Effect of paraquat on motility in *Drosophila melanogaster*

Singh Sushma Kumari, Gudadhe Shubham and Ahsan Jawaid*
Drosophila Behaviour Laboratory, Department of Biotechnology, Central University of South Bihar (CUSB), Gaya- 824 236, Bihar, INDIA
*jahsan@cub.ac.in

Abstract
Paraquat is a potential environmental toxin that contributes not only to cellular damage, but also causes specific neuronal level damage worldwide. The fruit fly Drosophila melanogaster is a dynamic model system used to study environmental toxin-induced parkinsonism and the mechanisms involved in neurodegenerative disorders. Paraquat, a dopaminergic neurotoxin, has been successfully used in Drosophila to produce characteristic features of Parkinson's disease and to suggest convenient studies of paraquat-induced oxidative stress. Pertaining to the similarity between Drosophila and humans, in this study we investigated the impact of paraquat-induced locomotor defects on third-instar larvae of Drosophila melanogaster as model.

The study was divided into two groups: the first batch of larvae served as the paraquat-treated group and received exposure at doses of 10mM and 20mM paraquat and the second group of untreated larvae served as the control group. Locomotory activity in both the treated and untreated larvae was monitored in a time-dependent manner. A significant decrease in locomotor ability of the treated third-instar larvae was observed with increasing concentrations of paraquat. The results of this study revealed the toxic effect of paraquat on larval motility. The study could lead to better understanding the toxic effects of paraquat on humans.

Keywords: Crawling, *Drosophila melanogaster,* Locomotion, Paraquat, Parkinson's disease, Third instar larvae.

Introduction

Herbicides are chemical agents used to control the growth of undesirable weeds in agriculture and they are considered as weed killers. Use of herbicides is increasing day by day throughout the world because of high demand of labour cost, various choice of application of herbicides, very fast weed control in crop field. Therefore, the extreme and repetitive use of this substance might potentially lead to issues related to residue and phytotoxicity in crop plants, lingering impacts on vulnerable intercrops or subsequent crops, negative effects on non-target organisms and finally health risks to both humans and animals. Currently, approximately 2 million metric tons of pesticides are used annually. This usage consists of 47.5% herbicides, 29.5% insecticides, 17.5% fungicides and 5.5% as other types of pesticides[5].

Herbicide usage for weed control in India has increased by 30% over the past decade. Recently, 51 herbicides have been officially registered for use in different crops in India. Among them, one belongs to category I of the pesticide classification, which is considered extremely harmful. Four fell under the highly hazardous category whereas 26 fell under the moderately hazardous category. Additionally, 24 fall under the fourth group, which is unlikely to have any detrimental consequences.

Paraquat, a broadly used herbicide, is a non-selective compound derived from quaternary ammonium ions[6]. It is soluble in water and poses a significant risk to human health due to its high toxicity. The molecular formula of paraquat is 1,1'-dimethyl-4,4'-bipyridinium. It has been classified as a dipyridyl herbicide. In plants, it interrupts photosynthesis by preventing the electron transport chain (ETC)[8] which belongs to category 2 pesticides. In India, the usage of this paraquat is allowed by the Central Insecticide Board and registration committee. It was available in the form of a concentrated solution with a concentration of 24%. Almost 20,000 people die each year because of the consumption of pesticides in food[13]. These pesticides affect the microbiome of the gastrointestinal area, leading to digestive issues, hormone imbalances and lung cancer.

Paraquat targets multiple organs in the body such as the eyes, skin, heart, liver, kidneys, respiratory system and the gastrointestinal tract. Particularly, deaths caused by mild poisoning were predominantly significant. These deaths are usually caused by pulmonary fibrosis, which is a condition where the lungs become damaged and a slow deterioration in lung function occurs in several weeks after consuming the poison[4]. In adult rodents, paraquat models have been used to produce Parkinsonian pathology and to examine the suppression of oxidative stress and inflammatory pathways. Intraperitoneal injection of low doses of PQ in adult rats and mice leads to the targeted destruction of dopaminergic neurons in the substantia nigra (SN), resulting in a decline in locomotor ability[1,3,11].

Additionally, paraquat, which affects pathogenic genetic processes, shows significant toxicity to *C. elegans* leading to harmful effects on their lifespan, egg production and body size. Embryo growth was reduced by approximately 15% at the lowest dose of 8µM paraquat, However, at 40µM paraquat, it declined by almost 60%[7]. Additional study found that extensive exposure of low levels of paraquat (0.035mM) caused oxidative damage, ultimately leading to cell death and total loss of embryo development in *C. elegans*. The organs and developmental pathways in mammals are comparable to those in humans. However, studies on toxicity in mammalian animals are ethically complicated, costly and time-consuming[10].

The genome content of *Drosophila* is identical that of humans; nearly 75–77% of the genes with human homologs are linked to diseases such as Alzheimer's disease, Parkinson's disease, immune disorders, cancer, cardiovascular diseases and intestinal infections. These genes have been commonly used in toxicity studies. *Drosophila* toxicity assays, on the other hand, give metabolically safe information from a full organism, including all its active nervous, digestive, reproductive and endocrine systems.

Drosophila larvae grow from embryo to adult stage in approximately 10 days in an ideal temperature of 25°C and it has three larval instars. The larva possesses a less complex brain network than adults, making it an excellent model organism for studying the physiology, circuitry and signalling tests related to associative plasticity. The effect of paraquat on locomotion (motility) in fruit fly model is not well understood. Therefore, the objective of this study was to investigate how the exposure of paraquat affects the motor activity in *Drosophila* third instar larvae.

Material and Methods

Fly stocks and media preparation: Wild-type *Drosophila melanogaster* strains Oregon-R, were kept in a temperature-controlled environment at 25°C with a 12-hour day/night cycle. The standard corn meal media containing of 8 gm/l agar (HiMedia; 9002-18-0), 15gm/l yeast extract, 80gm/l corn, 20gm/l dextrose (HiMedia; 50-99-7) and 40gm/l sucrose (HiMedia; 57-50-1). 4 ml/l of propionic acid (HiMedia; 79-09-4) and 0.6ml/l of ortho-phosphoric acid (HiMedia; 7664-38-2) were added to the media to prevent fungal growth.

Chemicals: The chemicals used were obtained from HiMedia, Thermo Fisher and GBiosciences. Paraquat was used for larval treatment. Polyethylene glycol – 6000 (PEG 6000), potassium chloride (KCl), sodium chloride (NaCl), calcium chloride ($CaCl_2$), disodium hydrogen phosphate (Na_2HPO_4) and monopotassium phosphate (KH_2PO_4) were used for larvae isolation.

Isolation of larvae and paraquat treatment: Around 200 fruit flies were placed in fresh corn meal media bottles and then placed in a BOD incubator at a temperature of 25°C for egg laying. After 18-20 hours, all the flies were transferred into another bottle and the bottles with eggs were maintained at 25°C temperature in the BOD incubator for three days to ensure their proper development. Early third-instar larvae were then developed after about 72 hours (three days) of the eggs being laid. For neurobehavioral experiments involving behavioural assays, early third-instar larvae were used. These third-instar larvae were extracted from the upper layer of corn media and carefully collected in a strainer with the help of a soft paintbrush, without harming the larvae. To separate the larvae from the corn media, the media (which contained the larvae) were transferred into a small vial with a 30% solution of PEG-6000 (made by dissolving 300g of PEG 6000 in 1000mL of distilled water).

The debris from the corn meal media settled to the bottom of the vial due to the difference in their relative density, while the early third instar larvae floated to the top. The upper most layer of the vial was emptied into the strainer and gently washed with running water to remove the PEG solution that had adhered to the larval body. After being removed from the cornmeal media and PEG solution, the early third instar larvae were transferred into a glass Petri plate with Ringer's solution (0.5mL). To maintain an osmotic balance, the larvae were protected from drying out while behavioural experiments were performed[9]. The Ringer's solution had 128 mM NaCl, 4.7 mM KCl, 1.8 mM $CaCl_2$, 0.9 mM Na_2HPO_4 and 0.37 mM KH_2PO_4.

After harvesting third-instar larvae from media bottles, the larvae were treated with specific paraquat concentrations. Approximately 20mL of 1% agar solution were poured into a Petri plate. The two concentrations of paraquat used for the treatment of larvae were 10mM and 20mM. In the agar Petri plate, 2.5mL of paraquat solution was added and harvested larvae were transferred into it for treatment overnight. These treated larvae were further used in larval crawling assay.

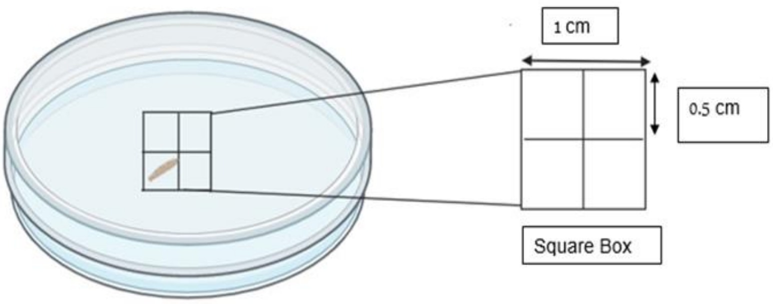

Figure 1: Schematic representation of larval crawling assay.
The number of smaller lines crawled by third instar larvae in 15 seconds are estimated to determine larva motility

Larval crawling assay: Agar petri dish was made by adding 20mL of 1% agar solution. A thin coating of agar was applied on a Petri dish to aid the larva's easy crawling. The agar Petri plate was put on normal graph paper. The locomotory abilities of treated and untreated larvae were examined. One larva at a time treated with a specific paraquat concentration was put in the centre of the square box depicted in the Petri plate graph (Figure 1). After a 3-second pause, larva movement was measured for 15 seconds. The lines they traversed in 15 seconds, were determined. The total number of smaller lines (0.5 cm) travelled by each larva was added together to measure the distance in centimetres (cm) they crawled in 15 minutes.

Statistics: Statistical significance of differences between distance crawled by untreated and treated larvae were evaluated by parametric tests of ANOVA. Similarly, the student's *t*-test analysed the significance of relative motility relative to untreated larvae motility. GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis.

Results and Discussion

The maximum and minimum distance crawled by 10mM paraquat treated larvae were 1.5cm and 1cm respectively within 15 sec. (Figure 2). 20mM paraquat treated larvae crawled an average distance of 1.25cm within the duration of 15 sec. (Figure 3). As the concentration of paraquat was increased, the crawling ability of third instar larvae was observed to be decreasing in a time dependent manner. There was about 55.33 percent decrease in crawling ability of 10 mM paraquat treated larva relative to control within the duration of 15 sec. There was a decrease of 78.66 percent in crawling ability of 20mM paraquat treated larva in comparison to untreated larva (Figure 4).

When the paraquat concentration was increased from 10mM to 20mM for treating third instar larvae, the crawling ability of ability drastically decreased. There was a 23.33 percentage difference in the locomotory activity between 10 mM and 20 mM paraquat treated larvae. The bar graph represents the average distance crawled by larva in 15 seconds for both the treated and untreated larva (Figure 2 and 3). The crawling of larva at higher concentration of paraquat was highly affected due to its toxic effects.

Parkinson's disease (PD) is second most common neurodegenerative disorder primarily characterized by motor symptoms such as rigidity, tremors, bradykinesia (slowness of movement) and postural instability. Locomotor deficits, which encompass difficulties in walking, balance and coordination, are common displays of PD and can significantly affect the quality of life of affected individuals. The age-dependency of locomotor deficits was utilized as a symptomatic marker for Parkinson's disease (PD)[2].

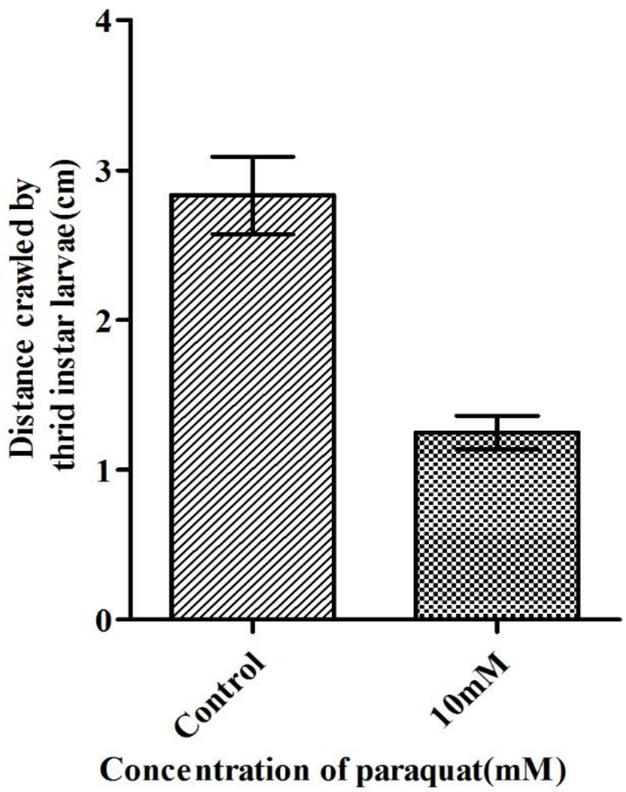

**Figure 2: The bar graph represents effect of 10mM paraquat on the crawling distances of larvae with respect to control, Significant decreases in larvae crawling distances were observed in treated larvae.
Error bar shows mean± S.D. n=10, ***p <0.0001, Student *t*- test**

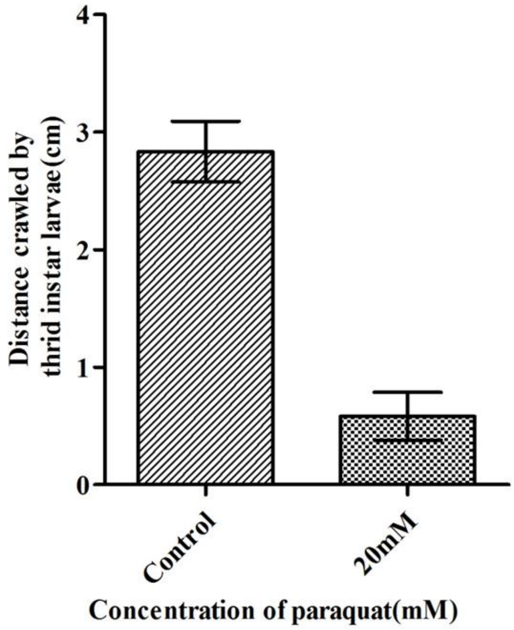

Figure 3: The bar graph represents effect of 20mM paraquat on the crawling distances of larvae with respect to control, Significant decreases in larvae crawling distances were observed in treated larvae. Error bar shows mean± S.D. n=10, ***p <0.0001, Student *t*- test

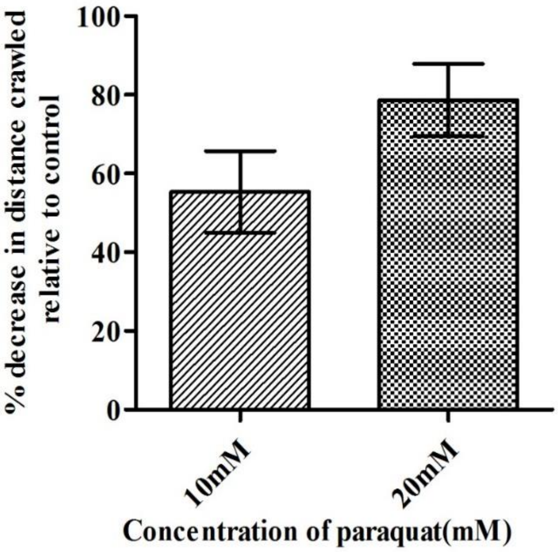

Figure 4: The bar graph represents relative percentage decrease in the crawling distances of 10mM and 20mM paraquat treated larvae with respect to control, Error bar shows mean± S.D. n=10, **p =0.002, Student *t*- test.

The larvae of the *Drosophila* system contain a full range of genetic tools, a nearly 4-day short life cycle and they also contain different defined groups of dopaminergic neurons and demonstrate age-dependent behavioural features of Parkinson, making them excellent models for environmental toxin-induced neurodegenerative disorders. Locomotion assays accessible to larvae provide quantitative measurements of speed and enable detailed analysis of crawling patterns. In contrast, the adult locomotion assay produces a simple binary score, distinguishing between passing the line. In the current study, untreated third instar larvae crawled greater distances than those treated with paraquat, suggesting that exposure to paraquat impaired the locomotor ability of larvae. It clearly indicates that as the concentration of paraquat increased, there was a notable reduction in larval crawling ability. *Drosophila* larvae exposed to paraquat exhibited significant decline in mobility, depending on the concentration used, compared to the untreated control group. This observation suggests an indication of the symptoms associated with neurological disorders. The findings of our study depend on earlier research which highlighted that the combination of paraquat and the fungicide maneb can lead to dopamine depletion in the striatum, loss of dopaminergic cells, aggregation of α-synuclein and motor deficits in rodents[16].

A different study suggested that the systemic intraperitoneal (i.p.) delivery of low doses of paraquat (PQ) in adult mice and rats leads to targeted depletion of dopaminergic neurons in the substantia nigra (SN), accompanied by a decline in locomotor function. Our research investigates into the toxicological effects of different paraquat doses on locomotor ability, offering valuable insights into the behavioural impairments associated with the phenotypes of neurodegenerative disorders.

Conclusion

Our study shows that acute exposure to various concentrations of paraquat leads to a dose-dependent reduction in locomotor ability. Also, interpreting paraquat toxicity in *Drosophila* will help in understanding the effect of paraquat on life processes including fertility, lifespan, motor ability of agricultural workers. Furthermore, the outcomes of our study will serve as a guide for assessing the measurable toxicological impact of paraquat, discovering natural alternatives to these chemicals, particularly through investigation of biological control methods and conducting toxicological research on other substances hazardous to human health.

Drosophila serve as a valuable tool for understanding the mechanisms underlying cancer and neurodegenerative diseases such as Alzheimer's and Parkinson's.

References

1. Bastías-Candia S. et al, Combined exposure to agriculture pesticides, paraquat and maneb, induces alterations in the N/OFQ—NOPr and PDYN/KOPr systems in rats: Relevance to sporadic Parkinson’s disease, *Environ Toxicol*., **30**, 656–663 **(2015)**

2. Bilen J. and Bonini N.M., *Drosophila* as a Model for Human Neurodegenerative Disease, *Annu Rev Genet*, **39**, 153–171 **(2005)**

3. Brooks A.I. et al, Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss, *Brain Res*, **823**, 1–10 **(1999)**

4. Casarett L.J., Casarett and Doull's toxicology: the basic science of poisons, McGraw-Hill, New York **(2008)**

5. De A., Bose R., Kumar A. and Mozumdar S., Targeted Delivery of Pesticides Using Biodegradable Polymeric Nanoparticles, Springer India, New Delhi **(2014)**

6. Grant H.C., Lantos P.L. and Parkinson C., Cerebral damage in paraquat poisoning, *Histopathology*, **4**, 185–195 **(1980)**

7. Hausburg M.A. et al, Effects of paraquat on development of preimplantation embryos *in vivo* and *in vitro*, *Reprod Toxicol*, **20**, 239–246 **(2005)**

8. Jiang T., Sun Q. and Chen S., Oxidative stress: A major pathogenesis and potential therapeutic target of antioxidative agents in Parkinson’s disease and Alzheimer’s disease, *Prog Neurobiol*, **147**, 1–19 **(2016)**

9. Khurana S., Li W.K. and Atkinson N.S., Image Enhancement for Tracking the Translucent Larvae of Drosophila melanogaster, *PLoS ONE*, **5**, 15259 **(2010)**

10. Knight A.W. et al, Evaluation of high-throughput genotoxicity assays used in profiling the US EPA ToxCast^{TM} chemicals, *Regul Toxicol Pharmacol*, **55**, 188–199 **(2009)**

11. McCormack A.L. et al, Environmental Risk Factors and Parkinson’s Disease: Selective Degeneration of Nigral Dopaminergic Neurons Caused by the Herbicide Paraquat, *Neurobiol Dis*, **10**, 119–127 **(2002)**

12. Raghu K. et al, Paraquat poisoning: A case report and review of literature, *J Fam Community Med*, **20**, 198 **(2013)**

13. Shaltiel-Karyo R. et al, A Novel, Sensitive Assay for Behavioral Defects in Parkinson’s Disease Model *Drosophila*, *Park Dis*, **2012**, 1–6 **(2012)**

14. Suntres Z., Role of antioxidants in paraquat toxicity, *Toxicology*, **180**, 65–77 **(2002)**

15. Thiruchelvam M. et al, The Nigrostriatal Dopaminergic System as a Preferential Target of Repeated Exposures to Combined Paraquat and Maneb: Implications for Parkinson’s Disease, *J Neurosci*, **20**, 9207–9214 **(2000)**.

(Received 07th January 2025, accepted 12th March 2025)