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EVALUATION OF AMELIORATIVE POTENTIAL OF DIFFERENT PLANT EXTRACTS ON GLYPHOSATE-INDUCED DOPAMINE NEURODEGENERATION AND ITS IMPACTS IN *CAENORHABDITIS ELEGANS*

Kisan B. Jadav[1*], Sachin S. Patil[1], Shaheen Jafri Ali[4*], Sanganna Sajjanar[2], Ayyangouda Patil[1], Kavita T. R.[3], Sharanbasappa Yerri[1] and K. Shruthi Nagarhal[1]

[1]Department of Molecular Biology and Agricultural Biotechnology, University of Agricultural Sciences, Raichur 584104, Karnataka, India.

[2]Department of Agricultural Entomology, University of Agricultural Sciences, Raichur 584104, Karnataka, India.

[3]Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

[4]Department of Biotechnology, Teresian College, Bannur Road, Siddarthanagar, Mysuru 570011, Karnataka, India

*Corresponding authors E-mail: shaheen.jafri@gmail.com / kisanb1@gmail.com

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ABSTRACT

Glyphosate (GLY) manages weeds by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP), the target pathway presents only in plants and used for synthesis of aromatic amino acids. This study evaluates if GLY induces dopaminergic toxicity in non-targeted organism like *C. elegans* and the ameliorative potentials of various plant extracts against it. GLY exposure with secondary non-targeted effects in non-targeted organisms is explored due to its secondary nonspecific impact. It is proved that GLY exposure leads to genetic damage, increased oxidative stress, interference with the estrogen pathway, and several types of cancers. This study utilizes BZ555 transgenic strain of *C. elegans* with Green fluoroscence proteins (GFP) tagged dopaminergic neurons to assess the ameliorative potentials of *Bacopa monnieri* (Brahmi), *Citrus limon* (Lemon) and *Withania somnifera* (Ashwagandha) plant extracts against GLY-induced dopaminergic neurodegeneration. BZ555 transgenic strain were treated with GLY and plant extract to understand possible amelioration from GLY-induced dopaminergic neuron degeneration. The different assays performed were dopamine neuron degeneration in the anterior dendrites visualized as (breaks, blebs and kinks), pharyngeal pumping, motility and percentage neuroprotection from the plant extract. Our results highlighted that as compared to GLY, Ashwagandha followed by Brahmi and then lemon significantly reduced dopaminergic degeneration in breaks, blebs and kink formations. Furthermore, there was increase in pharyngeal pumping (2.85 folds) and motility (1.5 folds). Overall Ashwagandha offered significant neuroprotection on exposure to GLY. Collectively the use of GLY is cautioned with Ashwagandha showing promising neuroprotection in *C. elegans*.

Key words: Glyphosate, *C. elegans*, dopamine, plant extracts, Arena Tracker, Pharyngeal

Introduction

According to a report by United Nations, weeds cause from 5-30% loss in agriculture globally(Junaid & Gokce, 2024). Traditional practices adopted to control weed included hand weeding, harrowing, ploughing or preparing the soil or land through mechanical or manual efforts. Glyphosate (GLY), an provides an effective, economic and a reliable solution for control of roughly 100 species of weeds and 60 species of perennial weed plants (Chaufan *et al.*, 2014; Ferrante *et al.*, 2023; Muñoz *et al.*, 2023). Principally it works by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) that is present in plants and microorganisms (Leino *et al.*, 2021; Peillex & Pelletier, 2020) and subsequently blocks the synthesis of aromatic amino acids like phenylalanine, tryptophan and tyrosine.

Globally, GLY is used both on genetically modified organisms (GMO- such as cereals and soybeans) (Bukowska *et al.*, 2022), non-GM crops and residential application. GMO's convert GLY, to aminomethylphosphonic acid (AMPA), traces of which have been reported both from soil (Annett *et al.*, 2014; Bukowska *et al.*, 2022) and groundwater (Battaglin *et al.*, 2014). Their ubiquitous application hints the important role GLY play in agriculture, contributing to the stability and resilience of the global food supply system. GLY contamination has been reported in biological fluids such as maternal milk, urine and blood, (Muñoz *et al.*, 2023; Cellier *et al.*, 2022; Filippi *et al.*, 2024)(Muñoz *et al.*, 2023)[4](4). In addition, it has been reported in beer and cereals, reiterating the non-occupational route of exposure (Pérez-Lucas *et al.*, 2024). There have also been reports of cases of non-Hodgkin's lymphomas in farm workers exposed to GLY (Acquavella, 2023). Additionally *in vitro* data have reported GLY exposure inducing genetic damage, increase oxidative stress and interfere with the oestrogen pathway, and several types of cancers as well (Bukowska *et al.*, 2022; Nagy *et al.*, 2019; Peillex & Pelletier, 2020; Portier *et al.*, 2016)

In India, GLY is approved for weed control in tea gardens and non-crop areas. It is sold in seven different formulations (of active ingredient along with the supportive adjuvants) that are registered under Insecticide Act 1968, of the country (http://ppqs.gov.in/divisions/cib-rc/registered-products). Unfortunately, it also finds indiscriminate use in production of many field crops such as soybeans, field corn, pasture, wheat and hay. Residues of GLY on fruit juices, vegetables like beans, wheat products, honey and meat are also reported. GLY has an acceptable daily intake of 0.05 mg/kg/day and acceptable residue concentration of 0.2 ppm (Massaro, 1997). Refuted in their judgment on the effect of GLY impact on human health, some Indian states like Andhra Pradesh, Punjab, Telangana and Kerala have imposed a temporary ban on the use of GLY -based herbicide (GBH).

Among the cellular and transgenic models available to study neurotoxicity, *C. elegans* offers distinct advantages with a transparent body, availability of transgenic green fluorescence protein (GFP) tagged signals and mutant strains to study pesticide induced dopaminergic toxicity. It further shares many conserved molecular pathways and cellular mechanisms with mammals and thus offers economical, but strategic experimental model, that allows rapid analyses of susceptibility factors for pathological mechanisms. Likewise, in the present study, we investigated if GLY had the potential to affect the non-target organism lacking the EPSP synthase pathway and further can we ameliorate such affects by the use of plant extracts like Bacopa monnieri (Brahmi), Citrus limon (Lemon) and Withania somnifera (Ashwagandha) was evaluated on GLY induced neurotoxicity in *C. elegans*. Analysis of neurotoxicity was done in terms of percentage mortality, estimation of dopamine neuron degeneration in the anterior dendrites visualized as (breaks, blebs and kinks), movement of pharyngeal pumping, motility screening and percentage neuroprotection offered by the plant extracts. Holistically, the present study offers new insights on the alternative targets sites of GLY toxicity which could have human translations due to conserved molecular and cellular mechanisms between nematodes and mammals.

Materials and Methods

Collection of Plant Material

Citrus limon (Lemon) fruit, *Bacopa monnieri* (Brahmi) leaves and *Withania somnifera* (Ashwagandha) roots were purchased from the local market and further identified at the Department of Studies in Agriculture, University of Agriculture, Raichur, Karnataka, India before use.

Preparation of plant extract

After collection, the leaves, fruit and roots were thoroughly washed with distilled water and dried in shade. The powdered plant parts were dissolved in distilled water (1g sample/10ml of water) in an electric blender for 10 min. After 72 hr, they were filtered through a Whatman filter paper-1 and refrigerated at 4ºC in falcon tubes till further analysis. Final concentration of plant extract used for experimentation was 600µg/ml.

Strain maintenance and synchronization

C. elegans strains such as wild-type N2 and transgenic BZ555 (dat-1p:: GFP; green fluorescent protein expression in DAergic neuronal soma and processes) were procured from the *Caenorhabditis* Genetics Center (CGC, University of Minnesota, USA). All procedures performed on *C. elegans* were carried out according to protocol number MUSC60-049-398 approved by the MUSC-ACUC (Nass & Blakely, 2003). Briefly, worms were cultured on nematode growth media (NGM) with OP50 strain of *Escherichia coli* (auxotrophic for uracil) as a food source and maintained on 20ºC in an incubator. Synchronised eggs were isolated from adult worms by a process of bleaching (12% NaOCl and 10% 1 M NaOH) and subsequently washed with M9 buffer before being plated on NGM plates without the food source. They were subsequently incubated overnight at 20ºC to obtain newly hatched larvae of the

L1 stage. The stock solution of GLY 41% SL was prepared in sterile distilled water at final concentration of 10mM ($1/7^{th}$ LC_{50} (71.54 mM) (Zhihang *et al.*, 2024). All experiments were repeated thrice at three different days using between 50-100 worms.

Dopamine neuron degeneration and protection assay

The neuron degeneration and neuroprotection assay were performed following protocols from (Anjaneyulu *et al.*, 2020; Bijwadia *et al.*, 2021), with slight modifications. Briefly, synchronized L-1 BZ555 larvae were exposed to GLY 41% SL (10mM) and GLY 41% SL (10mM) +the plant extracts (600µg/ml) in a 24-well plate. After 24hrs of incubation, worms were washed with M9 buffer and further processed for the imaging of the dopamine neurons. Before imaging worms were immobilized using 20 mM of sodium azide (Ali and Rajini, 2012). Imaging was processed in 96 well imaging plates and degeneration of neurons and possible neuroprotection by plant extracts was accessed through analysis of the breaks, blebs and kink seen in the worm dendrites. Images were captured through LUMASCOPE 620 fluorescence microscope with excitation wavelengths ranging from 784 - 490 nm at 80X magnification. Worm were considered exhibiting neurodegeneration (seven point scale neurodegeneration) if one of the four CEP dendrites exhibited partial or complete loss of green fluorescence signal either as breakage in the dendrites (breaks), small continuous dots seen in the degenerate neurons (blebs) or zig-zag orientation of neurons instead of straight orientation (kinks). The number of worms exhibiting degeneration was counted in each group and the results were expressed as the percentage neurodegeneration. This experiment was conducted in triplicate and 50 images were analysed in each group of single experimentation.

Calculations for neurodegeneration by GLY and neuroprotection by plant extracts was performed following protocol from (Anjaneyulu *et al.*, 2020)

Percentage of worms with neuron degeneration were calculated based on the formula:

Neuron degeneration = (number of worms with degenerated neurons/total number of worms) *100

Neuroprotection offered by application of different plant extracts was based on the formula:

Neuroprotection (%) = percentage of worms with neurodegeneration in the GLY-treated group - percentage of worms with neurodegeneration in the extract-treated group.

An average of 100 worms were analysed in each group. The experiments were repeated three times on three different days.

Mobility assay as quantified by the Arena Tracker

The mobility assay was done following protocols from (Bauer *et al.*, 2022; Kutzner *et al.*, 2024) that employs detecting the changes in the worm's positional movement through repeated scanning. Briefly worms were exposed to GLY 41% SL (10mM) and GLY 41% SL (10mM) + plant extracts (600µg/ml) in a 24-well plate for 6hrs. Infrared LED micro-beams from the WMicroTracker ARENA System tracked the movement and positioning of worms on the culture dishes and interruptions of the LED micro-beam by a worm's movement allowed real-time data to be processed at time points 0, 0.5, 1, 2, 4 and 6hrs. The accompanying software identified changes in the positions of the interrupted beams during scans and computed an activity score based on the successive scan differences. 70 worms were analysed in each group of experimentation which was repeated thrice at three different days.

Pharynx pumping assay

The pharyngeal pumping assay was done following protocol from previously published literature (J J. O'Brien, 2022). L4 stage worms were washed in M9 buffer to remove off the OP-50 bacteria, and further exposed to GLY 41% SL (10mM) and GLY 41% SL + plant extracts (600µg/ml). After 10 min of incubation, pharyngeal pumping was estimated by counting the number of grinder movements made by the worms in 30 sec of observation under a stereo-binocular microscope. An average of 50 worms were analysed in each group. The experiment was repeated thrice at three different days.

Statistical Analysis

Statistical analysis was done using GraphPad Prism version 9.0.0. Values analysed were taken from Mean ± S.E (n=50, 70 and 100). Data analysis was done through 1- way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test) ($p<0.0001$).

RESULTS

Effect of GLY-induced dopamine neuron degeneration

The impact of lemon fruit juice, Brahmi leaf extract, and Ashwagandha root extract on GLY 41% SL induced neurodegeneration was assessed in *C. elegans* by the analysis of neurodegeneration in any of the four CEP dendrites exhibiting partial or complete loss of GFP signal assessed as breakage in the dendrites (breaks), small

continuous dots seen in the degenerate neurons (blebs) or zig-zag orientation of neurons instead of straight orientation (kinks). In relation to breaks, highest neurodegeneration was observed in the GLY 41% SL arm - 15 folds higher as compared to the control group, followed by GLY + lemon, GLY + Brahmi and GLY + Ashwagandha arms. Comparing GLY vs GLY + plant extract groups, the GLY + lemon reported 4% insignificant decrease in toxicity, GLY + Brahmi reported 13% decrease and GLY + Ashwagandha 87% decrease in toxicity. Similarly, as compared to the three plant extracts maximum neuroprotective effect was seen in Ashwagandha followed by Brahmi and then lemon.

Similarly on analysis of the degeneration in terms of the blebs formation, highest neurodegeneration was observed in the GLY 41% SL arm - 14 folds higher as compared to the control group, followed by GLY + lemon then GLY + Brahmi arm and lastly GLY + Ashwagandha arm. Comparing GLY vs GLY + plant extract groups, the GLY + lemon reported 14% decrease in toxicity, and GLY + Brahmi reported 20% decrease and GLY + Ashwagandha 86% decrease in toxicity. Similarly, as compared to the three plant extract groups, maximum neuroprotective effect was seen in Ashwagandha followed by Brahmi and then lemon. Lastly on analysis of the kinks formation due to glyphosate toxicity, no statistical significance was observed between control vs GLY, control vs GLY + Brahmi and GLY + Ashwagandha arms for data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test). Holistically, GLY caused 90% neuron degeneration, GLY + lemon-70%, GLY + Brahmi-60% and GLY + Ashwagandha-10% as compared to the GLY arm.

Values represented are Mean ± S.E (n=100). Data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test). **:** *** significant degeneration as compared to control; á significant degeneration as compared to Lemon and Brahmi ($p<0.0001$).

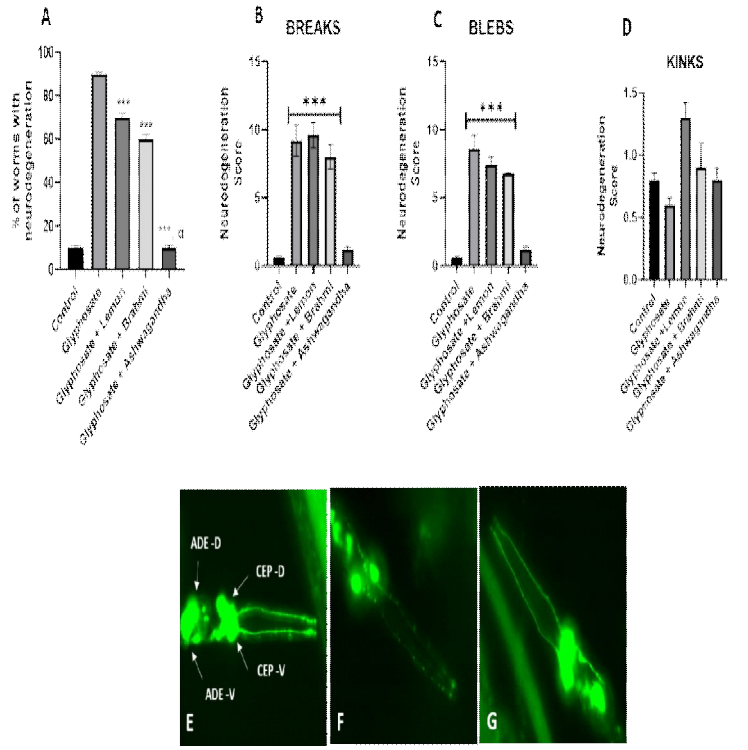

Fig. 1: Evaluating neurodegeneration and the effect of different plant extracts against glyphosate -induced toxicity in *C. elegans*
A: Percentage neurodegeneration in different treatment groups **B:** Breaks/ breakage in dopamine neuron dendrites **C:** Blebs/ small continuous dots seen in the degenerate neurons **D:** Kinks/ zig-zag oriented neurons **E:** Control **F:** Glyphosate treated **G:** Ashwagandha treated

Neuroprotective effect of plant extracts on GLY-induced dopamine neurodegeneration

The plant extracts of lemon fruit, Brahmi leaves and Ashwagandha roots used at a concentration of 600µg/ml/ along with GLY, showed varying degree of neuroprotection against GLY-induced dopamine neurodegeneration in worms. The highest neuroprotection was observed for Ashwagandha (80%) followed by Brahmi (30%) and then lemon (20%). Data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test) showed significant difference in neuroprotection by Ashwagandha, Brahmi and lemon between themselves and as compared to the GLY arm.

Values represented are Mean ± S.E (n=75). Data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test). **:** *** significant movement as compared to control using optical interferometry ($p<0.0001$).

Pharynx pumping visualisation in *C. elegans* on GLY-induced dopamine neurodegeneration

In the present study we found that GLY 41% SL arm – reported 3.33 folds lower (70% decrease) rate of pharyngeal pumping, GLY + lemon reported 27% decrease, GLY + Brahmi 24% decrease and GLY + Ashwagandha 6.6% decrease in the rate of pharyngeal pumping as compared to control. Comparing GLY vs GLY + plant extract groups, the GLY + lemon reported 2.4 fold increase, GLY + Brahmi 2.5 fold increase

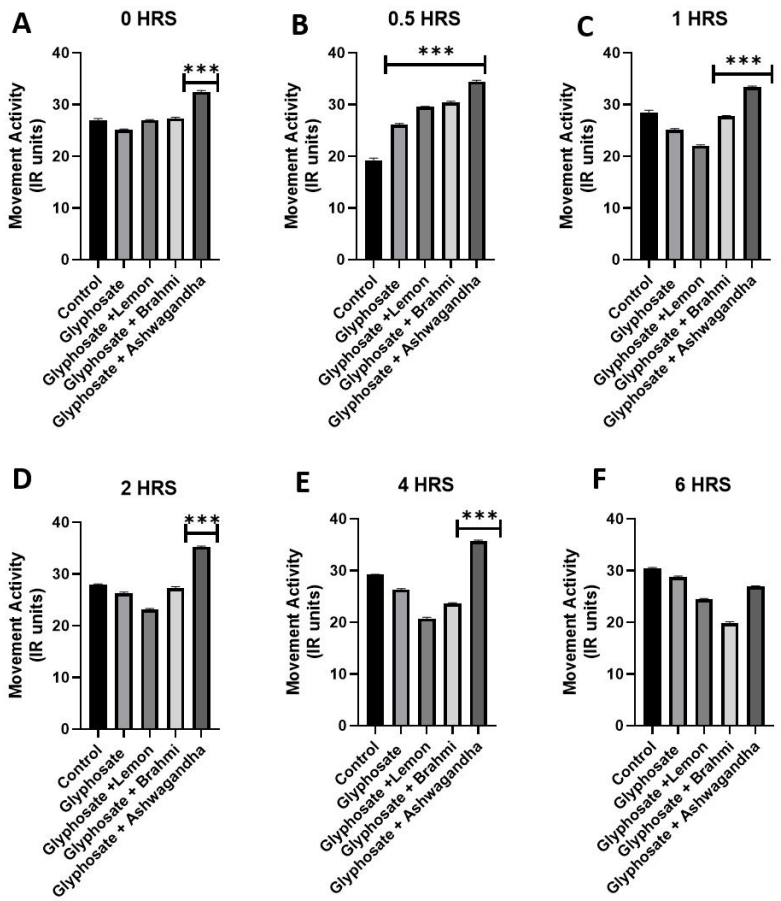

Fig. 2: Arena tracker-based screening of plant extracts and movement activity in different treatment groups of glyphosates induced toxicity in *C. elegans* (BZ-555) at different time points

Behaviour of worm population in different treatment groups at **A:** 0 hrs **B:** 0.5 hrs **C:** 1 hrs **D:** 2 hrs **E:** 4 hrs F: 6 hrs

and GLY + Ashwagandha 3.1fold increase in rate of pharyngeal pumping. Similarly maximum effect of the plant extract on feeding efficiency was observed in the following order: Ashwagandha > Brahmi > lemon.

Values represented are Mean ± S.E (n=100). Data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test). **:** *** significantly increased as compared to glyphosate ($p<0.0001$).

Effect of GLY-induced Dopamine neurodegeneration on motility

The mobility assay utilized scans generated from infrared LED micro-beams from the WMicroTracker ARENA System that tracked the movement and positions of the worms as activity units on the culture dishes and allowed real-time data to be processed at different time points as 0, 0.5, 1, 2, 4 and 6 hrs. Results of the motility activity established that except the Ashwagandha arm all other treatment groups showed a time dependent decrease in the static and dynamic movement activities of the worms. At 0.5 hr the erratic increase in motility activity could be because of the initial acclimatization to culture conditions. From 1-4 hr plant extract from Ashwagandha arm showed a steady statistically significant increase in their motility that ranged from (0 hr-27.05 units IR activity vs 4 hr-30.50 units IR activity). Since at 6 hr exposure, worms failed to show any significant motility activity as compared to control arm, further exposure was terminated.

Values represented are Mean ± S.E (n=100). Data analysed by 1 way ANOVA followed by post-hoc analysis (Tukey Multiple Comparison Test). **:** *** significant as compared to glyphosate ($p<0.0001$).

Discussion8

Herbicides plays a critical role in feeding a growing global population. Farmers around the world have experienced significant socio-economic benefits with herbicide use and GLY in particular. While every agricultural technology impacts the environment, the propensity of GLY's safety profile remains obscure with reports of contamination in biological fluids and possible cancer associations drawing much refutation from the world health organizations. Additional *in vitro* data linking GLY exposure to genetic, oxidative and estrogenic stress has significantly questioned a ban on the use of GLY in many parts of the world. Therefore, the primary objective of the present study was to further understand if GLY inhibits other target sites other than the shikimate pathway and the effect of GLY toxicity on the integrity of the dopaminergic circuitry and functions in *C. elegans* model. In furtherance to these questions, we also proposed to evaluate the effects of different plant extracts like Bacopa monnieri (Brahmi), Citrus limon (Lemon) and Withania somnifera (Ashwagandha) on *C. elegans* dopaminergic integrity on exposure to GLY. Our findings aimed to establish a scientific basis for integrating plant-based treatment regimens for management of dopaminergic toxicities as seen typically in PD.

Among the cellular and transgenic models available to study the pathology of PD, *C. elegans* has distinct advantages. The hermaphrodite has 302 neurons in its body, with a fully mapped neuronal circuitry (Bargmann,

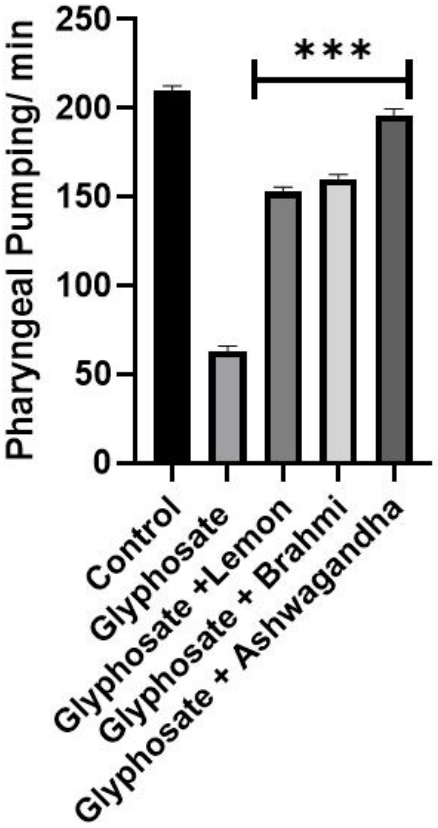

Fig. 3: Rate of pharyngeal pumping in different plant extract groups against glyphosate induced toxicity in *C. elegans*

1998). In the hermaphrodite, of the 302 neurons, 8 neurons are dopaminergic (DA), which include 6 anterior DA neurons (4 CEP neurons and 2 ADE neurons) and 2 posterior DA neurons PDE neurons (Sulston *et al.*, 1975). Since *C. elegans* is completely transparent, neurons are easily visualized by expressing a GFP signal. This is especially useful when studying neurodegenerative diseases, such as PD, since neuronal cell death can be readily visualized and quantified within the live organisms.

Our studies on impact of GLY on the integrity of the *C. elegans* DA neurons clearly established that GLY 41% SL was highly toxic to other target sites such as DA. Our results highlighted that GLY holistically induce 90% degeneration of the DA neuron dendrites. The neuroprotective effect of plant extracts was, however, quite encouraging with the highest neuroprotection observed for Ashwagandha (80%) followed by Brahmi (30%) and then lemon (20%) extracts. Many authors in the past have also investigated the potential of other medicinal plant extracts on the PD like outcomes. In 2022, (Nghi *et al.*, 2022) studied the antioxidant potential of Rumdul (Sphaerocoryne affinis) for PD outcomes. Similarly (Ly *et al.*, 2022) studied the therapeutic potential of *Polyscias fruticosa* (L.) Harms leaf extract for PD treatment on drosophila melanogaster model. Likewise (Arslan & Yýlmaz, 2023) also studied the neuroprotective effects of *Geranium robertianum* L. aqueous extract on the cellular PD modelling.

The pharynx of *C. elegans*, is a neuromuscular pump synonymous with the heart of vertebrates and invertebrates, which contracts and relaxes rhythmically for feeding. Average rate of pharyngeal pumping in normal healthy worms is reported between 250–300 pumps/min under normal conditions. Our results from pharynx pumping studies were concurrent with those obtained from neuroprotection studies. We found that GLY 41% SL arm reported a 70% decrease in pharyngeal pumping rate and the neuroprotective effect of the plant extracts were able to ameliorate this effect with Ashwagandha providing 3.1 fold increase in the rate of pharyngeal pumping followed by Brahmi and lemon extracts. Similar to our study, (Liu *et al.*, 2023) studied that pesticide fluopimomide induced an oxidative stress and mitochondrial damage with reduced pharyngeal pumping as compared to control in their studies in *C. elegans*. Likewise (Wei *et al.*, 2025) studied copper exposure in *C. elegans*. Their study revealed that the behaviour inhibition such as defecation interval, head

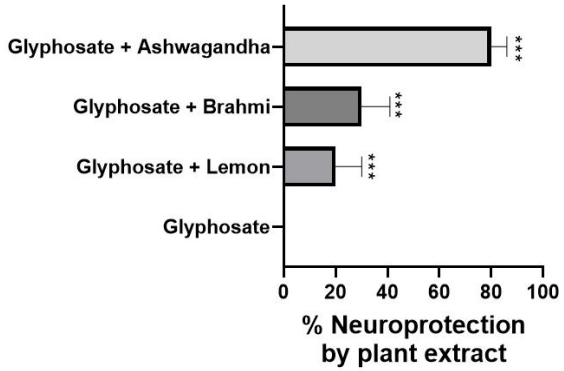

Fig. 4: Neuroprotection by different plant extracts against glyphosate induced toxicity in *C. elegans*

thrash, pumping frequency and neuronal degeneration of GABAergic neurons was caused by neurotoxicity induced through ferroptosis.

Automated worm monitoring is a useful and faster method for studying health span (Zavagno *et al.*, 2023). The effect of GLY-induced neurotoxicity on the worm motility was done through Worm Microtracker ARENA machine that comprehends its locomotion through infrared scattering by worm movements crossing the path of light which is taken as its locomotion. A software counts the number of activity events per time on the plate in IR activity units/time. In our study *C. elegans* that were

incubated for 6 hrs with GLY and different plant extracts clearly indicated that plant extract from Ashwagandha showed a steady statistically significant increase in motility till 4 hr of study which can be considered as an indication of good health of the worms. Similar to our findings another research group (Thakkar *et al.*, 2024) reported Ashwagandha root extract conferring beneficial effects on health and lifespan of the worms. Interestingly the concentration of Ashwagandha studied in both are overlapping in the 600-700µg/ml range. Taken together comparing the three plant extracts from Citrus limon (Lemon) fruit, Bacopa monnieri (Brahmi) leaves and Withania somnifera (Ashwagandha) roots, we find that Withania somnifera (Ashwagandha) roots showed higher neuroprotective potential in terms of amelioration of dopaminergic damage, pharyngeal pumping and motility activity.

Conclusion

Our studies substantiate that GLY inhibits other targets sites other than the shikimate pathway and that *C. elegans* model of neurotoxicity are vulnerable to GLY - induced dopamine neurodegerneration at 10mM concentration. *C. elegans* can be exploited as a valuable system for pesticide screening. Plant extracts tested for amelioration showed varying degree of neuroprotection of dopaminergic system. We conclude that Ashwagandha roots offer neuroprotection from GLY-induced toxicity and can be further investigated for their mechanism of protection in the worms.

Author Contributions

Kisan B Jadav: conceived, supervised, acquired grants and designed the experiments; Shaheen Jafri Ali: data analysis and drafting the final manuscript; Sachin S Patil and K Shruthi Nagarhal: design of experiments, procurement of plant samples and first draft of manuscript; Sanganna Sajjanar, Ayyangouda Patil, Kavita T.R Sharanbasappa Yerri performed experimental design, data interpretation and discussion.

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Conflict of interest

The authors have no conflict of interest to report.

References

Acquavella, J. (2023). Epidemiologic studies of glyphosate and non-Hodgkin's lymphoma: A review with consideration of exposure frequency, systemic dose, and study quality. *Global Epidemiology*, **5:** 100101. https://doi.org/10.1016/j.gloepi.2023.100101

Anjaneyulu, J., R, V. and Godbole, A. (2020). Differential effect of Ayurvedic nootropics on C. elegans models of Parkinson's disease. *Journal of Ayurveda and Integrative Medicine*, **11(4):** 440–447. https://doi.org/10.1016/j.jaim.2020.07.006

Annett, R., Habibi, H. R. and Hontela, A. (2014). Impact of glyphosate and glyphosate based herbicides on the freshwater environment. *Journal of Applied Toxicology*, **34(5):** 458–479. https://doi.org/10.1002/jat.2997

Arslan, M. E., & Yýlmaz, A. (2023). Neuroprotective effects of *Geranium Robertianum* L. Aqueous extract on the cellular Parkinson's disease model. *European Review for Medical and Pharmacological Sciences*, **27(2):** 570–579. https://doi.org/10.26355/eurrev_202301_31058

Bargmann, C. I. (1998). Neurobiology of the *Caenorhabditis elegans* Genome. *Science*, **282(5396):** 2028–2033. https://doi.org/10.1126/science.282.5396.2028

Battaglin, W. A., Meyer, M. T., Kuivila, K., & Dietze, J. E. (2014). Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. *Journal of the American Water Resources Association*, **50(2):** 275–290. USGS Publications Warehouse. https://doi.org/10.1111/jawr.12159

Bauer, K. C., York, E. M., Cirstea, M. S., Radisavljevic, N., Petersen, C., Huus, K. E., Brown, E. M., Bozorgmehr, T., Berdún, R., Bernier, L., Lee, A. H. Y., Woodward, S. E., Krekhno, Z., Han, J., Hancock, R. E. W., Ayala, V., MacVicar, B. A., & Finlay, B. B. (2022). Gut microbes shape microglia and cognitive function during malnutrition. *Glia*, **70(5):** 820–841. https://doi.org/10.1002/glia.24139

Bijwadia, S. R., Morton, K., & Meyer, J. N. (2021). Quantifying Levels of Dopaminergic Neuron Morphological Alteration and Degeneration in Caenorhabditis elegans. *Journal of Visualized Experiments*, **177:** 62894. https://doi.org/10.3791/62894

Bukowska, B., WoŸniak, E., Siciñska, P., Mokra, K., & Micha³owicz, J. (2022). Glyphosate disturbs various epigenetic processes in vitro and in vivo – A mini review. *Science of The Total Environment*, **851:** 158259. https://doi.org/10.1016/j.scitotenv.2022.158259

Cellier, M., Anthony, N., Bruneau, C., & Descatha, A. (2022). Determination of Glyphosate and AMPA in Blood Can Predict the Severity of Acute Glyphosate Herbicide Poisoning. *Laboratory Medicine*, **53(4):** 394–398. https://doi.org/10.1093/labmed/lmac002

Chaufan, G., Coalova, I., & Molina, M. D. C. R. D. (2014). Glyphosate Commercial Formulation Causes Cytotoxicity, Oxidative Effects, and Apoptosis on Human Cells:

Differences With its Active Ingredient. *International Journal of Toxicology*, **33(1):** 29–38. https://doi.org/10.1177/1091581813517906

Ferrante, M., Rapisarda, P., Grasso, A., Favara, C., & Oliveri Conti, G. (2023). Glyphosate and environmental toxicity with "One Health" approach, a review. *Environmental Research*, **235:** 116678. https://doi.org/10.1016/j.envres.2023.116678

Filippi, I., Fernández, P., Grimalt, J. O., Butinof, M., Amé, M. V., & Muñoz, S. E. (2024). Glyphosate and AMPA in saliva and other traditional human matrices. New findings for less invasive biomonitoring to the exposure to pesticides. *Environmental Advances*, **15:** 100474. https://doi.org/10.1016/j.envadv.2023.100474

J J. O'Brien, T. (2022). *Pharyngeal Pumping Assay v1*. https://doi.org/10.17504/protocols.io.b3hiqj4e

Junaid, M., & Gokce, A. (2024). GLOBAL AGRICULTURAL LOSSES AND THEIR CAUSES. *Bulletin of Biological and Allied Sciences Research*, **1:** 66. https://doi.org/10.54112/bbasr.v2024i1.66

Kutzner, C. E., Bauer, K. C., Lackmann, J.-W., Acton, R. J., Sarkar, A., Pokrzywa, W., & Hoppe, T. (2024). Optogenetic induction of mechanical muscle stress identifies myosin regulatory ubiquitin ligase NHL-1 in C. elegans. *Nature Communications*, **15(1):** 6879. https://doi.org/10.1038/s41467-024-51069-3

Leino, L., Tall, T., Helander, M., Saloniemi, I., Saikkonen, K., Ruuskanen, S., & Puigbò, P. (2021). Classification of the glyphosate target enzyme (5-enolpyruvylshikimate-3-phosphate synthase) for assessing sensitivity of organisms to the herbicide. *Journal of Hazardous Materials*, **408:** 124556. https://doi.org/10.1016/j.jhazmat.2020.124556

Liu, H., Fu, G., Li, W., Liu, B., Ji, X., Zhang, S., & Qiao, K. (2023). Oxidative stress and mitochondrial damage induced by a novel pesticide fluopimomide in Caenorhabditis elegans. *Environmental Science and Pollution Research*, **30(40):** 91794–91802. https://doi.org/10.1007/s11356-023-28893-z

Ly, H. T., Nguyen, T. T. H., Le, V. M., Lam, B. T., Mai, T. T. T., & Dang, T. P. T. (2022). Therapeutic Potential of *Polyscias fruticosa* (L.) Harms Leaf Extract for Parkinson's Disease Treatment by Drosophila melanogaster Model. *Oxidative Medicine and Cellular Longevity*, *2022*, 1–18. https://doi.org/10.1155/2022/5262677

Massaro, E. J. (Ed.). (1997). *Handbook of Human Toxicology* (0 ed.). CRC Press. https://doi.org/10.1201/9781439805756

Muñoz, J. P., Silva-Pavez, E., Carrillo-Beltrán, D., & Calaf, G. M. (2023). Occurrence and exposure assessment of glyphosate in the environment and its impact on human beings. *Environmental Research*, **231:** 116201. https://doi.org/10.1016/j.envres.2023.116201

Nagy, K., Tessema, R. A., Budnik, L. T., & Ádám, B. (2019). Comparative cyto- and genotoxicity assessment of glyphosate and glyphosate-based herbicides in human peripheral white blood cells. *Environmental Research*, **179:** 108851. https://doi.org/10.1016/j.envres.2019.108851

Nass, R., & Blakely, R. D. (2003). The *Caenorhabditis elegans* Dopaminergic System: Opportunities for Insights into Dopamine Transport and Neurodegeneration. *Annual Review of Pharmacology and Toxicology*, **43(1):** 521–544. https://doi.org/10.1146/annurev.pharmtox.43.100901.135934

Nghi, N. B. T., Uyen, T. T., Anh, H. M., Linh, D. M., & Thao, D. T. P. (2022). Rumdul (Sphaerocoryne affinis) Antioxidant Activity and Its Potential for Parkinson's Disease Treatment. *Oxidative Medicine and Cellular Longevity*, *2022*, 1–12. https://doi.org/10.1155/2022/8918966

Peillex, C., & Pelletier, M. (2020). The impact and toxicity of glyphosate and glyphosate-based herbicides on health and immunity. *Journal of Immunotoxicology*, **17(1):** 163–174. https://doi.org/10.1080/1547691X.2020.1804492

Pérez-Lucas, G., Navarro, G., & Navarro, S. (2024). Understanding How Chemical Pollutants Arise and Evolve in the Brewing Supply Chain: A Scoping Review. *Foods*, **13(11):** 1709. https://doi.org/10.3390/foods13111709

Portier, C. J., Armstrong, B. K., Baguley, B. C., Baur, X., Belyaev, I., Bellé, R., Belpoggi, F., Biggeri, A., Bosland, M. C., Bruzzi, P., Budnik, L. T., Bugge, M. D., Burns, K., Calaf, G. M., Carpenter, D. O., Carpenter, H. M., López-Carrillo, L., Clapp, R., Cocco, P., Zhou, S. F. (2016). Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA). *Journal of Epidemiology and Community Health*, **70(8):** 741–745. https://doi.org/10.1136/jech-2015-207005

Sulston, J., Dew, M., & Brenner, S. (1975). Dopaminergic neurons in the nematode *Caenorhabditis elegans*. *Journal of Comparative Neurology*, **163(2):** 215–226. https://doi.org/10.1002/cne.901630207

Thakkar, N., Gajera, G., Mehta, D., Nair, S., & Kothari, V. (2024). *Withania somnifera root extract (LongeFera-TM) confers beneficial effects on health and lifespan of the model worm Caenorhabditis elegans*. https://doi.org/10.1101/2024.08.01.606126

Wei, J., Zhang, Y., Shi, W., Lu, L., Zhou, Q., Pu, Y., & Yin, L. (2025). Copper exposure induces neurotoxicity through ferroptosis in C. elegans. *Chemico-Biological Interactions*, **407:** 111369. https://doi.org/10.1016/j.cbi.2024.111369

Zavagno, G., Raimundo, A., Kirby, A., Saunter, C., & Weinkove, D. (2023). Rapid measurement of ageing by automated monitoring of movement of C. elegans populations. *GeroScience*, **46(2):** 2281–2293. https://doi.org/10.1007/s11357-023-00998-w

Zhihang, H., Ezemaduka, A. N., Hongxia, C., Yan, P., Yiwen, G., Nan, Z., Xinrui, L., Shan, G., Guojun, L., Jing, Y., & Bo, X. (2024). The joint toxicity effect of glyphosate and cadmium in a concentration-dependent manner on nematode Caenorhabditis elegans. *Ecotoxicology and Environmental Safety*, **285:** 117081. https://doi.org/10.1016/j.ecoenv.2024.117081