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Research Paper

Hepatic and Renal Histopathological Changes and Oxidant-Sensitive Fluorescence After 28-Day Glyphosate Exposure in Mice

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ABSTRACT

Background: Repeated glyphosate exposure may affect hepatic and renal tissue integrity and redox homeostasis. This study evaluated histopathological changes and oxidant-sensitive fluorescence after a defined 28-day exposure in mice.

Methods: Twenty-four male Swiss albino mice received glyphosate by oral gavage at 50 mg/kg daily for 28 days or saline (n=12/group). We assessed liver and kidney sections for necrosis and inflammation using a semi-quantitative 0–3 scale. Dichlorofluorescein diacetate (DCFH-DA) probe oxidation was measured in tissue homogenates and normalized to protein. Histopathological scores were summarized descriptively; continuous fluorescence outcomes were evaluated using exploratory summary-data Welch comparisons with Holm adjustment.

Results: Mean liver and kidney inflammation scores were 2.0±0.6 and 1.8±0.5 in exposed mice, compared with 0.5±0.3 and 0.5±0.2 in controls. Corresponding necrosis scores were 1.5±0.5 and 1.3±0.4, whereas controls showed none. Liver fluorescence was 2.0±0.5 versus 0.0±0.1 units/mg protein, and kidney fluorescence was 1.8±0.6 versus 0.0±0.1. Mean differences were 2.00 (95% confidence interval [CI], 1.68–2.32) and 1.80 (95% CI, 1.42–2.18), respectively; both Holm-adjusted P values were <0.001.

Conclusion: Under the tested conditions, 28-day glyphosate exposure was associated with hepatic and renal histopathological injury and increased DCFH-DA probe oxidation. The concurrent findings are consistent with altered tissue redox activity but do not identify a specific reactive oxygen species or establish a causal mechanism.

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Introduction

Glyphosate is a common broad-spectrum herbicide utilized in agriculture. Global glyphosate use has grown with glyphosate-resistant cropping systems, increasing opportunities for occupational and environmental exposure [1, 2]. These organs are primarily involved in systemic homeostasis and xenobiotic handling, which determine hepatic and renal responses to repeated exposure and remain common experimental endpoints.

Experimental findings are inconsistent and vary with test material composition, dose, route, duration, animal model, and endpoint. Reviews emphasize that glyphosate and commercial glyphosate-based formulations are not considered the same class of product, even though they are chemically similar [3, 4]. Examining tissue morphology and redox-sensitive measures in defined-duration studies can clarify how organs respond to adjuvants in a specific glyphosate-based formulation under a defined experimental paradigm.

Glyphosate exposure has been associated with changes in glutathione status, lipid peroxidation, and antioxidant-enzyme activity [5, 6]. Redox imbalance can be associated with cellular injury and inflammation [7, 8]. However, an oxidant-sensitive fluorescent signal and a histopathological lesion provide different information: the former reflects probe oxidation under assay conditions, while the latter reflects structural tissue changes.

This study evaluated histopathological changes in the liver and kidney and protein-normalized DCFH-DA fluorescence in male Swiss albino mice treated with glyphosate at 50 mg/kg body weight by oral gavage for 28 consecutive days. Results included semi-quantitative necrosis and inflammation scores in both organs and tissue probe-oxidation fluorescence.

Materials and Methods

Experimental design and animals

We conducted a controlled parallel-group experiment with 24 male Swiss albino mice (Mus musculus), 8–10 weeks old. We acclimated animals for 7 days and housed them under a 12 h light/dark cycle, with food and water ad libitum, from a certified supplier. The unit of observation was the single mouse.

Inclusion and exclusion criteria

Eligible animals were healthy 8–10-week-old male Swiss albino mice that had completed the 7-day acclimatization period. We observed animals daily for signs of toxicity, distress, and changes in body weight. Humane endpoint and exclusion criteria were severe distress or body-weight loss > 20%. The results report animal disposition and the number included in each analysis.

Glyphosate exposure

Mice were randomly divided into two groups (n=12/group). The exposed group received glyphosate by oral gavage at 50 mg/kg body weight for 28 consecutive days. Controls received an equivalent saline administration. We selected the 28-day period to assess early effects of repeated exposure and did not intend to model lifelong or chronic exposure.

Sample collection and tissue processing

Mice were euthanized by isoflurane overdose at the end of the 28-day exposure period. We dissected the liver and kidney tissues, observed them, washed them with Phosphate-buffered saline (PBS), and divided them for histopathological and biochemical examination. For histology, we fixed samples in 10% neutral buffered formalin for at least 24 h, dehydrated them, cleared them in xylene, and embedded them in paraffin. Tissue for fluorescence analysis was held at −80°C until processing.

Histopathological assessment

Paraffin sections were cut at ~5 µm and stained with hematoxylin and eosin (H&E). Liver evaluation included lobular architecture, hepatocytes, central and midzonal areas, and portal areas. Kidney assessment included glomeruli, tubules, and the interstitium. Degeneration describes cellular swelling and altered cellular or tissue architecture; it is not synonymous with necrosis. Necrotic change refers to loss of cellular integrity and is considered together with tissue disorganization. We assessed inflammation by the extent of inflammatory-cell infiltration. We reported necrotic change and inflammation separately on a semi-quantitative scale from 0 (absent) to 3 (extensive). Fibrosis was not quantified because H&E is not a collagen-specific stain.

Measurement of DCFH-DA probe oxidation

We assessed oxidant-sensitive fluorescence using a dichlorofluorescein diacetate (DCFH-DA) assay. Liver and kidney samples were homogenized in ice-cold PBS and centrifuged at 10,000 rpm at 4 °C. Supernatants were incubated with 10 µM DCFH-DA at 37°C for 30 minutes. We measured fluorescence at excitation and

emission wavelengths of 485 and 535 nm, respectively. We determined protein concentration using the Bradford assay, normalized fluorescence to tissue-supernatant protein concentration, and expressed it as fluorescence units per milligram of protein. The endpoint is DCFH-DA probe oxidation under the stated assay conditions, rather than direct measurement of an individual reactive oxygen species.

Statistical analysis

The mouse was the biological replicate (n=12/group); summary-data calculations used the animal count rather than the number of sections, microscopic fields, or assay readings. Results are presented as mean±SD. Histopathological scores are ordinal and are described descriptively, with differences in group means (exposed minus control). Summary statistics were used to calculate non-rank-based P values or confidence intervals for ordinal effects. For the two continuous fluorescence outcomes, we performed exploratory two-sided Welch independent-samples t tests and calculated mean differences with nominal 95% confidence intervals (CIs) from the reported group means, SDs, and sample sizes. We applied Holm adjustment across the two tissue-fluorescence comparisons, using an adjusted $P<0.05$ threshold for significance. These calculations characterize the supplied summaries and do not constitute an animal-level reanalysis. We did not perform any between-organ, regression, or correlation analyses.

Results

Animal disposition

All 24 animals completed the 28-day protocol. No animal showed severe distress or a body-weight loss greater than 20%, and no animal was excluded from analysis.

Hepatic and renal histopathology

Liver sections from exposed mice showed hepatocellular degeneration and necrosis, predominantly in the central and mid-zonal regions, along with portal inflammatory-cell infiltration (Table 1; Figure 1A–D). The mean liver necrosis score was 1.5±0.5 in exposed mice and 0 in controls. Mean liver inflammation scores were 2.0±0.6 and 0.5±0.3, respectively, corresponding to a descriptive mean difference of 1.50 score units.

Kidney sections from exposed mice showed glomerular and tubular alterations, including tubular dilatation and epithelial-cell sloughing, with inflammatory-cell infiltration in interstitial areas (Table 1; Figure 1E–H). The mean kidney necrosis score was 1.3±0.4 in exposed mice and 0 in controls. Mean kidney inflammation scores were 1.8±0.5 and 0.5±0.2, respectively, for exposed and control mice, corresponding to a mean difference of 1.30 score units.

Tissue DCFH-DA probe oxidation

Mean liver DCFH-DA fluorescence was 2.0±0.5 fluorescence units/mg protein in exposed mice and 0.0±0.1 in controls. Corresponding kidney values were 1.8±0.6 and 0.0±0.1 fluorescence units/mg protein. The exposed-minus-control mean difference was 2.00 (95% CI, 1.68–2.32) for liver and 1.80 (95% CI, 1.42–2.18) for kidney. Both Holm-adjusted P values were <0.001 in the summary-data comparisons (Table 2; Figure 2). Control values are displayed at the originally reported precision.

Discussion

Repeated glyphosate exposure at 50 mg/kg for 28 days was associated with hepatic and renal degenerative, necrotic, and inflammatory changes, accompanied by increased DCFH-DA probe oxidation. Structural alterations and oxidant-sensitive fluorescence characterize complementary aspects of the tissue response. Their co-occurrence supports an association between tissue injury and altered oxidizing activity under the tested conditions, without establishing the temporal sequence or molecular cause of the lesions.

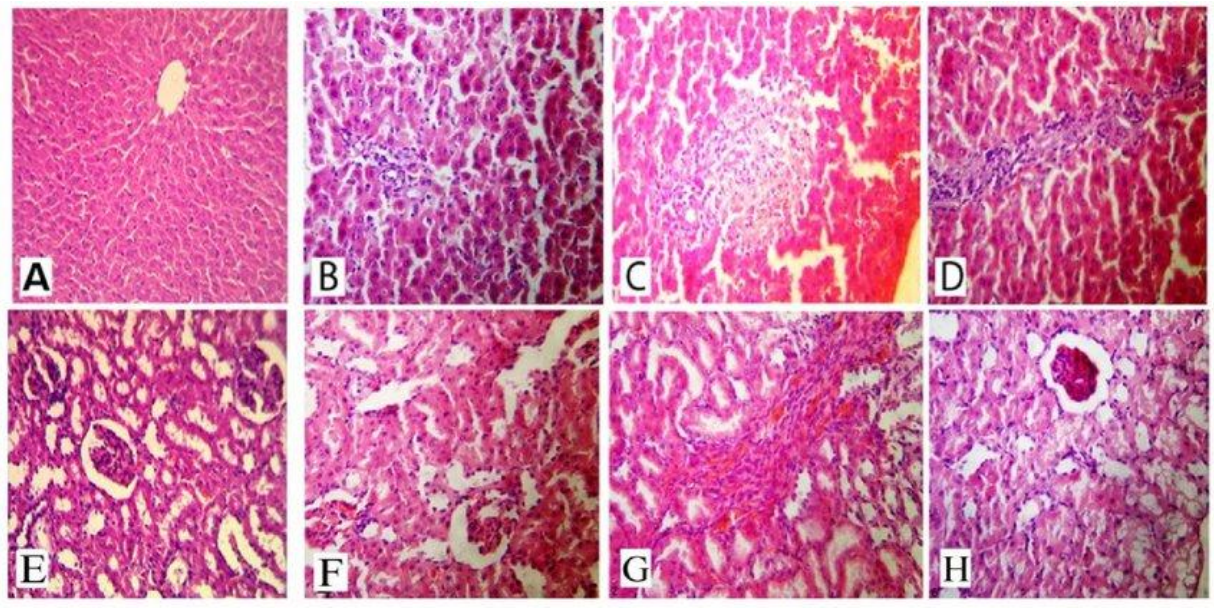
The hepatic findings are overall similar to those found in short-duration rodent studies. Qi et al. reported that 30-day exposure to a glyphosate-based herbicide

Table 1. Hepatic and renal histopathological scores after 28-day glyphosate exposure.

Organ	Outcome	Glyphosate (n=12)	Control (n=12)	Mean difference
Liver	Necrosis	1.5 ± 0.5	0	1.50
Liver	Inflammation	2.0 ± 0.6	0.5 ± 0.3	1.50
Kidney	Necrosis	1.3 ± 0.4	0	1.30
Kidney	Inflammation	1.8 ± 0.5	0.5 ± 0.2	1.30

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Values are mean±SD; n=12 mice/group. Scores range from 0 (absent) to 3 (extensive). Mean differences are exposed minus control and are descriptive summaries of ordinal scores, not rank-based effect estimates. Controls were reported as showing no necrotic change; control-group necrosis SDs were not reported. Inferential P values and ordinal-effect CIs are not presented.



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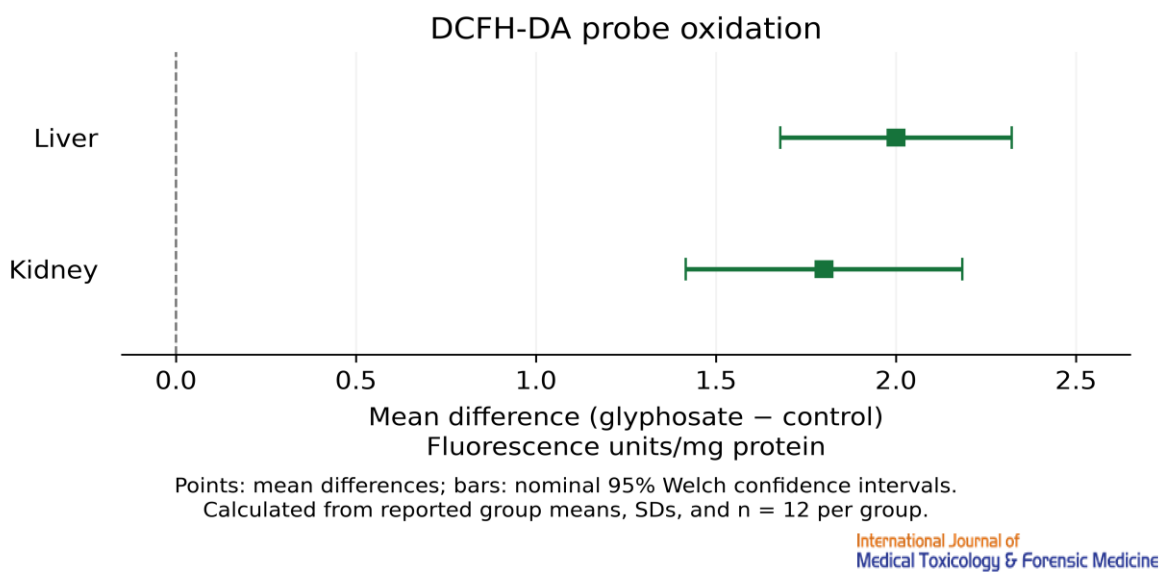
Figure 1. A, control liver with preserved lobular architecture; B, exposed liver with altered hepatocellular architecture and cellular infiltration; C, exposed liver with focal tissue alteration; D, exposed liver with inflammatory-cell accumulation and adjacent architectural disturbance; E, exposed kidney showing glomerular and tubular profiles; F, exposed kidney showing tubular dilatation and epithelial disorganization; G, exposed kidney showing dense interstitial cellular infiltration; H, exposed kidney showing glomerular and surrounding tubular alterations. All sections were stained with hematoxylin and eosin.

Table 2. Protein-normalized DCFH-DA fluorescence and summary-data effect estimates.

Organ	Glyphosate (n=12)	Control (n=12)	Mean difference (95% CI)	Holm-adjusted P value
Liver	2.0 ± 0.5	0.0 ± 0.1	2.00 (1.68–2.32)	<0.001
Kidney	1.8 ± 0.6	0.0 ± 0.1	1.80 (1.42–2.18)	<0.001

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Values are mean±SD fluorescence units/mg protein; n=12 mice/group. Mean differences and nominal, non-multiplicity-adjusted 95% CIs were calculated from reported summaries using the Welch method. P values were Holm-adjusted across the two continuous fluorescence outcomes. The assay endpoint is nonspecific probe oxidation. Displayed control values retain the originally reported precision; they do not establish an absolute absence of tissue oxidants.



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Figure 2. Squares indicate exposed-minus-control mean differences; horizontal bars indicate nominal 95% Welch CIs calculated from the reported group means, SDs, and n=12 mice/group. The vertical dashed line denotes zero mean difference. Both tissue comparisons had Holm-adjusted P<0.001.

(including at 50 mg/kg) in male mice resulted in structural changes, oxidative stress, energy metabolism imbalance, and inflammatory responses [9]. The comparable duration and dose make it an apt comparison, but formulation-specific effects cannot be assumed to be due exclusively to glyphosate itself. Tang et al. reported hepatic oxidative damage, ion imbalance, and morphological abnormalities in the liver after 35 days of glyphosate exposure in rats [10]. As in other studies, we recommend evaluating liver structure alongside redox-sensitive endpoints, despite species and protocol differences.

Biochemical oxidative responses are not necessarily reflected in conventional histology. Jasper et al. orally administered Roundup at 50 or 500 mg/kg/day for 15 days to male and female Swiss albino mice and found elevated hepatic enzymes and sex-dependent effects on lipid peroxidation and nonprotein thiols, without significant differences in liver histology [11]. The current 28-day observations extend morphological characterization to a longer exposure window. Thus, differences in duration, sex, and formulation can affect how well biochemical and structural endpoints correspond.

The renal findings have a relevant experimental context: Wang et al. found renal vacuolar degeneration and interstitial changes in male ICR mice after 90-day oral exposure to glyphosate, with increased injury in hard water [12]. Nacano et al. described proximal tubular vacuolation, elevated kidney injury molecule-1 and urinary oxidative markers in male Wistar rats given glyphosate at 5 mg/kg/day by oral gavage for 25 days [13]. These findings align with current renal changes. Differences in species, exposure conditions, and endpoints preclude a common dose-response interpretation. The current endpoints also do not establish changes in renal filtration or excretion.

Test-material composition remains central to cross-study interpretation. Reviews distinguish glyphosate from glyphosate-based herbicides because other formulation constituents can have biological effects [3, 4]. El-Shenawy reported hepatic glutathione depletion and increased lipid peroxidation, with a stronger oxidative effect from Roundup than glyphosate in a two-week intraperitoneal rat experiment [14]. Antioxidant and inflammatory responses differ across experimental systems [5, 6]. Agreement in the direction of organ changes is informative but does not establish a common quantitative dose response across disparate formulations, species, and schedules. The 28-day design is for early repeated exposure effects, not lifetime exposure or chronic disease.

The fluorescence endpoint requires assay-specific interpretation. Milić et al. reported tissue- and dose-specific glutathione-system changes after 28-day glyphosate exposure, while bulk oxidant-sensitive measurements were generally unchanged [15], illustrating that redox endpoints can differ. Bonini et al. [16] reported redox amplification in a chemical model during dichlorofluorescin oxidation. Forman et al. stress that nonspecific probe oxidation is insufficient to identify a given reactive oxygen species [17]. The enhanced protein-normalized signal is thus termed an enhanced DCFH-DA probe oxidation under the experimental conditions. It is consistent with a change in tissue redox activity in addition to the histologic injury but does not prove that a specific oxidant caused cell death. Protein normalization does not provide oxidant specificity.

The near-zero control means represent reported assay values, not absence of physiological oxidant activity. Documentation of their relation to background in the assay. Complementary redox processes can be defined by glutathione status, antioxidant enzyme activity, lipid peroxidation, or chemically selective probe products [5, 6, 17]. These were not endpoints in the present study and were not derived from DCFH-DA fluorescence.

Histopathological grades describe such structural changes. Degeneration is to be distinguished from irreversible necrotic injury. Ordinal grades are not continuous measures of tissue loss. Published guidance stresses the need to define scoring anchors and report sampling and observer procedures [18]. Group summaries suggest more necrotic and inflammatory change with exposure. Rank-based comparisons or exposure-injury regression need animal-level measures. Higher liver scores are numeric descriptors, not indicative of a greater hepatic than renal susceptibility.

H&E morphology or probe oxidation do not establish fibrosis. Characterization of extracellular-matrix deposition requires collagen-specific stains and standardized morphometry. Street et al. described an automated quantification of renal fibrosis based on Sirius Red [19]. Multiple doses in both sexes, longer follow-up, organ-function markers, and complementary oxidative endpoints would further characterize the persistence and biological significance of the observed tissue responses.

We examined one dose and one 28-day exposure period in male mice; dose dependence, sex-related variation, lesion persistence, and chronic outcomes were outside the scope of the experiment. DCFH-DA fluorescence is a nonspecific probe-oxidation endpoint, not oxidant identity, and concurrent endpoints do not

provide causality. Histopathological scores are reported descriptively, while fluorescence inferences are based on group summaries.

Conclusion

Glyphosate exposure at 50 mg/kg daily for 28 days was associated with hepatic and renal necrotic and inflammatory changes and increased protein-normalized DCFH-DA probe oxidation in male Swiss albino mice. These findings characterize early tissue responses to repeated exposure and support further investigation of the relationship between redox activity and organ injury. Interpret these findings within the tested dose, duration, and assay conditions, without inferring oxidant specificity, fibrosis, or chronic toxicity.

Ethical Approval

All animal experiments were approved by the institutional animal care and use committee of the University of Babylon (reference 14623/12/6/2024). We performed procedures in accordance with institutional and international guidelines for animal welfare.

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Conflicts of Interest

The authors report there are no competing interests to declare.

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