T- and B-cell numbers, and decreased spleen and thymus weights [23].

Erythrocytes are considered key biomarkers of oxidative stress due to their sensitivity to physical and chemical changes caused by various pesticide classes [24]. Despite earlier beliefs that glyphosate has minimal impact on liver histology or blood biochemistry [25], emerging studies suggest otherwise. In light of this, the current study aimed to conduct a comprehensive evaluation of the effects of varying levels of prolonged glyphosate exposure on the genotoxic and hematological parameters in Japanese quail.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 24). All data were expressed as mean ± standard deviation (SD). The independent sample t-test was used to determine significant differences between the control and treatment groups. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

MATERIALS AND METHODS

Chemicals

Glyphosate was obtained from a local agrochemical market in Peshawar.

Experimental Design

Ethical approval for the experiment was granted by the Advanced Studies & Research Board (ASRB) and the Ethical Research Board of the University of Peshawar.

A total of 18 healthy, sexually mature Japanese quails were procured from Nadir Khalil (NK) Quail Farm, located at Bara Gate near Peshawar Cantt. The birds were housed in stainless steel cages and acclimatized for two weeks under controlled environmental conditions before beginning the experimental trial. Throughout the study, the quails were maintained under standardized housing conditions, with a consistent light-dark cycle, ambient temperature, and unrestricted access to clean drinking water and a standard poultry diet (National Feed SP 14).

Following acclimatization, the birds were randomly assigned to four groups (A–D), each containing 4–5 quails. The treatment groups were exposed to oral doses of glyphosate for 21 consecutive days. Group A served as the control and received no glyphosate. Groups B, C, and D received sub-lethal doses of glyphosate at 334.0 mg/kg, 33.40 mg/kg, and 3.340 mg/kg body weight, respectively. These doses were calculated based on the LD_{50} of glyphosate (LD_{50} = 3340 mg/kg), determined using the Probit Analysis method [26]. The 21-day experimental period was divided into three treatment intervals: Day 7, Day 14, and Day 21.

Note: Birds were closely monitored throughout the trial for any signs of abnormal physical or behavioral changes.

Experimental Procedure

Hematological Analysis

At the end of each treatment interval (Day 7, Day 14, and Day 21), blood samples were collected from all groups. Sampling was conducted via the jugular vein on the right side of the neck, a safe and non-lethal procedure, using 3 mL disposable syringes fitted with 23G-1 TW needles (0.6 × 25 mm). The collected blood was immediately transferred into EDTA-coated tubes (light blue, ATLAS-LABOVAC Italiano, K2EDTA).

Micronucleus Assay

Micronucleus assays were performed within 4 hours of sample collection, while hematological parameters were analyzed within 24 hours. The following parameters were assessed using an automated hematology analyzer: total erythrocyte count (RBC), total leukocyte count (WBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count.

Micronucleus (MN) formation in red blood cells was assessed to evaluate the genotoxic effects of glyphosate, following the protocol described by Yin [27]. For each bird, a thin blood smear was prepared on a clean glass slide and air-dried at room temperature. The slides were then stained using 10% Giemsa solution for 45–50 minutes. After staining, the slides were gently rinsed with tap water or phosphate-buffered saline (PBS), air-dried, and examined under a light microscope (Olympus DP71, U-CMAD3, Japan) using an oil immersion lens (100×/1.25).

Micronuclei were identified in erythrocytes based on the morphological criteria defined by Fenech [28]. A total of 1,000 erythrocytes were randomly observed per slide to determine the presence of micronuclei in each sample.

The frequency of micronuclei was calculated using the following formula:

$$\text{MN frequency (\%)} = \frac{\text{Number of cells with MN}}{\text{Total number of counted cells}} X\ 100$$

RESULTS

Interpretation of Hematological Changes

Red blood cell (RBC) levels initially increased at Days 7 and 14 across all treatment groups, possibly reflecting a physiological compensatory response. However, by Day 21, RBC counts significantly decreased, particularly in Group B (1.53 ± 1.0) and Group D (1.59 ± 0.90), as shown

in Table 1. This sharp decline suggests the onset of anemia, which aligns with previous findings in mice treated with glyphosate at 500 mg/kg body weight, where reduced erythrocyte counts were reported [29].

Hemoglobin (Hb) concentrations also declined progressively across all exposed groups. The most notable reduction was observed in Group B by Day 21 (9.70 ± 2.55). While an initial increase in Hb at Day 7 (16.2 ± 3.54) could represent an adaptive mechanism to oxidative stress or transient hypoxia, the significant drop by Day 21 suggests impaired hemoglobin synthesis or enhanced RBC destruction, ultimately reducing oxygen-carrying capacity.

Hematocrit (HCT%) levels on Day 7 in all exposed groups were comparable to the control group, indicating no immediate hematological disruption from short-term glyphosate exposure. However, by Day 14, a sharp decline in HCT was observed across all treated groups, with the lowest in Group B (18.50 ± 11.60), suggesting the development of anemia, potentially due to hemolysis or suppressed erythropoiesis. A partial recovery in HCT by Day 21 (45.10 ± 15.80) may reflect compensatory erythropoietic activity.

Mean corpuscular volume (MCV) was elevated on Day 7 in all treatment groups—B (159.00 ± 17.16), C (150.00 ± 12.68), and D (153.00 ± 15.77)—compared to the control (146 ± 8.5), indicating macrocytosis. This may represent a compensatory response to oxidative damage or hemolysis. However, a decline in MCV by Day 21 (121.01 ± 13.23 in Group B) reflects microcytosis, which could result from iron deficiency or continued oxidative injury to developing erythrocytes.

Elevated mean corpuscular hemoglobin (MCH) values at Day 7 suggest the presence of fewer, larger, hemoglobin-rich RBCs—again, likely a compensatory response. By Day 14, MCH declined in some groups, notably in Group D (43.7 ± 7.07), indicating a transition toward microcytic anemia. A rebound increase by Day 21 may point to bone marrow adaptation, with increased hemoglobin loading in fewer cells to counteract chronic anemia. However, this rebound was not accompanied by normalization of RBC or Hb levels, indicating incomplete hematological recovery.

Mean corpuscular hemoglobin concentration (MCHC) showed elevated values at Day 7 in Group C, suggesting hyperchromic cells possibly due to membrane damage or dehydration. By Day 21, MCHC values further increased (52.6 ± 10.87 in Group B), which could signify hemoglobin over-concentration in damaged or fragile cells, potentially leading to hemolysis or ineffective oxygen delivery.

Platelet counts showed consistent and significant declines across all exposed groups throughout the study period. The sharpest drop was observed in Group B (2.45 ± 2.86 at Day 21). This progressive thrombocytopenia may be due to bone marrow suppression, toxic effects on megakaryocytes, or increased platelet destruction, and is indicative of hematological toxicity. Similar findings have been reported by Galli [30], linking glyphosate exposure to alterations in platelet dynamics and immune suppression.

Overall, these findings support the hypothesis that glyphosate, even at sub-lethal doses, induces significant hematological disturbances in Japanese quail. The observed patterns initial compensatory responses followed by progressive decline highlight both the acute and chronic toxic effects of glyphosate exposure.

Table 1. Hematological Parameters of Japanese Quail Exposed to Varying Doses of Glyphosate

Parameter / Day	Control (A)	Group B (334.0 mg/kg)	Group C (33.40 mg/kg)	Group D (3.340 mg/kg)
RBC (million/cmm)				
Day 7	2.9 ± 0.4	3.08 ± 0.90	2.00 ± 0.65	2.91 ± 0.70
Day 14	2.9 ± 0.4	3.10 ± 0.70	3.26 ± 0.75	3.32 ± 0.50
Day 21	2.9 ± 0.4	1.53 ± 1.00	1.90 ± 0.85	1.59 ± 0.90
Hb (g/dl)				
Day 7	14.8 ± 2.4	16.2 ± 3.54	14.10 ± 0.60	15.20 ± 2.70
Day 14	15.9 ± 2.7	15.40 ± 2.56	14.80 ± 1.60	14.50 ± 1.90
Day 21	16.8 ± 6.5	9.70 ± 2.55	11.70 ± 1.40	10.10 ± 1.60
HCT (%)				
Day 7	48.0 ± 9.0	47.10 ± 15.80	44.02 ± 10.50	43.90 ± 11.06
Day 14	49.0 ± 2.1	18.50 ± 11.60	23.70 ± 9.40	19.08 ± 14.06
Day 21	49.0 ± 9.2	45.10 ± 15.80	40.02 ± 10.50	39.90 ± 11.06
MCV (fl)				
Day 7	146 ± 8.5	159.00 ± 17.16	150.00 ± 12.68	153.00 ± 15.77
Day 14	147 ± 1.6	152.00 ± 20.17	136.00 ± 8.88	132.00 ± 10.59
Day 21	149 ± 8.7	121.01 ± 13.23	124.80 ± 13.62	124.00 ± 14.89
MCH (pg)				
Day 7	46.7 ± 1.3	55.0 ± 7.06	70.0 ± 9.53	55.0 ± 8.07
Day 14	47.7 ± 1.4	49.7 ± 7.07	45.4 ± 12.93	43.7 ± 7.07
Day 21	48.7 ± 2.5	63.7 ± 7.07	61.9 ± 10.55	63.8 ± 8.08
MCHC (g/dl)				
Day 7	32.2 ± 2.6	33.1 ± 14.37	47.0 ± 8.60	34.2 ± 7.29
Day 14	33.2 ± 1.7	32.7 ± 11.38	33.5 ± 8.67	33.00 ± 10.24

Parameter / Day	Control (A)	Group B (334.0 mg/kg)	Group C (33.40 mg/kg)	Group D (3.340 mg/kg)
Day 21	33.9 ± 2.8	52.6 ± 10.87	49.5 ± 8.85	51.3 ± 11.77
PLT ($\times 10^3/\mu L$)				
Day 7	13.0 ± 1.9	8.0 ± 1.96	4.5 ± 2.23	5.0 ± 2.13
Day 14	14.0 ± 2.10	4.0 ± 2.80	1.0 ± 0.94	2.0 ± 1.82
Day 21	13.0 ± 1.11	2.45 ± 2.86	3.8 ± 2.23	–

Genotoxic Effects of Glyphosate Exposure

The frequency of micronucleus formation and other nuclear abnormalities in erythrocytes increased significantly in glyphosate-exposed groups compared to the control group (Table 1). The control group showed minimal micronucleus frequency (0.2%) and negligible nuclear deformities, indicating no baseline genotoxic stress.

Micronucleus Frequency

A dose- and time-dependent increase in **micronucleus frequency** was observed in all treated groups. At the **highest dose (334.0 mg/kg)**, micronucleus frequency reached **3.8% by day 21**, compared to **0.2% in controls**, indicating clear genotoxic potential. Even at **lower doses (3.34 and 33.40 mg/kg)**, MN frequency increased significantly with prolonged exposure (up to **2.5%** at day 21 for 3.34 mg/kg). The double micronucleus frequency also showed a similar increasing trend, with the highest observed at **2.4%** (334.0 mg/kg, day 21).

Nuclear Abnormalities

Glyphosate exposure led to various **nuclear deformities**, including: **Deformed nucleus**, **nuclear shift**, and **lobed or irregular nuclei**, all of which progressively increased over time and dose. At the highest dose, **lobed nuclei** and **irregular nuclei** were markedly elevated by day 21 (1.8% and 1.5%, respectively), suggesting nuclear fragmentation and chromatin disruption.

Cellular Abnormalities

A consistent increase in **deformed cells, vacuolated cells, swollen cells, and microcytes** was observed across all glyphosate-treated groups. Notably, at **334.0 mg/kg**, **swollen cells** reached **3.1%** by day 21, and **microcytes** reached **1.9%**, pointing to cytoplasmic damage and oxidative stress effects on erythrocytes.

Table 2

Glyphosate (mg/Kg)	Treated Groups	Single Micronucleus Frequency %	Double Micronucleus Frequency %	Deformed nucleus %	Nuclear shift %	Lobed nucleus %	Irregular nucleus %	Deformed cells %	Microcytes %	Vacuolated Cells %	Swollen Cells %
	Control	0.2	0.0	0.2	0.3	0.0	0.0	3.5	0.2	0.1	0.5
Sublethal Dose (334.0)	7 day	1.5	1.4	1.7	1.8	0.6	0.8	5.5	0.8	1	1.1
	14 day	2.2	1.8	1.9	2	1	1.3	6.1	1.4	1.6	2
	21 day	3.8	2.4	2.7	2.8	1.8	1.5	7.3	1.9	2.1	3.1
Sublethal Dose (33.40)	7 day	1.1	0.5	0.4	0.6	1	0.4	3.7	0.7	0.6	1.1
	14 day	2.2	0.8	0.8	1	1	1	4.2	1.1	1.2	2.1
	21 day	2.4	1.3	1.2	1.4	1.3	1.5	4.9	1.8	1.7	2.6

Sublethal Dose (3.340)	7 day	1.2	0.7	0.5	1	1.1	0.5	4	0.8	0.7	1
	14 day	2.3	1	1	1.6	1.1	1.1	4.3	1	1	2
	21 day	2.5	1.6	1.6	1.5	1.2	1.7	5	2	1.6	3

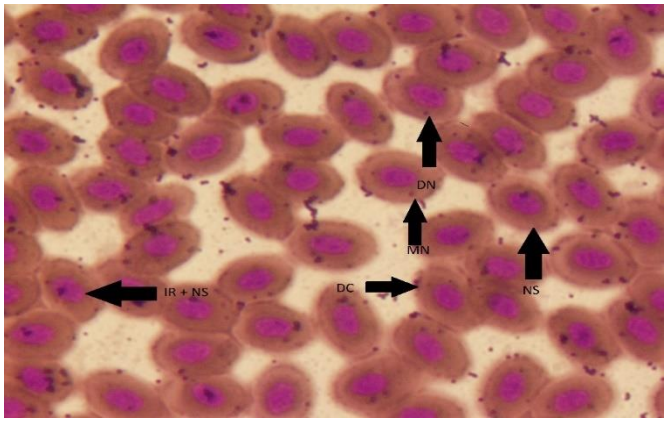
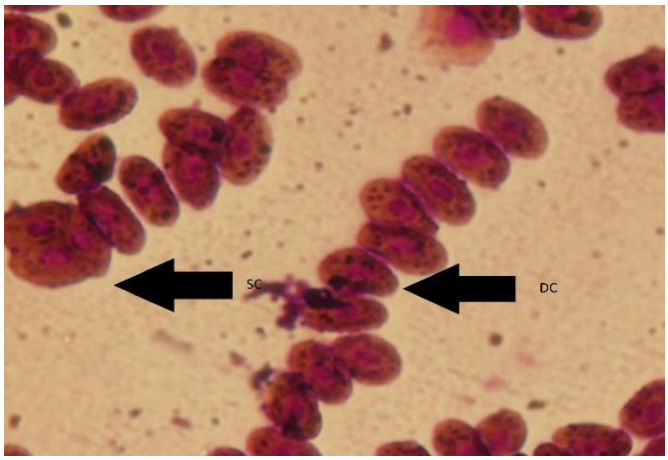


Fig 1.a. Blood smears of Coturnix Japonica exposed to Glyphosate showing micronucleus (MN), nucleus shift (NS), and Deformed Cell(DC)



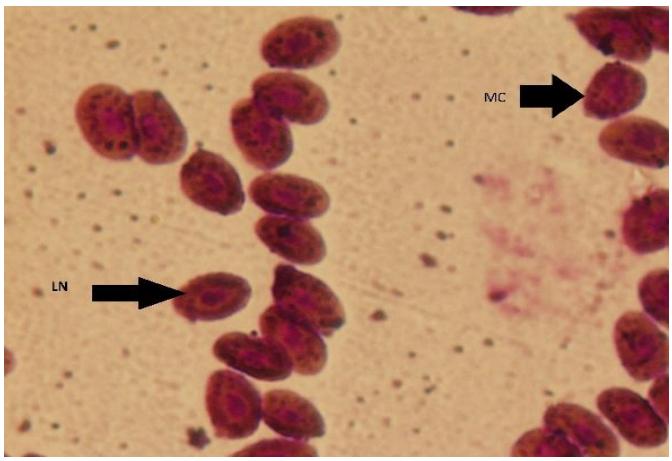


Fig 1 b. Swollen Cell (SC)and Deformed Cell (DC) Fig 1 c. Lobed Nucleus (LN), Micronucleus Cell(MC)

These genotoxic alterations are in line with previous findings [27] [28], indicating that even sub lethal doses of glyphosate can induce significant DNA and cellular damage in avian species.

DISCUSSION

Previous studies have suggested that glyphosate-containing formulations are associated with hepatic and oxidative stress-related changes[31, 32]. The present study aimed to evaluate the hematological and genotoxic effects of glyphosate in *Coturnix japonica* (Japanese quail). The primary effects observed included altered blood profiles, increased micronucleus formation, nuclear and cellular deformities, and abnormal behavioral responses, indicating systemic toxicity.

Consistent with earlier research, other studies have reported histopathological changes in the liver, thymus, and kidneys following glyphosate exposure. These include hepatocyte alterations, congestion of portal blood vessels, inflammatory infiltration, and sporadic necrosis of hepatocytes [33]. In the current investigation, significant cytotoxic and genotoxic effects were observed in erythrocytes, with the highest sublethal dose (334.0 mg/kg) inducing a 19-fold increase in micronucleus frequency by day 21. This indicates extensive DNA fragmentation and chromosomal damage.

Additionally, nuclear abnormalities—such as lobed and irregular nuclei—and the frequency of deformed cells increased progressively in a dose- and time-dependent manner. These alterations are likely the result of impaired mitotic processes, oxidative damage, and compromised membrane integrity.

Crucially, even the lowest tested dose (3.340 mg/kg) produced statistically significant genotoxic effects after 21 days of exposure. This finding suggests that glyphosate residues, even at low environmental concentrations, may pose long-term risks to avian species, especially ground-feeding or seed-eating birds frequently exposed to contaminated habitats.

Our results align with similar findings in other animal models. Studies have shown glyphosate-induced genotoxicity in rats [34], fish [35], and birds including quail [36]. Likewise, the observed increase in micronucleus frequency correlates with the findings of Campos-Ventura [37], emphasizing the broad genotoxic potential of glyphosate across taxa.

CONCLUSION

This study demonstrates that sublethal exposure to glyphosate causes significant hematological and genotoxic alterations in Japanese quail. The herbicide induces anemia, oxidative stress, membrane damage, and DNA fragmentation—highlighting its cytotoxic and immunosuppressive potential even at low doses.

These findings underscore the vulnerability of birds to agricultural chemicals and call for stricter pesticide regulations, improved ecological risk assessments, and long-term wildlife biomonitoring programs. Protecting avian health is essential not only for species conservation

but also for preserving the ecological balance of terrestrial and agro-ecosystems.

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