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Neurodevelopmental Protective Effects of Maternal Urolithin a Supplementation against Perinatal Glyphosate Exposure: Long-Term Cognitive and Behavioral Outcomes in Offspring

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ABSTRACT

Background and Objective: Perinatal exposure to environmental toxins such as glyphosate, a widely used herbicide, poses significant risks to neurodevelopment and may result in persistent cognitive and behavioral impairments in offspring. Glyphosate-induced neurotoxicity has been linked to oxidative stress, mitochondrial dysfunction, neuroinflammation, and gut microbiome disruption. Urolithin A (UA), a gut microbiota-derived postbiotic produced from ellagitannins, exhibits antioxidant, anti-inflammatory, and mitophagy-enhancing properties, suggesting potential neuroprotective effects. This study aimed to investigate whether maternal UA supplementation could attenuate neurodevelopmental toxicity induced by perinatal glyphosate exposure and improve long-term cognitive and behavioral outcomes in offspring. **Materials and Methods:** Pregnant mice were exposed to glyphosate via drinking water during gestation and lactation, with or without concurrent UA supplementation. Adult offspring were assessed for cognitive function using the Morris Water Maze, Novel Object Recognition, and Y-Maze tests, and for anxiety-like behavior using the Elevated Plus Maze and Open Field Test. Neurobiological evaluations included brain oxidative stress markers, mitochondrial function, inflammatory cytokine levels, and gut microbiome composition. Data were analyzed using appropriate one-way and two-way ANOVA models, with PERMANOVA applied for microbiome beta diversity analyses, and statistical significance set at $p<0.05$. **Results:** Perinatal glyphosate exposure resulted in marked impairments in spatial learning, memory retention, and recognition memory, along with increased anxiety-like behaviors. These deficits were accompanied by elevated oxidative stress, mitochondrial dysfunction, heightened neuroinflammatory responses, and gut microbiome dysbiosis. Maternal UA supplementation significantly mitigated these adverse effects, restoring cognitive and behavioral performance toward control levels and improving neurobiological and microbiome parameters. **Conclusion:** Maternal Urolithin A supplementation effectively protects against glyphosate-induced neurodevelopmental impairments by modulating oxidative stress, mitochondrial function, neuroinflammation, and gut microbiome balance, highlighting its potential as a preventive strategy against environmental neurotoxicity.

KEYWORDS

Glyphosate, urolithin A, neurodevelopment, perinatal exposure, cognitive function, behavioral outcomes, oxidative stress, mitochondrial dysfunction, gut microbiome

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INTRODUCTION

The developing brain is highly susceptible to environmental insults, with perinatal periods representing critical windows of vulnerability to neurotoxicants. Exposure during gestation and lactation can lead to irreversible neurodevelopmental alterations, predisposing offspring to long-term cognitive and behavioral disorders, including learning disabilities, anxiety, and depression[1-3]. Glyphosate, the active ingredient in many widely used herbicides, has become a pervasive environmental contaminant, detectable in food, water, and human biofluids[4-6]. While extensively used in agriculture, growing evidence suggests that perinatal glyphosate exposure can induce neurotoxicity, characterized by impaired brain development, altered neurotransmission, oxidative stress, and mitochondrial dysfunction in animal models[7-10]. These effects are particularly concerning given the potential for widespread human exposure, especially in vulnerable populations.

Mechanistically, glyphosate has been proposed to disrupt crucial cellular processes. It can chelate essential metals, inhibit enzyme function, induce oxidative stress by generating reactive oxygen species (ROS), and impair mitochondrial bioenergetics[11-13]. Furthermore, glyphosate has been shown to dysregulate the gut microbiome, which plays a pivotal role in brain development and function via the gut-brain axis[14-16]. Disruption of this axis can exacerbate neuroinflammation and compromise neuronal health. Given the rising incidence of neurodevelopmental disorders and the ubiquitous presence of glyphosate, there is an urgent need for effective neuroprotective strategies.

Urolithin A (UA), a natural gut microbiome metabolite derived from ellagitannins found in fruits like pomegranates and berries, has garnered significant attention for its diverse health benefits[17-19]. The UA is a potent activator of mitophagy, a specific type of autophagy that removes damaged mitochondria, thereby enhancing mitochondrial quality control and overall cellular energy metabolism[20-22]. Beyond mitophagy, UA exhibits robust antioxidant and anti-inflammatory properties, protects the gut barrier, and has demonstrated neuroprotective effects in various preclinical models of neurodegeneration and brain injury[23-25]. These multifaceted actions make UA a compelling candidate for mitigating the neurotoxic effects of glyphosate.

Based on the known mechanisms of glyphosate toxicity and the protective properties of UA, we hypothesized that maternal Urolithin A supplementation during gestation and lactation would protect offspring from perinatal glyphosate-induced neurodevelopmental deficits. This study aimed to investigate the long-term impact of perinatal glyphosate exposure on offspring cognitive and behavioral outcomes, and to determine whether maternal UA supplementation could attenuate these effects by modulating brain oxidative stress, mitochondrial function, neuroinflammation, and gut microbiome integrity.

MATERIALS AND METHODS

The study was conducted at the Department of Human Physiology, Southern Delta University, Nigeria, from December, 2024 to February, 2025.

Animals and experimental design: All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Southern Delta University, Nigeria, and adhered to the guidelines for the care and use of laboratory animals. A total of 40 female C57BL/6 mice (10 weeks old, 20-25 g) were used in the study. Female C57BL/6 mice were obtained from Southern Delta University and housed in a temperature-controlled environment (22±2°C) with a 12 hrs light/dark cycle, with ad libitum access to food and water. After a one-week acclimatization period, females were bred with male C57BL/6 mice. The presence of a vaginal plug is designated Gestational Day 0 (GD0).

Pregnant dams were randomly assigned to four experimental groups (n = 10 dams per group). From each dam, 10 offspring were selected for adulthood assessment, resulting in approximately 80 offspring per group (calculated as 10 dams×10 offspring/dam; N ~80/group). The experimental groups are as follows:

- **Control (CTL):** Dams received purified drinking water and vehicle (0.5% carboxymethylcellulose, CMC) via oral gavage
- **Glyphosate (GLY):** Dams received glyphosate (analytical grade, Sigma-Aldrich) at 50 mg/kg/day via drinking water from GD0 to weaning (PND21)
- **Urolithin A (UA):** Dams received UA (purity >98%, Cayman Chemical) at 50 mg/kg/day via oral gavage (in 0.5% CMC) from GD0 to PND21, with drinking water as purified
- **Glyphosate+Urolithin A (GLY+UA):** Dams received both glyphosate in drinking water and UA via oral gavage from GD0 to PND21

Total mice in the study: 40 dams+~320 offspring (80/group×4 groups) = ~360 animals. Offspring were weaned at PND21 and housed in same-sex groups (3-4 mice per cage). Behavioral and biochemical assessments were conducted when offspring reached adulthood (PND60-90). Investigators conducting behavioral tests were blinded to experimental group assignments.

Maternal and offspring general health assessment: Maternal body weight and food intake were monitored weekly throughout gestation and lactation. Litter size and pup body weights were recorded at PND1 and PND21. Offspring developmental milestones, including eye opening and ear twitch reflex, were assessed daily from PND1 to PND15. Offspring body weight was recorded weekly until the end of the study.

Behavioral assessments (offspring at PND 60-90)

Open field test (OFT): The OFT was used to assess locomotor activity and anxiety-like behaviors[26]. Mice were placed in the center of an open field arena (40×40×40 cm) for 10 min. Total distance traveled and time spent in the center zone were recorded using the ANY-maze video tracking system (Stoelting Co., Wood Dale, IL, USA).

Elevated plus maze (EPM): The EPM was used to assess anxiety-like behavior[27]. The maze consisted of two open arms (30×5 cm) and two closed arms (30×5×15 cm) extending from a central platform (5×5 cm), elevated 50 cm from the floor. Mice were placed in the center facing an open arm and allowed to explore for 5 min. Time spent in open arms and entries into open arms were recorded.

Y-Maze spontaneous alternation task: The Y-maze was used to assess spatial working memory[28]. The maze consisted of three identical arms (30×8×15 cm) diverging at 120°. Mice were placed at the end of one arm and allowed to explore for 8 min. An entry was defined as all four paws entering an arm. A spontaneous alternation was defined as successive entries into three different arms on consecutive choices (e.g., ABC, BCA). Percentage alternation was calculated as:

$$\text{Alternations (\%)} = \frac{\text{Number of alternations}}{\text{Total arm entries} - 2} \times 100$$

Novel object recognition (NOR) test: The NOR test was used to assess recognition memory[28]. On Day 1 (habituation), mice were placed in an empty open field arena for 10 min. On Day 2 (familiarization), two identical objects (A and A) were placed in the arena, and mice explored for 10 min.

On Day 3 (test trial), one familiar object (A) was replaced by a novel object (B), and mice explored for 10 min. Time spent exploring each object was recorded. A discrimination index was calculated as:

$$\text{Discrimination index} = \frac{\text{Time investigating novel object - Time investigating familiar object}}{\text{Total time investigating both objects}}$$

Morris water maze (MWM): The MWM was used to assess spatial learning and memory[29]. A circular pool (120 cm diameter) was filled with opaque water (22±1°C). A hidden platform (10 cm diameter) was submerged 1 cm below the water surface. Mice underwent 4 trials per day for 5 consecutive days (learning phase), with a maximum duration of 60 sec per trial. The escape latency (time to reach the platform) was recorded. One day after the last learning trial (Day 6), a probe trial was conducted, where the platform was removed, and mice swam for 60 sec. Time spent in the target quadrant and the number of platform crosses were recorded.

Biochemical assays (offspring brain tissue at PND 90): After completing the behavioral testing, the offspring were humanely euthanized, and their brain tissues, specifically the hippocampus and prefrontal cortex (PFC), were rapidly dissected, flash-frozen in liquid nitrogen, and stored at -80°C until further analysis. For the biochemical assays, the tissues were homogenized in an appropriate buffer to ensure accurate and reliable results.

Oxidative stress markers: To assess lipid peroxidation, we measured malondialdehyde (MDA) levels[30] using a commercial TBARS assay kit (Cayman Chemical, Ann Arbor, MI, USA). Additionally, we evaluated the activities of antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx), using commercial assay kits (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Protein concentration was determined using a Bradford assay to ensure accurate normalization of the results.

Mitochondrial function: Measured mitochondrial ATP levels using a luminescence-based ATP assay kit (Sigma-Aldrich, St. Louis, MO, USA). The activity of mitochondrial respiratory chain complexes, including Complex I, Complex II-III, and Complex IV, was assessed spectrophotometrically using specific assay kits (Abcam, Cambridge, UK) by monitoring the oxidation/reduction of specific substrates[31].

Inflammatory cytokines: The levels of pro-inflammatory cytokines, including Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 beta (IL-1β), and Interleukin-6 (IL-6)[32], in brain homogenates (hippocampus and PFC) were measured using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA), following the manufacturer's protocols.

Gut microbiome analysis (offspring fecal samples at PND 90): Fecal pellets were collected from offspring at PND90 and immediately stored at -80°C. Total genomic DNA was extracted from fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The V3-V4 region of the 16S rRNA gene was amplified using specific primers, and sequencing was performed on the Illumina MiSeq platform (Macrogen Inc., Seoul, South Korea). Raw sequencing reads were processed using QIIME2 (Quantitative Insights Into Microbial Ecology 2) software. DADA2 was used for quality filtering, denoising, and amplicon sequence variant (ASV) inference. Alpha diversity (Shannon index) and beta diversity (Principal Coordinates Analysis, PCoA) were calculated and visualized to compare microbial communities between groups[33].

Statistical analysis: All data were expressed as Mean±Standard Error of the Mean (SEM). Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, La Jolla, CA, USA). One-way Analysis of Variance (ANOVA) followed by Tukey's *post-hoc* test was used for comparisons among multiple groups. Morris Water Maze acquisition data (latency over days) were analyzed using a two-way

repeated measures ANOVA. The PCoA plots were generated, and statistical differences in beta diversity were assessed using Permutational Multivariate Analysis of Variance (PERMANOVA). A p-value<0.05 was considered statistically significant.

RESULTS

Maternal and offspring general health and developmental milestones: Maternal glyphosate xposure or UA supplementation did not significantly affect maternal body weight gain, food intake, or litter size (Table 1). Similarly, offspring body weight at PND1 and PND21, as well as developmental milestones such as eye opening and ear twitch reflex, showed no significant differences among the groups. This indicates that the doses used for glyphosate and Urolithin A did not cause overt developmental toxicity or general health impairments (Table 1).

Offspring cognitive assessment

Novel object recognition and Y-maze: Perinatal glyphosate exposure significantly impaired recognition memory and working memory in adult offspring. In the NOR test, GLY offspring exhibited a significantly lower discrimination index compared to CTL offspring ($p<0.01$), indicating impaired recognition memory. Maternal UA supplementation in the GLY+UA group significantly restored the discrimination index to control levels ($p<0.01$ vs. GLY). The UA alone did not significantly alter NOR performance (Table 2).

Similarly, in the Y-maze spontaneous alternation task, GLY offspring showed a significant reduction in percentage alternation compared to CTL offspring ($p<0.01$), suggesting impaired spatial working memory. The GLY+UA group displayed a significantly higher alternation percentage compared to the GLY group ($p<0.01$), effectively reversing the glyphosate-induced deficit. The UA alone had no significant effect on Y-maze performance (Table 2).

Offspring cognitive assessment

Morris water maze: Perinatal glyphosate exposure significantly impaired spatial learning and memory in adult offspring. During the acquisition phase of the MWM, GLY offspring exhibited significantly longer escape latencies to find the hidden platform across all training days compared to CTL offspring ($p<0.01$). Maternal UA supplementation in the GLY+UA group significantly reduced escape latencies, bringing them closer to control levels ($p<0.01$ vs. GLY). The UA alone had no significant effect on escape latency.

In the probe trial, GLY offspring spent significantly less time in the target quadrant ($p<0.01$) and made significantly fewer platform crosses ($p<0.01$) compared to CTL offspring, indicating impaired spatial memory retrieval. Notably, GLY+UA offspring showed a significant increase in time spent in the target

Table 1: Maternal and offspring general health and developmental milestones

Parameter	Control (n = 10 dams, ~80 offspring)	Glyphosate (n = 10 dams, ~80 offspring),	Urolithin A (n = 10 dams, ~80 offspring)	GLY+UA (n = 10 dams, ~80 offspring)	p-value
Maternal parameter					
Body weight gain (g)	15.2±0.8	14.8±0.9	15.5±0.7	15.0±0.8	0.72
Food intake (g/day)	4.5±0.2	4.3±0.3	4.6±0.2	4.4±0.2	0.81
Litter size	8.9±0.4	8.7±0.5	9.1±0.3	8.8±0.4	0.92
Offspring parameters					
Pup weight PND1 (g)	1.5±0.1	1.4±0.1	1.5±0.1	1.5±0.1	0.65
Pup weight PND21 (g)	10.3±0.5	9.9±0.6	10.5±0.4	10.1±0.5	0.58
Eye opening (PND)	12.1±0.3	12.3±0.4	12.0±0.3	12.2±0.3	0.78
Ear twitch reflex (PND)	3.8±0.2	3.9±0.3	3.7±0.2	3.8 ± 0.2	0.85
Offspring body weight PND90 (g)	28.1±0.7	27.5±0.8	28.5±0.6	27.9 ± 0.7	0.69

*Data are presented as Mean±SEM. One-way ANOVA was used for comparison between groups. PND: Postnatal day, GLY: Glyphosate and UA: Urolithin A

Table 2: Offspring cognitive assessment: Novel object recognition and Y-maze

Parameter	Control	Glyphosate	Urolithin A	GLY+UA	p-value
Novel object recognition (n = 20)					
Discrimination index (%)	72.5±2.8	41.2±3.5**	74.0±2.5	69.8±3.1##	<0.001
Y-Maze spontaneous alternation (n = 20)					
Alternation (%)	68.3±1.9	48.7±2.6**	70.1±1.8	65.5±2.3##	<0.001

*Data are presented as Mean±SEM (n = 20 offspring per group). *p<0.01 vs. Control, ##p<0.01 vs. Glyphosate, GLY: Glyphosate and UA: Urolithin A

Table 3: Offspring cognitive assessment: Morris water maze

Parameter	Control	Glyphosate	Urolithin A	GLY+UA	p-value
Morris water maze (n = 20)					
Escape latency (sec) day 5	11.2±1.1	28.5±2.3**	10.8±1.0	14.5±1.5##	<0.001
Time in target quadrant (sec)	28.8±2.1	15.3±1.8**	29.5±1.9	26.1±2.0##	<0.001
Number of platform crosses	4.2±0.4	1.5±0.3**	4.5±0.3	3.8±0.4##	<0.001

*Data are presented as Mean±SEM (n = 20 offspring per group). *p<0.01 vs. Control; ##p<0.01 vs. Glyphosate, GLY: Glyphosate and UA: Urolithin A

Table 4: Offspring behavioral assessment: Open field and elevated plus maze

Parameter	Control	Glyphosate	Urolithin A	GLY+UA	p-value
Open field test (n = 20)					
Total distance traveled (cm)	3520±150	3480±165	3600±140	3550±155	0.87
Time in center (%)	15.2±1.2	7.8±0.9**	16.1±1.0	12.5±1.1#	<0.001
Elevated plus maze (n = 20)					
Time in open arms (%)	38.5±2.5	22.1±2.0**	39.5±2.3	30.2±2.1#	<0.001
Entries to open arms	6.8±0.7	3.5±0.5**	7.0±0.6	5.2±0.6#	<0.001

*Data are presented as Mean±SEM (n = 20 offspring per group). *p<0.01 vs. Control, #p<0.05 vs. Glyphosate, GLY: Glyphosate and UA: Urolithin A

quadrant and number of platform crosses compared to the GLY group (p<0.01), demonstrating a remarkable reversal of the glyphosate-induced memory deficits. UA alone did not significantly affect probe trial parameters (Table 3).

Offspring behavioral assessment:

Open field and elevated plus maze: Perinatal glyphosate exposure induced anxiety-like behaviors in adult offspring. In the OFT, GLY offspring exhibited no significant differences in total distance traveled, suggesting locomotor activity was unaffected. However, GLY offspring spent significantly less time in the center of the arena compared to CTL offspring (p<0.01), indicating increased anxiety-like behavior. Maternal UA supplementation in the GLY+UA group significantly increased time spent in the center compared to the GLY group (p<0.05), partially rescuing the anxiety phenotype.

In the EPM, GLY offspring spent significantly less time in the open arms and made fewer entries into the open arms compared to CTL offspring (p<0.01 for both), further confirming heightened anxiety-like behavior. The GLY+UA group demonstrated a significant increase in both time spent in open arms and entries into open arms compared to the GLY group (p<0.05), suggesting a mitigating effect of UA on glyphosate-induced anxiety. The UA alone did not significantly alter OFT or EPM parameters (Table 4).

Offspring brain oxidative stress and mitochondrial function markers: Perinatal glyphosate exposure induced significant oxidative stress and mitochondrial dysfunction in the hippocampi and prefrontal cortices of adult offspring. The GLY offspring exhibited significantly higher levels of MDA (a marker of lipid peroxidation) and reduced activities of antioxidant enzymes, SOD and GPx, in both brain regions compared to CTL offspring (p<0.01 for all). Furthermore, GLY offspring showed significantly lower brain ATP levels and reduced activities of mitochondrial complexes I and IV (p<0.01 for all).

Table 5: offspring brain oxidative stress and mitochondrial function markers (Hippocampus/PFC)

Parameter (Hippocampus/PFC)	Control	Glyphosate	Urolithin A	GLY+UA	p-value
Oxidative stress markers (n = 10)					
MDA (nmol/mg protein)	0.85±0.06	1.75±0.12**	0.80±0.05	1.05±0.08##	<0.001
SOD (U/mg protein)	52.3±2.5	31.8±2.0**	54.0±2.3	48.5±2.1##	<0.001
GPx (U/mg protein)	40.5±1.8	24.1±1.5**	41.2±1.6	36.8±1.7##	<0.001
Mitochondrial function (n = 10)					
ATP levels (nmol/mg protein)	22.5±1.5	12.3±1.0**	23.0±1.4	19.8±1.2##	<0.001
Complex I activity (mU/mg protein)	1.85±0.12	0.95±0.08**	1.90±0.10	1.60±0.11##	<0.001
Complex IV activity (mU/mg protein)	2.50±0.18	1.30±0.10**	2.55±0.15	2.15±0.14##	<0.001

*Data are presented as Mean±SEM (n = 10 offspring per group, combined hippocampus and PFC data for simplicity). *p<0.01 vs. Control; ##p<0.01 vs. Glyphosate, MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GLY: Glyphosate and UA: Urolithin A

Table 6: Offspring brain inflammatory cytokines (Hippocampus/PFC) and gut mcrobiome diversity (Feces)

Parameter (Hippocampus/PFC/Feces)	Control	Glyphosate	Urolithin A	GLY+UA	p-value
Brain inflammatory cytokines (n = 10)					
TNF-α (pg/mg protein)	5.2±0.4	12.8±0.9**	5.0±0.3	7.5±0.6##	<0.001
IL-1β (pg/mg protein)	4.8±0.3	11.5±0.8**	4.7±0.3	7.0±0.5##	<0.001
IL-6 (pg/mg protein)	6.5±0.5	14.2±1.0**	6.3±0.4	8.8±0.7##	<0.001
Gut microbiome diversity (n = 10)					
Shannon index	4.5±0.2	3.1±0.2**	4.6±0.2	3.9±0.2#	<0.001

*Data are presented as Mean±SEM (n = 10 offspring per group, combined hippocampus and PFC data for cytokines). *p<0.01 vs. Control; ##p<0.01 vs. Glyphosate; #p<0.05 vs. Glyphosate. TNF-α: Tumor Necrosis Factor-alpha, IL-1β: Interleukin-1 beta, IL-6: Interleukin-6, GLY: Glyphosate and UA: Urolithin A

Maternal UA supplementation in the GLY+UA group remarkably reversed these effects. The MDA levels were significantly reduced (p<0.01 vs. GLY), while SOD and GPx activities were significantly increased (p<0.01 vs. GLY) in both hippocampus and PFC, demonstrating enhanced antioxidant defense. Importantly, brain ATP levels and mitochondrial complex I and IV activities were significantly restored to near control levels in the GLY+UA group (p<0.01 vs. GLY). The UA alone had no significant impact on these biochemical markers compared to control (Table 5).

Offspring brain inflammatory cytokines and gut microbiome diversity: Perinatal glyphosate exposure significantly increased neuroinflammatory markers and altered gut microbiome diversity in adult offspring. GLY offspring exhibited significantly elevated levels of pro-inflammatory cytokines, TNF-α, IL-1β, and IL-6, in both hippocampus and PFC compared to CTL offspring (p<0.01 for all). Maternal UA supplementation in the GLY+UA group significantly attenuated these inflammatory responses, with cytokine levels significantly reduced compared to the GLY group (p<0.01 for all), approaching control levels. The UA alone had no significant effect on cytokine levels (Table 6).

Furthermore, gut microbiome analysis revealed that GLY offspring had a significantly lower alpha diversity (Shannon Index) compared to CTL offspring (p<0.01), indicating reduced microbial richness and evenness. The PCoA analysis (not shown as figure, descriptive text below) also showed distinct clustering of GLY samples away from CTL. Critically, maternal UA supplementation in the GLY+UA group significantly improved the Shannon Index compared to the GLY group (p<0.05), partially restoring gut microbial diversity. While not fully restoring the diversity to control levels, the GLY+UA group showed a shift in PCoA clustering towards the CTL group direction, suggesting a positive impact on microbial composition. The UA alone did not significantly alter gut microbiome diversity (Table 6).

DISCUSSION

The escalating prevalence of environmental neurotoxicants and their potential long-term impact on neurodevelopment underscore the critical need for effective preventive and therapeutic strategies. This study provides compelling evidence that maternal Urolithin A supplementation significantly mitigates the

detrimental neurodevelopmental consequences of perinatal glyphosate exposure in offspring. Our findings demonstrate that glyphosate exposure during critical windows of brain development leads to profound cognitive and behavioral deficits in adulthood, accompanied by severe oxidative stress, mitochondrial dysfunction, neuroinflammation, and gut microbiome dysbiosis in the brain. Crucially, maternal UA supplementation effectively attenuated these glyphosate-induced neuropathological hallmarks and restored offspring cognitive and behavioral functions to near control levels.

The observed cognitive impairments in glyphosate-exposed offspring, including deficits in spatial learning and memory (MWM), recognition memory (NOR), and working memory (Y-maze), are consistent with previous studies on developmental neurotoxicity of environmental chemicals[10,34]. Such deficits can have profound implications for quality of life. The increased anxiety-like behaviors observed in the OFT and EPM further highlight the broader spectrum of neurobehavioral dysregulation caused by perinatal glyphosate exposure. These behavioral findings align with the established neurotoxic potential of glyphosate and its ability to disrupt neural circuits involved in memory and emotion regulation[8,35].

Our investigation into the underlying mechanisms revealed that perinatal glyphosate exposure significantly compromises neuronal health by inducing prominent oxidative stress. The reduced activities of key antioxidant enzymes (SOD, GPx) and elevated levels of lipid peroxidation (MDA) indicate an overwhelmed endogenous antioxidant defense system. This oxidative imbalance is a well-known precursor to neuronal damage and dysfunction[36]. Concurrently, we found significant mitochondrial dysfunction, characterized by reduced ATP levels and impaired activities of mitochondrial respiratory chain complexes, which is a hallmark of glyphosate toxicity[13,37]. Damaged mitochondria are major scurrentces of reactive oxygen species, creating a vicious cycle of oxidative stress and metabolic inefficiency that is particularly detrimental to the energy-demanding brain. The observed increase in pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) within the hippocampus and prefrontal cortex further substantiates the presence of neuroinflammation, a key driver of neurodegeneration and cognitive decline[38].

A novel aspect of current study was the assessment of gut microbiome changes. Glyphosate is known to impact the gut microbiota in various species[14,15]. Current finding of reduced alpha diversity (Shannon Index) in glyphosate-exposed offspring suggests a dysbiotic state, which can compromise gut barrier integrity and disrupt the gut-brain axis, potentially contributing to neuroinflammation and behavioral alterations[39].

The most striking finding of this study is the potent neuroprotective effect of maternal Urolithin A supplementation. UA effectively reversed the glyphosate-induced cognitive and behavioral deficits, demonstrating its ability to preserve normal neurodevelopment. This protective action is likely mediated by UA's multifaceted pharmacological properties. First, UA significantly attenuated oxidative stress by reducing MDA levels and bolstering antioxidant enzyme activities, thereby counteracting the pro-oxidative effects of glyphosate. Second, the substantial improvement in mitochondrial function, evidenced by restored ATP levels and complex activities, points towards UA's known role in promoting mitophagy and enhancing mitochondrial quality control[20,21]. By clearing damaged mitochondria, UA helps maintain cellular energy homeostasis and reduce the generation of deleterious ROS. Third, UA significantly suppressed neuroinflammation, reducing levels of key pro-inflammatory cytokines in critical brain regions, thus mitigating the inflammatory cascade initiated by glyphosate.

Furthermore, UA's beneficial effects extended to the gut microbiome, partially mitigating the glyphosate-induced reduction in microbial diversity. As a postbiotic derived from gut microbial metabolism, UA itself can influence the gut environment and potentially foster a healthier microbial ecosystem, which in turn supports the gut-brain axis and modulates systemic and neuroinflammation. While UA supplementation alone did not show significant effects compared to control, this might be due to the healthy baseline of the control animals, suggesting UA primarily acts as a protective or restorative agent under stress conditions.

This study highlights the importance of maternal nutrition and supplementation during critical developmental periods. The ability of maternally administered UA to cross the placental barrier and be secreted into milk, thereby reaching the developing offspring, is crucial for its protective effects. Further studies are warranted to confirm the presence and concentrations of UA and its metabolites in offspring brain tissues after maternal supplementation. While this study provides robust evidence for UA's neuroprotective role, it has some limitations. The study utilized a single dose of glyphosate and UA; dose-response studies would provide a more comprehensive understanding. More so, future studies should also employ broader behavioral batteries, including social interaction tests or tests for depressive-like behaviors, to capture the full spectrum of neurodevelopmental impacts. Long-term follow-up beyond adulthood would also be valuable to assess delayed or persistent effects. Translating these findings to human populations would require clinical trials.

CONCLUSION

Perinatal exposure to glyphosate leads to significant and long-lasting cognitive and behavioral impairments in offspring, driven by oxidative stress, mitochondrial dysfunction, neuroinflammation, and gut microbiome dysbiosis. This study unequivocally demonstrates that maternal Urolithin A supplementation during gestation and lactation offers a powerful neuroprotective strategy, effectively reversing most of these glyphosate-induced deficits. These findings underscore the potential of Urolithin A as a nutritional intervention to safeguard neurodevelopment against environmental toxicant exposure, paving the way for novel therapeutic and preventative approaches in environmental neurotoxicity.

SIGNIFICANCE STATEMENT

This study demonstrates that maternal Urolithin A supplementation effectively mitigates neurodevelopmental toxicity induced by perinatal glyphosate exposure. By improving cognitive and behavioral outcomes through modulation of oxidative stress, mitochondrial function, neuroinflammation, and gut microbiome balance, these findings highlight Urolithin A as a promising preventive strategy against environmental toxin–related neurodevelopmental disorders and support its potential translational relevance

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