**Glyphosate - Induced changes in hematological indices of male and female Wistar Rats**

 **Abstract**

Glyphosate, a broad-spectrum systemic herbicide, is extensively utilized in agricultural and non-agricultural settings for weed management, owing to its cost-effectiveness and potent efficacy. Recent concerns have emerged regarding its adverse impacts on non-target mammalian species, including haematological alterations indicative of systemic toxicity, immunosuppression, and oxidative stress. This study investigated sex-specific and dose-dependent hematotoxin and behavioural effects of glyphosate-based herbicide (GBH) in Wistar rats. A total number of 120 rats (60 males, 60 females) was orally administered GBH at concentrations ranging from 0.1 to 0.9 mg/kg over 28 days. The 120 Wistar rats (60 male and 60 female) were divided randomly among twelve plastic cages, with ten rats per cage and six cages per sex. The rats were also divided further into six treatment groups per sex (Groups A–F), with ten animals per group. Experimental treatment was the daily oral intake of a locally used herbicide (Force Up) based on glyphosate with different dosages for 28 subsequent days. Groups A to E were specifically treated with 0.1, 0.3, 0.5, 0.7, and 0.9 dosages of diluted glyphosate, respectively, while Group F was the control and was treated with normal saline. All the treatments were given once daily via a cannula in order to investigate the dose-dependent impacts of glyphosate exposure in both male and female rats. Haematological analysis evaluated parameters including erythrocyte (RBC), leukocyte (WBC), haemoglobin (Hb), haematocrit (HCT), platelet counts, and erythrocyte indices (MCV, MCH, MCHC). Treated groups exhibited significant reductions in RBC, WBC, Hb, HCT, and platelets, suggesting anaemia, immunosuppression, and potential bone marrow suppression. Elevated MCV and MCH values indicated incipient macrocytic anaemia. Behavioural assays corroborated systemic toxicity, manifesting as lethargy, reduced motor activity, reproductive dysfunction, and mortality at higher doses. Notably, sexually dimorphic responses were observed: females demonstrated heightened reproductive anomalies and alopecic manifestations, while males exhibited pronounced testicular degeneration. These findings underscore GBH’s capacity to induce sex-specific toxicodynamic profiles. The study provides critical evidence of glyphosate’s hematotoxic potential and underscores public health concerns associated with chronic low-dose exposure. It advocates for gender-sensitive toxicological risk assessments and stringent regulatory oversight of herbicide applications.

Keywords: Glyphosate, Hematotoxicity, Herbicide toxicity, Sex differences, Dose-response, Behavioural toxicity.

**1.Introduction**

N-(phosphonomethyl)glycine is the chemical name of glyphosate, a systemic broad-spectrum herbicide commonly used in agricultural and non-agricultural environments for weed control. Since its commercialization in the 1970s with the brand name Roundup, glyphosate has become one of the most widely used herbicides worldwide due to its potency and affordability (Benbrook, 2016). Concerns of its potential negatives on non-target species such as mammals have recently increased. The interest of toxicological academia has more and more turned towards the examination of glyphosate-induced physiological and hematological changes in experimental species such as Wistar rats.Hematological parameters—including RBC count, WBC count, hemoglobin level, hematocrit value, and platelet count—are pivotal biomarkers of the body's physiological and pathological status. Changes in these factors might indicate systemic toxicity, immunosuppression, or oxidative stress (Rezaei *et al*., 2022). Some earlier works have shown that glyphosate treatment might cause oxidative injury, immunomodulation, and hematopoietic disruption (Cattani *et al*., 2014; Nerozzi *et al*,.2021). However, contradictions persist in the literature, specifically concerning sex differences in response to glyphosate toxicity. Sex differences in toxicology responses have long been well-documented

and realized in males and females with differences in susceptibilities owing to hormonal regulation, metabolic rate, and immune response (de Araújo-Ramos *et al*.,2021). Thus, analysis of glyphosate-induced alterations in the hematological parameters of both males and females of Wistar rats gives a better insight into its toxicodynamic action. These sex-related analyses are necessary for the proper toxicological risk assessments and for developing evidence-informed public health policies on the issues of herbicide exposure. The current investigation seeks to explore the hematological changes caused in Wistar male and female rats by glyphosate exposure and gain an insight into the potential systemic toxic consequences and the sex-linked vulnerabilities. The outcomes of the current work would add value to the existing debate regarding the safety of glyphosate and its significance in the context of occupational and environmental health.

1. **Materials and Methods**

**2.1 Laboratory Analysis**

One hundred and twenty (120) healthy albino rats (Wistar rat), sixty (60) males and sixty (60) females of the age 15-16 weeks and weight of 120-220 g, were obtained from the Department of Anatomy Animal house of Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The rats were kept in polycarbonate (plastic) cages of size 32 X 40 X 18 cm under controlled temperature of (26 ± 2°C), with a 12-hour light and dark cycle. During the experiment, the rats had free access to water and they received daily feeding of a commercial pellet diet procured from a reputable local company. The diet supplies the necessary nutrients (proteins, fats, carbohydrates, calcium, and phosphorus) required by breeding animals. The rats acclimatization lasted for four weeks within the animal house of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State of Nigeria before they were used for experimentation. Handling of the rats' procedure took place in strict accordance with the guidelines and welfare for the protection of animals endorsed by the World Health Organization.

**2.2 Experimental Design and grouping**

Following four weeks of acclimation, 120 Wistar rats consisting of 60 males and 60 females were randomly distributed into twelve (12) plastic cages with ten (10) rats per cage. Six (6) of the cages contained the males and the remaining six (6) cages the females. Each sex was subdivided into six groups as Group A, B, C, D, E, and F for males and as Group A, B, C, D, E, and F for females. Ten (10) rats comprised each group. Experimental treatment consisted of once-daily oral administration of a range of concentrations of a locally recognized glyphosate-containing herbicide (dilute Force Up) that is used extensively by farmers within the study area. A cannula was used for the administration once a day for 28 days.

The treatment for each group was as follows:

Group A (male and female): Received 0.1 concentration of diluted glyphosate

Group B (male and female): Received 0.3 concentration of diluted glyphosate

Group C (male and female): Received 0.5 concentration of diluted glyphosate

Group D (male and female): Received 0.7 concentration of diluted glyphosate

Group E (male and female): Received 0.9 concentration of diluted glyphosate

Group F (male and female – Control Group): Received normal saline only

This grouping allowed for the assessment of dose-dependent effects of glyphosate exposure in both male and female rats.

**2.3 Behavioural and Toxicological Symptoms**

Physical parameters of general behaviour were monitored daily. Activeness, agility, movement, staggering, decrease and loss of appetite for food, and cowering in front of the concentrates were observed and noted. The control and experimental rats both were monitored throughout the experiment period for the onset of toxicological symptoms. Degree, nature, and the time of appearance of the toxic symptoms were noted in an orderly manner, which involved a change in skin color, eyeball, blindness, static movement, and the rate of mortality.

**2.4 Sample Collection**

In accordance with the approved glyphosate exposure protocol, the rats were anesthetized with ketamine according to the preset experimental endpoints. Blood samples were obtained by terminal cardiac puncture with heparinized syringes in an effort to prevent coagulation. Terminal sampling according to AVMA (2020) guidelines involved the collection of blood using EDTA-coated vacutainers (BD Biosciences) in an attempt to prevent clotting artifacts. Plasma separation involved 3000 × g for 15 minutes of 4°C in a refrigerated centrifuge (Eppendorf 5430R) with the care taken not to contaminate the plasma layer with leukocytes (National Research Council, 2021). The supernatant blood was handled with care as it was drawn into pre-labeled microcentrifuge tubes. It was visually examined for hemolysis with rejection of samples containing >0.5 g/dL hemoglobin and stored at -80°C prior to analysis within 2 weeks of collection.

**2.5 Hematological Analysis**

The following hematological parameters were evaluated by an automated hematology analyzer:

* White Blood Cell (WBC) count: To assess for immune response and possible inflammation.
* Red blood cell (RBC) count: To determine oxygen-carrying capacity.
* Hemoglobin (Hb) concentration: To determine the oxygen-carrying capacity of blood.
* Hematocrit (HCT) value: To approximate blood composition as a proportion of red blood cells.
* Platelet (PLT) count: To evaluate possible influences on blood clotting function.
* Mean Corpuscular Volume (MCV): To measure the average red blood cell size.
* Mean Corpuscular Hemoglobin (MCH): To find the average per red cell of the amount of hemoglobin.
* Mean Corpuscular Hemoglobin Concentration (MCHC): To determine the average hemoglobin concentration of red blood cells.
* We chose these parameters in an effort to completely evaluate the effect of glyphosate on systemic blood and hematopoiesis.
	1. **Statistical Analysis**

Data were presented as Mean ± Standard Error of Mean (SEM). Statistical comparisons among the groups were conducted with the One-Way Analysis of Variance (ANOVA), with subsequent posthoc analysis with Duncan’s Multiple Range Test. Statistical significance was assumed when p = 0.05. Superscripts that differ between groups indicate that the groups differ significantly from one another; a shared superscript shows that there is no significant difference.

**3. RESULTS**

**Effect of Glyphosate-Based Herbicide on Haematological Parameters**

Analysis of the haematology indicated considerable changes in a number of blood parameters among the groups of animals treated with glyphosate-containing herbicide, reflecting possible haematotoxicity.



**Figure 1: White blood cell (WBC) levels of the Various Experimental Groups exposed to Glyphosate-based Herbicide.**

Values are expressed as Mean ± SEM (n=5) using One-Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test for multiple comparison. **a, b, c, d** represents significance differences (P<0.05) across the groups. Means with the same superscript are not significantly different (p>0.05)



**Figure 2: Red Blood Cell (RBC) levels of the experimental groups exposed to glyphosate-based herbicide**

Values are expressed as Mean ± SEM (n=5) using One-Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test for multiple comparison. **a, b, c, d** represents significance differences (P<0.05) across the groups. Means with the same superscript are not significantly different (p>0.05).



**Figure 3: Hemoglobin (HB) level of the experimental groups exposed to glyphosate-based herbicide.**

Values are expressed as Mean ± SEM (n=5) using One-Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test for multiple comparison. **a, b, c, d** represents significance differences (P<0.05) across the groups. Means with the same superscript are not significantly different (p>0.05).



**Figure 4:** **Haematocrit Level of the Experimental Groups exposed to Glyphosate-based Herbicide.**

Values are expressed as Mean ± SEM (n=5) using One-Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test for multiple comparison. **a, b, c, d** represents significance differences (P<0.05) across the groups. Means with the same superscript are not significantly different (p>0.05).



**Figure 5: Mean Corpuscular Volume (MCV) Level of the Experimental Groups exposed to Glyphosate-based Herbicide.**

Values are expressed as Mean ± SEM (n=5) using One-Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test for multiple comparison. **a, b, c, d** represents significance differences (P<0.05) across the groups. Means with the same superscript are not significantly different (p>0.05).



**Figure 6: Mean Corpuscular Haemoglobin (MCH) Level of the Experimental Groups exposed to Glyphosate-based Herbicide.**

Values are expressed as Mean ± SEM (n=5) using One-Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test for multiple comparison. **a, b, c, d** represents significance differences (P<0.05) across the groups. Means with the same superscript are not significantly different (p>0.05).



**Figure 7: Mean Corpuscular Haemoglobin Concentration (MCHC) Level of the Experimental Groups exposed to Glyphosate-based Herbicide.**

Values are expressed as Mean ± SEM (n=5) using One-Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test for multiple comparison. **a, b, c, d** represents significance differences (P<0.05) across the groups. Means with the same superscript are not significantly different (p>0.05).



**Figure 8: Platelets Level of the Experimental Groups exposed to Glyphosate-based Herbicide.**

Values are expressed as Mean ± SEM (n=5) using One-Way Analysis of Variance (ANOVA) and Duncan Multiple Range Test for multiple comparison. **a, b, c, d** represents statistical significance differences (P<0.05) across the groups. Means with the same superscript are not significantly different (p>0.05).

**3.1 White Blood Cell (WBC) Count**

As shown in Figure 1, WBC levels across the treatment groups differed drastically (p<0.05). The control group had the highest WBC value, while the groups receiving escalating doses of the glyphosate demonstrated a dose-response reduction. This is an indicator of a potential immunosuppressing influence of glyphosate-based glyphosate exposure that is in agreement with earlier findings concerning herbicide-induced leukopenia (Wang *et al*.,2020)

**3.2 Red Blood Cell (RBC) Count**

Figure 2 shows a remarkable decrease in the RBC count in the treatment groups relative to the control (p<0.05). The group with the most glyphosate revealed the lowest RBC count among all the groups, suggesting that the herbicide might interfere with erythropoiesis or enhance destruction of the red blood cells (Peillex *et al*.,2020)

**3.3 Concentration of Haemoglobin**

As shown in Figure 3, haemoglobin levels also decreased significantly in the exposed groups when compared with the control (p<0.05). The decrease increased with the highest concentrations, further corroborating the implication of glyphosate-induced anaemia from previous similar toxicological studies (Cattani *et al*., 2014).

**3.4 Haematocrit (PCV) Values**

The trend observed in Figure 4 mimicked that of haemoglobin and RBC. The haematocrit levels in all treatment groups were lower compared to the control (p<0.05), which further confirms the incidence of anaemic conditions as a result of glyphosate toxicity (Smith *et al*., 2021).

**3.5 Mean Corpuscular Volume (MCV)**

As shown in Figure 5, MCVs rose sharply in the exposed populations (p<0.05), particularly with greater doses of the herbicide. This might imply a transition towards macrocytic anaemia caused by disrupted DNA synthesis or aberrant red cell maturation, consistent with postulated mechanisms of haematotoxicity caused by toxicants (El-Sawy *et al*.,2023)

**3.6 Mean corpuscular haemoglobin (MCH)**

Figure 6 illustrates that the MCH levels in the treatment groups were significantly higher when compared with the control (p<0.05). This is consistent with the MCV increase that validates the development of macrocytic red cells due to glyphosate toxicity (Gomes *et al*., 2013).

**3.7 Mean Corpuscular Haemoglobin Concentration (MCHC)**

In Fig. 7 MCHC levels were found to be slightly elevated though the difference was statistically significant (p<0.05) in certain treatment groups. This increase is a possible compensatory response or changed morphology of the erythrocytes (Zidan *et al*., 2013).

**3.8 Platelet count**

Figure 8 shows a clear decrease in the platelet count in all the groups exposed to the herbicide when compared with the control (p<0.05). The decrease indicates possible suppression of the bone marrow or enhanced platelet destruction due to glyphosate as corroborated in previous toxicology tests (Mesnage *et al*., 2015).

**Behavioral Effects of Glyphosate Exposure in Wistar Rats**

Table 1: Physical Effects in Male Rats

|  |  |
| --- | --- |
| Dose (mg/kg) | Observed Physical Effects |
| 0.1 | No major physical signs; slight weight changes |
| 0.3 | Slight weight loss; mild reduction in food intake |
| 0.5 | Noticeable weight reduction, decreased activity, fur roughness |
| 0.7 | Reduced testicular size, dry skin, lethargy, dull fur |
| 0.9 | Systemic toxicity, testicular atrophy, dehydration, pale eyes, soft stool, mortality |

Table 2: Physical Effects in Female Rats

|  |  |
| --- | --- |
| Dose (mg/kg) | Observed Physical Effects |
| 0.1 | No visible changes; slight decrease in appetite |
| 0.3 | Slight lethargy; mild fur changes |
| 0.5 | Decreased appetite; early estrous cycle disruption |
| 0.7 | Significant weight loss, irregular estrous cycles, fur thinning, mortality |
| 0.9 | Vaginal bleeding, alopecia, body weight loss, lethargy, mortality |

Table 3: Severity of Behavioral Symptoms by Sex

|  |  |  |  |
| --- | --- | --- | --- |
| Symptom | Male Severity | Female Severity | Severity Scale |
| Weight Change | Moderate | Moderate | 1–3 |
| Appetite Loss | Moderate | Moderate | 1–3 |
| Lethargy | Moderate | Moderate | 1–3 |
| Fur Changes | Moderate | Severe | 1–3 |
| Reproductive Effects | Severe | Severe | 1–3 |
| Gastrointestinal Issues | Moderate | Moderate | 1–3 |
| Mortality | Moderate | Moderate | 1–3 |

Table 4: Estimated Activity Levels by Dose

|  |  |  |
| --- | --- | --- |
| Dose (mg/kg) | Male Rats | Female Rats |
| 0.1 | 5 | 5 |
| 0.3 | 4 | 4 |
| 0.5 | 3 | 3 |
| 0.7 | 2 | 2 |
| 0.9 | 1 | 1 |

**3.9.1 Overview of Observed Physical and Behavioral Changes**

The dose-dependent behavioral and physiological effects of a glyphosate-containing herbicide (Force Up) on the Wistar rats of both sexes were observed for 28 days in this study. In line with earlier observations, glyphosate treatment caused a range of toxicities that increased with escalating concentration as well as duration (Cook *et al*., 2019; Cattani *et al*., 2014).

**3.9.2 Physical Effects in Male Rats**

As presented in Table 1, males in 0.1 mg/kg exhibited no adverse observed effects except for body weight variations. Consistent with findings of Cook *et al* (2019), glyphosate-induced changes were more evident with 0.3 mg/kg of reduced body weight gain and lowered food consumption. At 0.5 mg/kg more significant signs appeared with less activity and roughening of the coat that is indicative of systemic stress reactions.Signs of reproductive toxicity such as the decreased testicular weight appeared at 0.7 mg/kg as per observations of Romano *et al*. (2012), reporting glyphosate-induced reproductive toxicity. Rats exhibited extreme distress with testicular atrophy, dehydration, pallor, and high mortality at the highest dose (0.9 mg/kg), which emphasized the systemic toxicity of the herbicide (Guyton *et al*., 2015).

**3.9.3 Physical Effects in Female Rats**

As shown in Table 2, female rats exhibited a similar course. At 0.1 mg/kg, appetite was slightly suppressed with no other apparent changes. At 0.3 mg/kg, there occurred lethargy and dullness of the fur—findings consistent with the hypothesis of early oxidative stress and endocrine involvement (Mesnage *et al*., 2015).At 0.5 mg/kg, there were disruptions in estrous cycles that have been associated with glyphosate's endocrine-disrupting potential (Talenezhad *et al*., 2020). 0.7 mg/kg caused significant reproductive impairments, thinning of the fur, and mortality. Vaginal bleeding, alopecia, and extreme sleepiness appeared in 0.9 mg/kg and confirm previous findings of extreme glyphosate-induced reproductive and systemic toxicity (Akhtar *et al*., 2021).

**3.9.4 Comparative Analysis of Behavioral Symptoms**

Table 3 presents a comparative picture of symptom severity for the two sexes. Lethargy, appetite suppression, and weight loss were moderate and similar across the sexes. More significant fur deterioration and reproductive dysfunction in the females indicate potential sex-specific vulnerabilities, as reported by Mac *et al* (2016). Gastrointestinal distress in the form of diarrhea and mortality occurred with increased doses in both the sexes as per previous glyphosate toxicology experiments (Cook *et al*., 2019).

**3.9.5 Activity Level Trends**

The reduction of physical activity shown in Table 4 is supported by observations from Cattani *et al*. (2014), as neurobehavioral suppression occurred among rats that had been exposed to glyphosate. Both the males and females had a recurring decrease in activity across moderate and high doses, which indicates glyphosate’s neurotoxicity as well as its cumulative nature over time.

**3.9.6 Summary of General Observations**

The common signs between treatment groups included reduced water consumption and appetite, progressive weight reduction, dull coat, and lethargy. Of significance is that these signs most often appeared in the second week and later, implying a latent period of evident toxicity (Benbrook, 2016). The additive nature of the effects is consistent with earlier evidence that implicates glyphosate as a cause of systemic oxidative injury, endocrine disruption, and behavioral changes (Mesnage *et al*., 2015; George *et al*., 2021).

**4 DISCUSSION**

The findings of this study provide convincing evidence of dose-dependent behavioral and hematophysiological toxicity in Wistar rats following glyphosate exposure. Not only does the current study corroborate the earlier toxicological evidence but it adds a new dimension as well by highlighting sex-specific responses and symptom development over a 28-day period of exposure.

**4.1 Impact on White Blood Cells (WBC)**

The treatment groups' WBC counts varied significantly (p<0.05). The WBC value was greatest in the control group, and it decreased in the groups that received increasing dosages of glyphosate.White blood cells play an important role in immunity against pathogens, with suppression potentially compromising the efficiency of the organism's response to infections (Benedetti *et al*., 2004). Results align with findings by Romano *et al*. (2010), citing leukopenia as well as immune dysfunction in glyphosate-exposed mice. The decrease might result from direct toxicity of the bone marrow cells or from apoptosis in immune cells due to oxidative stress.

**4.2 Alteration in Red Blood Cells, Haemoglobin, and Haematocrit**

The development of anemia is shown by the considerable decrease in RBC, hemoglobin (HB), and haematocrit (PCV) levels in the treatment groups receiving higher doses of glyphosate as compared to the control group. These variables are crucial markers of blood oxygen-carrying capacity, and it is known that suppressing them results in tissue hypoxia and metabolic impairment (Jasper et al., 2012). Other research looking into the negative effects of glyphosate have found similar anemic effects, which might be linked to peroxidative damage to the erythrocyte membrane (Cattani *et al*., 2014).

**4.3 Changes in MCV, MCH, and MCHC**

The increase in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values suggests a tendency toward macrocytic anemia, which was low in the control group and high in the group treated with high doses of glyphosate. This might be a sign of abnormal erythrocyte maturation or compromised DNA synthesis brought on by toxic impairment of folate or B12 metabolism (Oguntibeju *et a*l., 2003). Even though it is less consistent, the little increase in the MCHC value might indicate modifications to the erythrocyte's shape or the amount of hemoglobin present in the cells. These findings are consistent with previous glyphosate-related studies showing alterations in red blood cell indices as a sign of toxic distress (Gomes *et al*., 2013; Zidan *et al*., 2013).

**4.4 Reduction in Platelet Count**

An evident decrease in platelet count appeared in all the exposed groups with reduction on the control, the implication of thrombocytopenia. Platelets play a key role in haemostasis and vascular stability; their depletion might augment the risk of hemorrhage and vascular weakness. The decreased levels of plates that appeared might result simply from the suppression of the bone marrow or enhanced peripheral destruction as already reported in glyphosate models of toxicity (Mesnage *et al*., 2014). Further, the oxidative strain and the inflammation might contribute towards the damage of the megakaryocytes and platelet survival.

**4.4 General Implications**

As a whole, the haematological derangements found in the current experiment corroborate the hypothesis that glyphosate herbicide has dose-dependent haemotoxic actions. The sequence of anaemia, leukopenia, and thrombocytopenia indicates that glyphosate is capable of affecting both myeloid and erythroid lineages, possibly through pathways involving oxidative stress mechanisms, mitochondrial disruption, or suppression of the bone marrow. Impaired immunity, oxygen delivery capacity, and clotting effectiveness might ensue with these actions, particularly following chronic or high-dose exposures.In summary, glyphosate exposure resulted in marked alterations in key haematological indices, including reductions in WBC, RBC, HB, PCV, and platelet counts, alongside increases in MCV and MCH. These findings underscore the potential of glyphosate-based herbicides to compromise haematological health, particularly under prolonged or repeated exposure. Further studies, particularly in humans and under environmentally relevant conditions, are essential to fully elucidate the risk profile of glyphosate and its long-term impacts on blood physiology.

**4.6 Dose-Dependent Effects of Glyphosate**

The evidence indicates a consistent trend towards escalating behavioral and physiological toxicity with growing doses of glyphosate. At lower dosing levels (0.1–0.3 mg/kg), the responses were slight and generally consisted of merely minimal decreases in food consumption and weight gain. These slight side effects evolved into more severe responses—affecting systemic drowsiness, coat condition, and reproductive abnormalities—at moderate doses (0.4–0.7 mg/kg), ending in systemic toxicity and death with the highest dose (0.9 mg/kg). These findings corroborate earlier work by Benedetti *et al*. (2004) and Cattani *et al*. (2014), both of which described similar dose-response alterations in hematological and markers of oxidative stress. The worsening of the level of activity and body condition in all treatment groups is a further indicator of glyphosate’s cumulative toxicity potential, particularly in the context of subchronic exposures.

**4.7 Reproductive and Systemic Toxicity**

One of the most remarkable findings of this work is the expression of reproductive toxicity in both the sexes. In the males, testicular atrophy and lower testicular weight appeared with increased doses, similar to the findings of Romano *et al*. (2012), which associated glyphosate exposure with gonadotropin expression disruption. Likewise, the females had estrous cycle abnormalities, vaginal bleeding, and compromised reproductive health, augmenting earlier works demonstrating glyphosate as an endocrine-disrupting agent (Abarikwu *et al*., 2016). These findings imply that glyphosate might act on hormonal pathways or reproductive organ systems directly or indirectly by mechanisms like the generation of oxidative stress or disruption of steroidogenesis.

**4.8 Behavioral and Neurological Symptoms**

The progressive decrease in activity level and behavioral responsiveness, mainly at doses of C≥0.4 mg/kg, is indicative of probable neurotoxic action of glyphosate. Lethargy and dull eyes with reduced physical activity indicate possible disruptions of neuromuscular coordination or of the central nervous system functioning, as observed by Cattani *et al*. (2014). The cumulative behavioral observations imply that even when not associated with overt physical abnormalities, glyphosate is capable of having a fine but meaningful action on neurological functioning over time.

**4.9 Sex-Based Differences in Toxicity**

While a variety of toxicities were found in both genders, the evidence indicates sex-specific variability with more susceptibility of the fur and disruption of the estrous cycle in females and more pronounced reproductive organ involvement in males. These variations might find their basis in sex differences in the regulation of hormones, in the metabolism of xenobiotics, and in immunocompetence ((Abarikwu *et al*., 2016)). It is crucial that these differences are identified when determining accurate toxicological evaluations, specifically when establishing regulatory policies that account for both male and female biological responses.

**4.10 Public Health and Environmental Implications**

In light of the fact that glyphosate is the most used of all herbicides worldwide, these results raise significant questions regarding low-dose chronic exposure both in the workplace as well as in the environment. The spectrum of observed effects range from decreased food consumption up to death and highlight the need for more stringent regulation as well as more thorough risk assessments. In addition, the late onset of toxicity (from week 2 and thereafter) indicates the insidious cumulative nature of glyphosate’s action and the need for long-term exposure experiments.The findings of this study corroborate previous demands of scientists like Mesnage *et al*. (2014) and Benbrook (2016) that the classification and utilization of glyphosate be reconsidered, especially in environments of long-term exposure of either human beings or animals.

**5. Conclusion**

The current study has presented clear evidence of glyphosate-containing herbicide (Force Up) dose-dependent hematotoxicity and behavioral toxicity in both female and male *Wistar rats*. From slight changes in body physiology at low doses up to extreme systemic and reproductive damage with high doses, the evidence is clear that glyphosate is by no means a 'biologically inert' substance within mammalian systems. Importantly, both the female and the male rats displayed evidence of toxicity such as less body weight gain, changed activity levels, deterioration of the fur coat, reproductive aberrations, and eventual death from the highest dose examined.The research also illustrates sex-based variations in glyphosate sensitivity. Female rats proved more susceptible to reproductive and dermalic consequences, with the males suffering severe testicular and general impairment. These observations support the requirement of gender-sensitive toxicological analysis and risk assessments.The cumulative expression of the symptoms—which is typically not until the second or third week of exposure—stresses the insidious but insidious character of glyphosate toxicity. This trend has implications that long-term exposure even at sublethal levels might have serious health consequences for non-target species such as humans.The current study proved that glyphosate-based herbicide produces significant changes in haematological parameters in a dose-dependent manner. Significant drops in white blood cells, red blood cells, haemoglobin, haematocrit, and platelets indicate immunosuppression, anaemia, and possible suppression of the bone marrow. Parallel rises of MCV and MCH imply macrocytic anaemia due to impaired erythropoiesis and/or toxic disruption of red cell maturation.These results show the possible haematotoxicity of glyphosate and raise significant issues regarding its safety, particularly in situations of high- and/or repeat exposures. The evidence is indicative of the necessary need for safe handling of glyphosate-containing herbicides and of stricter regulation. Additional studies are justified in investigating the mechanisms behind these findings, measuring long-term consequences, and determining the applicability of these observations to human health and the environment.Scientists can develop a more comprehensive picture of the action of glyphosate in living systems and thereby inform safer use policies and public health action.

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1 Original Research Article

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