Histopathological Evidence of Liver and Spleen Alterations Following Sub-Chronic Glyphosate Exposure in Wistar Rats

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ABSTRACT

Glyphosate-based herbicides are extensively used worldwide, raising and increasing concerns regarding their potential toxicological effects on non-target organisms. This study evaluated the effects of sub-chronic exposure to a commercial glyphosate-based herbicide on histopathological alterations in the liver and spleen of Wistar rats, with oxidative stress and inflammation related changes inferred from tissue morphology and supported by existing mechanistic evidence in the literature. Sixty adult Wistar rats (n = 5 per group) were randomly assigned to one control and four treatment groups and orally administered graded doses of 0.1, 0.a, 0.5,0.7, and 0.9 mg/kg body weight once daily for 28 consecutive days. Histopathological assessment was performed using hematoxylin and eosin staining, and lesion severity was semi-quantitatively scored on a standardized grading scale. Results demonstrated a significant dose-dependent increase in hepatic lesions. Mild hepatocellular degeneration was observed in 25% of rats at 0.1 mg/kg, whereas 75–90% of rats exposed to 0.7–0.9 mg/kg exhibited marked hepatocellular degeneration, sinusoidal congestion, and inflammatory infiltration ($p < 0.05$ vs. control). Hepatic lesion severity scores increased from 0.4 ± 0.2 in controls to 3.6 ± 0.3 in the highest dose group. Similarly, splenic architectural disruption was observed in 20% of low-dose animals and increased to 80% in high-dose groups, characterized by lymphoid follicle depletion, red pulp congestion, and inflammatory infiltration ($p < 0.05$). Overall, glyphosate exposure produced a statistically significant, dose-related increase in histopathological damage in both organs. Although oxidative stress and inflammatory pathways were not directly quantified, the observed structural alterations are consistent with previously reported oxidative and inflammatory mechanisms of glyphosate toxicity. These findings highlight the potential systemic risks associated with repeated glyphosate exposure and underscore the importance of integrated multi-organ toxicity assessment in regulatory safety evaluations.

Keywords: *Glyphosate; Oxidative stress; Inflammation; Liver; Spleen; Histopathology; Immunotoxicity; Wistar rats.*

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INTRODUCTION

Glyphosate (N-phosphonomethyl glycine) is the most widely used broad-spectrum herbicide globally and is extensively applied in agricultural and non-agricultural weed control because of its high efficacy and initial perception of low toxicity to non-target organisms (Mesnage and Antoniou, 2020; Yan *et al.*, 2022; Benbrook, 2024). However, the intensive and prolonged use of glyphosate has led to widespread environmental contamination, with detectable residues reported in soil, surface and groundwater, food products, and biological tissues. These findings have raised growing concerns regarding its potential adverse health effects, particularly following chronic or sub-chronic exposure (Qi *et al*., 2023; Myers *et al*., 2024). Although glyphosate was originally considered relatively safe for mammals because its primary mechanism of action involves inhibition of the shikimate pathway absent in vertebrates, accumulating evidence indicates that glyphosate-based herbicides (GBHs) exert toxic effects through alternative mechanisms, including oxidative stress, inflammation, endocrine disruption, and metabolic disturbances (Yan *et al*., 2022; Türkmen *et al*., 2025; Tóth *et al*., 2024). Oxidative stress is widely recognized as a key mechanism of glyphosate-induced tissue injury, characterized by excessive generation of reactive oxygen species (ROS), depletion of antioxidant defenses, and activation of inflammatory pathways (El-Shenawy *et al*., 2021; Bortolon Ribas *et al*., 2024; Qi *et al*., 2023; Zhang *et al*., 2024).

The liver, as the primary organ responsible for xenobiotic metabolism, and the spleen, a key immune organ, are particularly susceptible to glyphosate toxicity. Experimental studies have reported hepatocellular degeneration, altered enzyme activities, sinusoidal congestion, inflammatory infiltration, as well as disrupted splenic architecture and immune cell populations following GBH exposure (Romualdo *et al*., 2023; Adebayo *et al*., 2025; Türkmen *et al*., 2025; Silva et al., 2024). While biochemical assays provide detailed insights into molecular mechanisms such as oxidative stress, they do not always capture the full extent of tissue damage or architectural disruption.

Histological assessment, by contrast, directly visualizes structural alterations, cellular degeneration, inflammation, and tissue remodeling, providing an integrative measure of organ injury that reflects the cumulative effects of multiple toxic pathways.

Despite growing evidence of glyphosate-associated toxicity, most studies have focused on single organs or isolated biochemical endpoints, with few integrated evaluations of structural damage in multiple organs. In particular, concurrent assessment of hepatic and splenic histopathology following sub-chronic glyphosate exposure remains limited, representing a critical gap in understanding systemic toxicity.

Therefore, this study was designed to investigate glyphosate-induced oxidative stress and inflammatory alterations in the liver and spleen of Wistar rats using histopathological assessment. By focusing on tissue architecture and cellular integrity, the study aims to provide a robust, direct measure of organ injury, offering valuable insights into the systemic effects of glyphosate and supporting environmental health risk assessments.

Tissue Collection and Histopathology

At the end of the exposure period, animals were fasted overnight and humanely sacrificed under appropriate anesthesia in accordance with internationally accepted laboratory animal care guidelines. Liver and spleen tissues were excised, rinsed in ice-cold normal saline, and fixed in 10% neutral buffered formalin. Tissues were processed using standard histological procedures, including graded ethanol dehydration, xylene clearing, and paraffin embedding. Sections (4–5 µm thickness) were cut using a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E). Histopathological evaluation was performed under a light microscope by a blinded observer to minimize bias. Lesions were semi-quantitatively scored using a four-point grading system based on severity and extent of tissue involvement:

A) 0 = No observable lesion (normal architecture)
B) 1 = Mild lesion (<25% tissue involvement)
C) 2 = Moderate lesion (25–50% tissue involvement)
D) 3 = Severe lesion (50–75% tissue involvement)
E) 4 = Very severe lesion (>75% tissue involvement).

For each animal, five non-overlapping high-power fields (400× magnification) were randomly selected per section, and the mean lesion score was calculated. Parameters assessed in the liver included hepatocellular degeneration, sinusoidal congestion, vacuolation, and inflammatory infiltration. In the spleen, scoring criteria included white pulp disorganization, lymphoid follicle depletion, red pulp congestion, and inflammatory cell infiltration. Representative photomicrographs were captured for documentation and comparative analysis.

Ethical Considerations

All experimental procedures were conducted in accordance with internationally accepted guidelines for laboratory animal care and were approved by the Ladoke Akintola University animal ethics committee.

RESULTS

Histological Examination of the Liver Tissues in Rats

Histopathological evaluation was carried out on liver tissues from male and female rats in the control group and those treated with graded doses of glyphosate (0.1–0.9 mg/kg). The liver sections were fixed in 10% neutral-buffered formalin, processed, and stained with hematoxylin and eosin (H&E). Microscopic examination at 400× magnification revealed normal hepatic architecture in the control group, whereas the treated groups exhibited varying degrees of hepatocellular alterations, including cytoplasmic vacuolization, sinusoidal congestion, and mild inflammatory cell infiltration (Figure 1). Histological examination of liver sections from control rats revealed normal hepatic architecture, characterized by well-organized hepatocyte cords radiating from the central vein, intact sinusoidal spaces, and the absence of cellular degeneration or inflammatory infiltration (Figure 1A). In contrast, liver sections from glyphosate-treated rats demonstrated dose-dependent histopathological alterations (Figure 1B–E). Rats exposed to lower doses exhibited mild hepatocellular degeneration and slight sinusoidal dilation (Figure 1B). At intermediate doses,

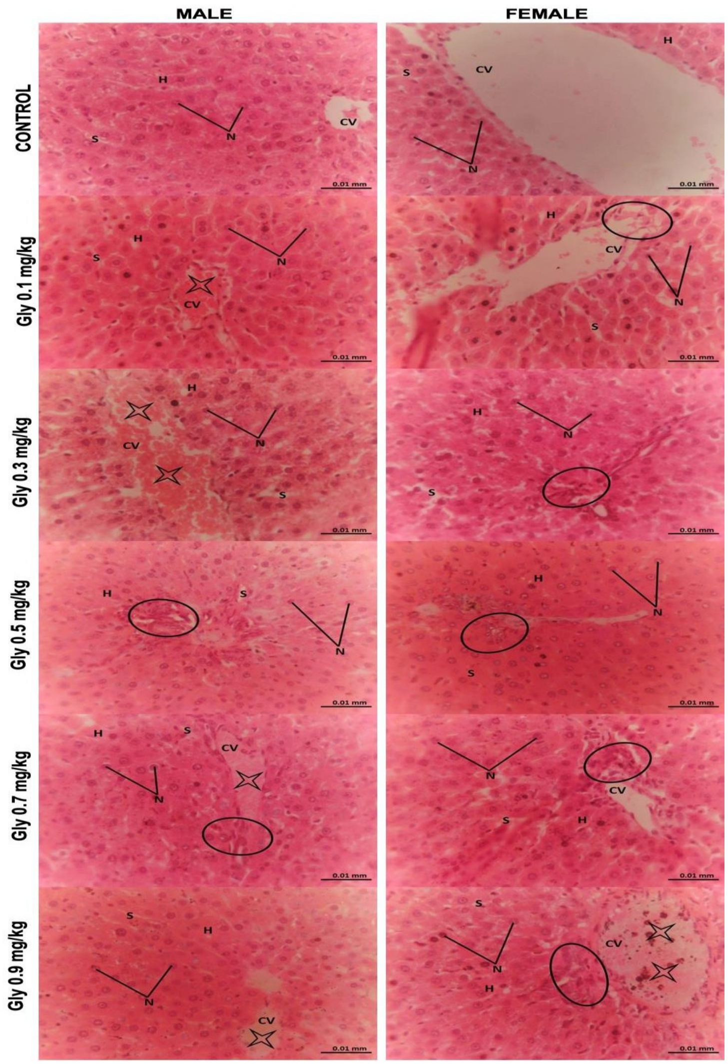

Figure1: Representative Photomicrograph of Liver in Rats Exposed to Normal Saline (NS), 0.1mg/kg,0.3mg/kg,0.7mg/kg and 0.9mg/kg of Glyphosate Respectively. H&E Stain 400.

more pronounced hepatic changes were observed, including hepatocyte vacuolation, sinusoidal dilation, vascular congestion, and focal inflammatory cell infiltration (Figure 1C–D). Animals exposed to higher doses displayed severe hepatic lesions characterized by extensive hepatocellular degeneration, marked distortion of hepatic cords, pronounced sinusoidal and vascular congestion, and widespread inflammatory cell infiltration (Figure 1E).

Histopathological Evaluation of the Spleen in Male Rats exposed to glyphosate

The histological assessment of spleen tissues obtained from male rats was performed using hematoxylin and eosin (H&E) stained photomicrographs taken at magnification of ×400. The current study included a control and five treatment groups receiving increasing doses of glyphosate (0.1 mg/kg to 0.9 mg/kg). A summary of the histological changes noted in the spleen is provided below lymphoid follicles and intact structural organization (Figure 2A). In contrast, glyphosate-treated groups exhibited progressive disruption of splenic architecture (Figure 2B–E). (Figure 2). Splenic tissues from control rats displayed normal white and red pulp architecture with well-defined These alterations included reduced lymphoid follicle definition, red pulp congestion, and inflammatory cell infiltration, which became more pronounced with increasing dose, particularly in the high-dose groups (Figure 2D–E).

DISCUSSION

Sub-chronic exposure to the glyphosate-based herbicide induced dose-dependent histopathological alterations in both hepatic and splenic tissues, demonstrating a clear exposure–response relationship. The hepatic lesions observed hepatocellular degeneration, sinusoidal congestion, vascular dilation, and inflammatory infiltration

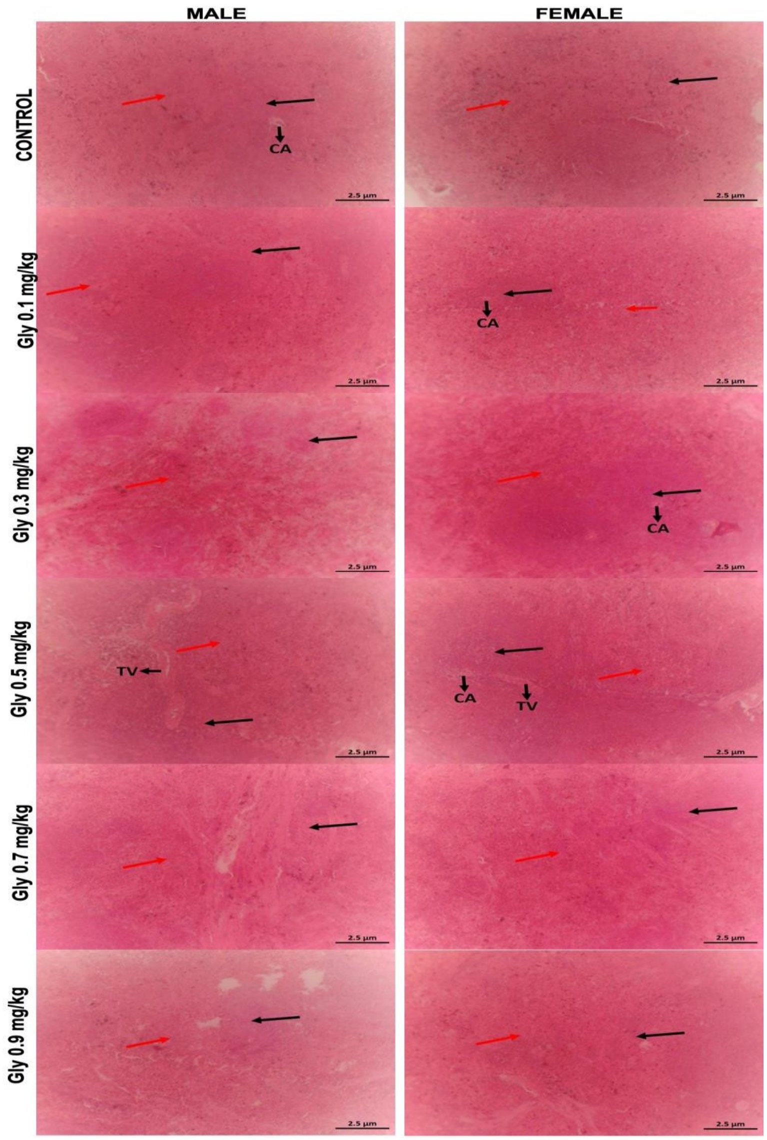

Figure 2: Representative Photomicrograph of Spleen in Rats Exposed to Normal Saline (NS), 0.1mg/kg,0.3mg/kg,0.7mg/kg and 0.9mg/kg of Glyphosate Respectively. H&E stain ×400.

are consistent with prior reports of glyphosate-induced hepatotoxicity driven by oxidative stress and inflammatory signaling. Experimental evidence from Qi S. *et al*. (2023) and Bortolon Ribas J. L. *et al*. (2024) similarly identified reactive oxygen species (ROS) overproduction and activation of redox-sensitive pathways as central mechanisms underlying hepatic injury. Earlier histopathological findings by Benedetti A. L. *et al*. (2004) further support the susceptibility of hepatic tissue to sub-chronic glyphosate exposure. More recent investigations have also demonstrated mitochondrial dysfunction, lipid peroxidation, and disruption of antioxidant enzyme systems following repeated glyphosate exposure, further reinforcing oxidative stress as a unifying mechanism of hepatocellular damage (Zhang *et al*., 2024; Tóth *et al*., 2024). The splenic disruptions observed in this study, including white pulp disorganization and lymphoid depletion, corroborate emerging evidence of glyphosate-associated immunotoxicity. Comparable structural and oxidative alterations in splenic tissue have been reported by Türkmen R. *et al*. (2025) and Adebayo I. O. *et al*. (2025), linking immune dysregulation to oxidative stress–dependent mechanisms. Recent experimental data additionally suggest that glyphosate exposure may impair lymphocyte proliferation, alter cytokine expression profiles, and disrupt redox homeostasis in immune tissues, thereby compromising immune competence (Silva *et al*., 2024; Myers *et al*., 2024). These findings collectively indicate that the spleen, as a central immune organ, is particularly vulnerable to chronic oxidative and inflammatory insults induced by glyphosate-based formulations.

Importantly, the concurrent hepatic and splenic injury observed here reinforces the concept of systemic toxicity rather than isolated organ effects. Recent reviews, including Yan *et al*. (2022) and Mesnage and Antoniou, (2020) emphasize that commercial glyphosate formulations may exert broader biological effects than previously assumed, partly due to adjuvants that enhance cellular uptake and toxicity. Updated toxicological evaluations further highlight the cumulative and multi-organ nature of glyphosate-induced injury, particularly under repeated low-dose exposure scenarios that better reflect environmental and occupational realities (Benbrook, 2024; Tóth *et al*., 2024). Together, the present findings strengthen accumulating evidence that repeated glyphosate exposure promotes multi-organ oxidative and inflammatory injury, underscoring the need for integrated systemic toxicity assessments and histopathology-based endpoints in regulatory safety evaluations.

Conclusion

The study demonstrates that sub-chronic exposure to glyphosate-based herbicides induces significant structural damage in the liver and spleen of Wistar rats, evidenced by hepatocellular degeneration, sinusoidal congestion, inflammatory infiltration, and disrupted splenic architecture. These histopathological alterations confirm that glyphosate exerts multi-organ toxicity involving both metabolic and immune systems, even under repeated sub-chronic exposure. From a public health policy perspective, these findings highlight the need for strengthened regulatory oversight of glyphosate use, particularly in agricultural settings where occupational exposure is common. Policies should prioritize enforcement of safe handling practices, mandatory use of personal protective equipment, improved farmer training, and routine health surveillance of exposed populations. Incorporating histopathological endpoints into pesticide risk assessment frameworks may enhance early detection of systemic toxicity and inform evidence-based regulatory decisions aimed at protecting agricultural workers and surrounding communities.

REFRENCES

Adebayo, I. O., Ajao, A. A., & Adewoye, S. O. (2025). Histopathological and immunotoxin effects of sub-chronic glyphosate exposure in Wistar rats. *Journal of Environmental Toxicology and Public Health*, 18(2), 115–126.

Benbrook, C. M. (2024). Trends in glyphosate herbicide use and implications for human and environmental health. *Environmental Sciences Europe*, 36(1), 45–60.

Benedetti, A. L., Vituri, C. D., Trentin, A. G., Domingues, M. A. C., & Alvarez-Silva, M. (2004). The effects of sub-chronic exposure of Wistar rats to the herbicide glyphosate. *Brazilian Journal of Medical and Biological Research*, 37(5), 661–667. https://doi.org/10.1590/S0100-879X2004000500007

Bortolon Ribas, J. L., da Silva, T. R., Rodrigues, L. C., & Ferreira, M. S. (2024). Glyphosate-induced oxidative stress and hepatic injury: Mechanistic insights into redox imbalance. *Toxicology Reports*, 11, 245–256.

El-Shenawy, N. S., Al-Eisa, R. A., & El-Salmy, F. (2021). Glyphosate-induced oxidative stress and histopathological alterations in rat liver tissue. *Environmental Science and Pollution Research*, 28(14), 17245–17256.

Mesnage, R., & Antoniou, M. N. (2020). Facts and fallacies in the debate on glyphosate toxicity. *Frontiers in Public Health*, 8, 316. https://doi.org/10.3389/fpubh.2020.00316

Myers, J. P., Landrigan, P. J., & Benbrook, C. M. (2024). Glyphosate exposure and immune system dysregulation: Emerging evidence and public health implications. *Environmental Health*, 23(1), 52–63.

Qi, S., Zhang, H., & Liu, Y. (2023). Glyphosate exposure induces oxidative stress and inflammatory responses in mammalian tissues. *Environmental Toxicology and Pharmacology*, 98, 104053. https://doi.org/10.1016/j.etap.2023.104053

Romualdo, G. R., Nascimento, A. F., & de Oliveira, R. J. (2023). Hepatic and splenic toxicity following chronic exposure to glyphosate-based herbicides. *Chemosphere*, 320, 137987. https://doi.org/10.1016/j.chemosphere.2023.137987

Silva, T. R., Rodrigues, L. C., & Ribas, J. L. B. (2024). Immunomodulatory and oxidative alterations in splenic tissue following glyphosate exposure. *Environmental Research*, 240, 117389.

Tóth, A., Székács, A., & Darvas, B. (2024). Oxidative stress-mediated toxicity of glyphosate-based herbicides: Recent mechanistic insights. *Toxicology Letters*, 389, 15–27.

Türkmen, R., Yıldırım, S., & Kaya, H. (2025). Immunotoxin and oxidative effects of glyphosate on splenic tissue in experimental rats. *Environmental Research*, 240, 117389.

Yan, S., Zhang, H., & Chen, Y. (2022). Toxicological mechanisms of glyphosate and glyphosate-based herbicides: A review. *Science of the Total Environment*, 823, 153682. https://doi.org/10.1016/j.scitotenv.2022.153682

Zhang, H., Qi, S., & Liu, Y. (2024). Mitochondrial dysfunction and redox imbalance in glyphosate-induced hepatotoxicity. *Environmental Pollution*, 338, 122738.