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Effect of Acute Exposure to Paraquat on Eye and Wing Development in *Drosophila melanogaster*

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Abstract

Paraquat is a quaternary herbicide commonly applied to different crop fields for broad control of weeds. It is associated with neurodegenerative disease such as Parkinson's disease. *Drosophila melanogaster* is a valuable key model due to its well-characterized genetic framework and brief lifespan. The present study examined the morphological and structural integrity of the wing and compound eye of flies exposed to varying concentrations of paraquat treatment. Analyses of the morphology of paraquat-treated flies and control groups were performed using a stereomicroscope. Paraquat exposure to *D. melanogaster* led to a dose-dependent effect on the morphology of the eyes, affecting both length and width of the eyes. Exposure to 5 mM paraquat showed moderate decrease, whereas exposure to 10 mM paraquat showed significant reduction in length and width of eyes, as compared to the control group with no exposure to paraquat, indicating more severe damage at higher concentration of paraquat. Although, there was no significant variation on the size or shape of wings when compared between the 2 experimental groups. Collectively, the data show that exposure to paraquat adversely affects the size of the eyes in dose-dependent manner, whereas no significant effect on the morphology of the wings was observed due to paraquat exposure in *D. melanogaster*.

Major Findings: Our present research data show that the acute exposure to paraquat affects the morphology of compound eyes in *D. melanogaster.*

Keywords: Compound Eye, *D. melanogaster*, Paraquat, Structural Damage, Wing

1. Introduction

Paraquat is chemically known as 1,1'-dimethyl-4,4'-bipyridinium dichloride, which is an over-the-counter broad-spectrum herbicide whose toxicity is dysfunction of the mitochondrial oxidative phosphorylation process via generation of reactive oxygen species[1]. WHO has rated paraquat as a highly toxic herbicide which, when taken internally, may cause death in humans, although this compound has been very effective in weed control in various crops[2]. The paraquat toxicity of primary molecular pathways includes oxidative stress, mitochondrial disorders, and interference with cell signal transduction[3]. It has been proved that the toxicity of paraquat comes from the compound getting reduced to a free radical that can be reoxidized to give a superoxide anion and cation[4]. They can generate superoxide anion, hydrogen peroxide, and hydroxyl radicals and react with cellular membranes, proteins, and biomolecules. This process generates Reactive Oxygen Species (ROS) that can directly harm cellular membranes, proteins, and other biomolecules. The resulting lipid peroxidation and protein modifications can have significant consequences for the structural integrity and functionality of various

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organs and tissues, including the compound eyes and wings in different organisms.

D. melanogaster is a recently emerging model system for studying toxicants in general and paraquat due to its well-defined genetics, short life cycle, overt phenotypes, availability of robust behavioural assays and manipulative flexibility. The fruit fly has the potential to be used as a powerful model to study the toxic effects of paraquat exposure on the structure and function in an organism[5]. *D. melanogaster* is a comparatively simple model system, but they contain highly developed neuronal connections and have been very helpful for studying some of the most complicated human neurological disorders[6]. Previous studies have reported that paraquat exposure can lead to neurodegeneration, locomotor impairment, and reduced lifespan in *Drosophila*[7].

Few researchers have examined the toxic effects of paraquat on the structural organization in *Drosophila*, but there is limited or no information regarding the impact of this herbicide on the morphology of its compound eyes and wing development or few other organs systems, it has been shown to generate parkinsonism-like symptoms in *Drosophila*, with age-related differences in susceptibility[8]. *Drosophila's* compound eye and wing are suitable for evaluating the structural alterations and developmental defects resulting from exposure to environmental stress factors[9]. The compound eye, containing of numerous ommatidia, is very efficient for detecting disturbances in cellular organization and the function of cells. In the same way, wings with regularly arranged veins and bristles can measure developmental integrity[10]. The present investigation aimed to explore the effects of paraquat on the morphological arrangement of the compound eye and wing of *D. melanogaster*. To this end, morphological measurements using a stereomicroscope were performed to determine the size and morphological alteration that may have been caused by paraquat exposure. The findings of this study can add to the existing literature on potential development and tissue damage consequences of paraquat toxicity and may provide insights into the mechanisms underlying paraquat-induced toxicity. The fly model is an advantage due to the presence of molecular aspects of toxicology and neurobehavioral diseases, as the functional research required to identify diverse mechanisms is expediting.

2. Materials and Methods

Wild-type strain Oregon-R flies and paraquat from Himedia were used in this study. Flies were maintained in BOD incubator on standard cornmeal agar medium at 25° C with a 12-hour light or dark cycle. The cornmeal media used for this experiment contained corn, dextrose, sucrose, agar, and yeast powder and antifungal antibacterial agents such as propionic acid and orthophosphoric acid, which were purchased from Himedia, India. Paraquat exposure was done by adding the required concentration of paraquat dichloride directly to the fly food (corn meal media) after cooking, just before pouring to the media bottles. This was called the experimental bottle. In the control bottle, there was standard corn meal media without paraquat. After the media solidified, 10 male and 10 female flies (same-age flies) were introduced in each bottle for egg lying overnight in the BOD incubator. The next day, flies were removed from the bottles and the eggs laid were allowed to hatch and complete their life cycle keeping in the BOD incubator. The larvae pupated and finally the flies emerged out of the pupae in both the experimental (media containing paraquat) and control bottles (media without paraquat). The newly emerged flies were collected in glass vials, anaesthetized on ice and were kept on the bottom of inverted glass petri plate with blotting sheet on top, and ice bath under the petri plate, for keeping the flies anaesthetized throughout the observation and study under the stereomicroscope. The flies were handled gently using soft camel hair brush. The morphology and dimensions of the compound eyes and wings of both the experimental and control flies were calibrated under the stereomicroscope. The morphological analysis was done using Magnus software of the stereomicroscope.

2.1 Statistical Analysis

Further data analysis was done via a statistical tool (GraphPad Prism). Data from the control and paraquat-treated groups were compared using the students t-test or one-way ANOVA and followed by Dunnett's multiple comparisons test. The significance level of $p < 0.05$ was considered statistically significant.

3. Results and Discussions

We examined the impact of paraquat on the compound eye and wing structures in flies. The quantitative measurement of the compound eye size yielded significantly lower

values in the paraquat-treated group than in the control group. The mean length of the compound eye was 15.1 mm (control), 13.3 mm and 12.9 mm for the 5 mM and 10 mM paraquat-treated flies, respectively (Figure 1).

The compound eye length was reduced by 9.7% and 13.8% for 5 mM and 10 mM paraquat-treated flies as compared to the control group (Figure 2).

Further, the mean width of the control, 5 mM and 10 mM paraquat treated were 11.5 mm,10.2 mm and 10.2 mm respectively as shown (Figure 3).

A significant reduction in width of 5 mM and 10 mM paraquat treated flies was observed and found to be 10.9% and 11.6% decreased as compared to the control group (Figure 4).

These findings show that paraquat exposure to the flies adversely affects the morphogenesis and maintenance of compound eyes in *Drosophila*. Moreover, it was observed that higher concentrations of paraquat significantly reduced the compound eye length and width of flies in a dose-dependent manner (Figure 5).

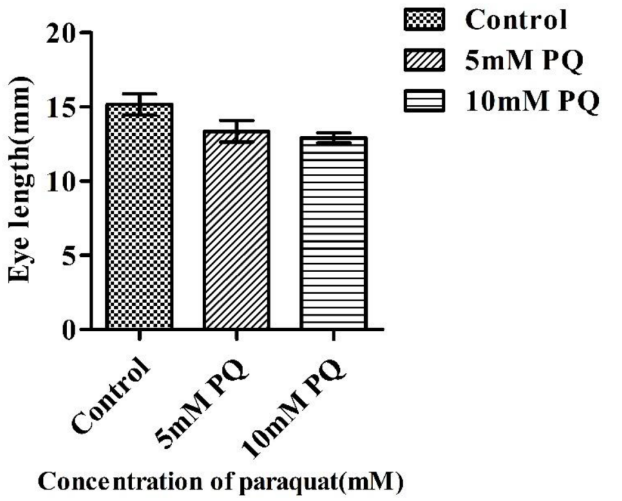


Figure 1. The bar graph represents effect of paraquat on the eye length with respect to control.
Error bar shows Mean± S.D. n=6, ***p=0.0002, one-way ANOVA.

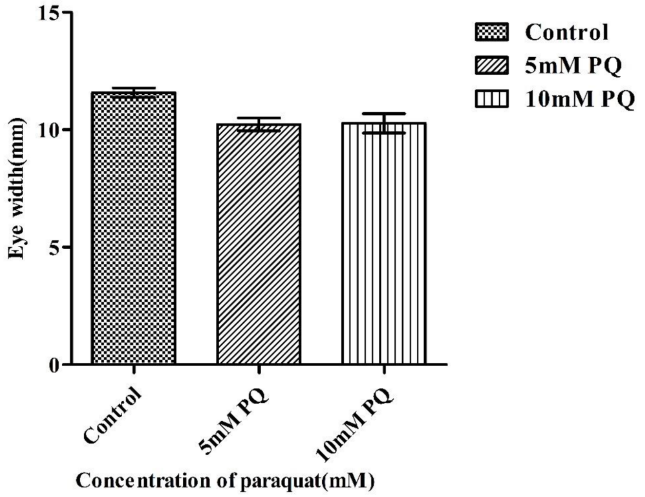


Figure 3. The bar graph represents effect of paraquat on the eye width with respect to control.
Error bar shows mean± S.D. n=6, ***p<0.0001, one-way ANOVA

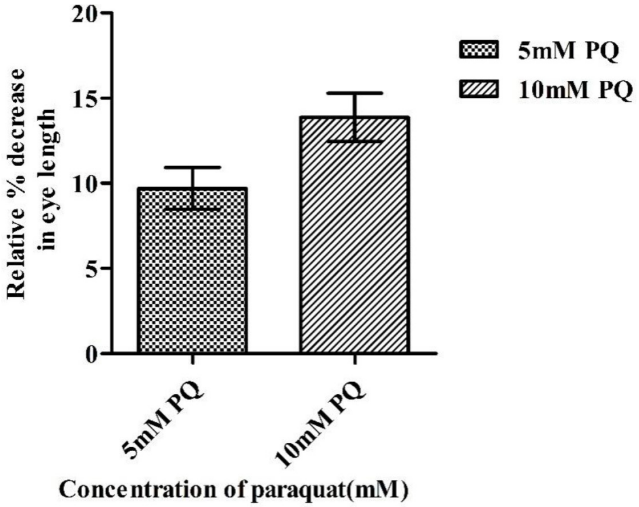


Figure 2. The bar graph represents relative percentage decrease in the eye length of 5 mM and 10 mM paraquat treated flies with respect to control.
Error bar shows mean± S.D. n=6, **p =0.004, Student t- test.

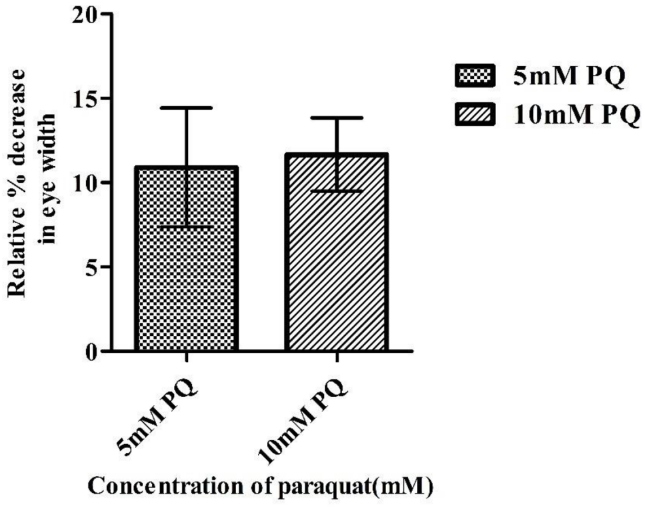


Figure 4. The bar graph represents relative percentage decrease in the eye width of 5 mM and 10 mM paraquat treated flies with respect to control.
Error bar shows Mean± S.D. n=6, p =0.6, Student t- test.

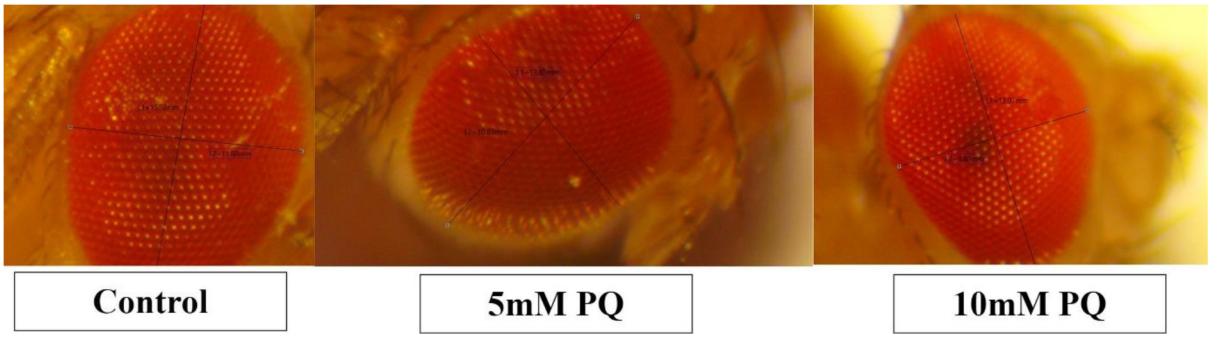


Figure 5. Compound eyes length and width measurement of *D. melanogaster* using stereomicroscope.

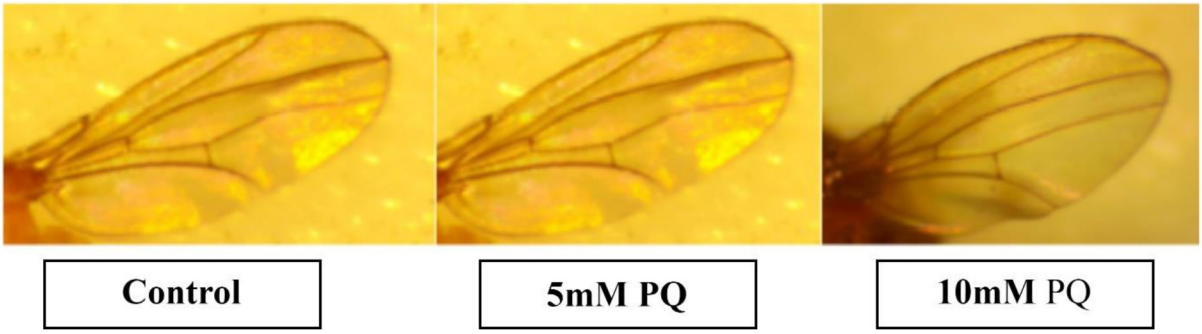


Figure 6. Wing size measurement of *D. melanogaster* using stereomicroscope.

Interestingly, no significant disparity was observed in the size or shape of wings in paraquat treated or untreated flies (Figure 6).

Few studies have been dedicated to understanding the mechanisms of paraquat toxicity in *D. melanogaster*. The present study aimed to evaluate the effects of paraquat on the morphology of compound eyes and wings of young adult flies exposed during embryonic stages to understand the mechanisms of toxicity. Present study discovered the adverse effect of paraquat on the compound eye structure and wing of a non-target insect, *D. melanogaster*. Adults were introduced in corn-meal media containing 5mM and 10mM of paraquat. Our results indicate that paraquat causes reductions in eye size and no significant alterations to wing size. Paraquat, a widely used herbicide, is known for its potent oxidative stress-inducing properties, which can lead to various morphological and physiological changes in biological systems. Similar studies show paraquat exposure leads to retinal degeneration in *Drosophila,* which is characterized by loss of photoreceptor cells and ultra-structural damage[11,12]. Paraquat's oxidative stress-inducing properties may also affect eye morphology in mosquitoes (*Anopheles gambiae*)[13]. Another previous investigation shows that acute paraquat poisoning in mice models produces substantial acute retinal capillary non-perfusion as well as extensive pan-retinal degeneration especially in CX3CR1-deficient mice while demonstrating the importance of inflammatory mechanisms in retinal damage[14]. In addition, the authors observed that paraquat exposure caused lung damage in rats, possibly linked to oxidative stress in the eyes[15]. The combination of abamectin and fipronil caused substantial damage to compound eyes along with dimensional changes to optic lobes which ultimately resulted in eye and optic nerve shape abnormalities[16]. It also says that Flubendiamide affects morphology of *D. Melanogaster's* compound eyes and causes instances of structural changes over four generations. However, it does not deal with morphology of the wings or with other insect species[17].

This implies that paraquat does not impair wing formation under these conditions, although the eye appears to be sensitive to paraquat toxicity. Future studies should be conducted to establish the mediators of this effect or process, and to investigate the possibilities of risk mitigation. The effect of paraquat on the morphological changes of compound eyes and wings in D. melanogaster was assessed in this study. Paraquat has previously been reported to affect few organs development or maintenance in the fruit fly, and based on morphological differences,

we have determined that the compound eye surface area was smaller in paraquat-exposed flies compared to control flies18. This agrees with other studies that show paraquat to be toxic to other organisms. However, the main reason for the toxicity of paraquat has been defined as its potential to produce Reactive Oxygen Species (ROS) that causes oxidative stress and cell damage. The compound eye with highly ordered tissues and high energetic demands may be sensitive to oxidative stress damage[19,20]. Surprisingly, regarding wing size or shape, there was no difference between the paraquat treated and control group of flies. This suggests that paraquat toxicity might affect the formation or differentiation of the compound eye of Drosophila while the wing development remains largely unimpaired under the described conditions. Such differences in susceptibility could be due to differences in antioxidant enzyme induction or other antioxidant defences between the 2 tissues. The oxidative stress markers, such as malondialdehyde and hydroperoxide levels, significantly increase following paraquat exposure, indicating potential damage to eye tissues[21]. Reduction in size of compound eyes observed in paraquat-treated flies might affect vision and fitness levels. Further studies are required to look at the functional implications of paraquat-induced toxicity on eye development and to look at other organs for the long-term toxicity of paraquat. For additional work, the possible impact of this on cells within the eye, as well as oxidative stress, apoptosis, and so on, could be investigated[22]. Several research directions are regarded as promising for future investigations; one of the priority areas is the search for probable protecting factors against the toxicity of paraquat.

4. Conclusion

Therefore, the finding of the current study showed that paraquat treatment caused a decrease in the size of the compound eye of *D. Melanogaster* with the severity increasing at higher concentrations. The control group maintained normal eye length and width, whereas the 5 mM PQ and 10 mM PQ exposed experimental group show a progressive decline in a concentration dependent manner. The exposure to paraquat did not affect the morphology of wings. Thus, the results show that the compound eyes of *D. melanogaster* were more susceptible to paraquat exposure as compared to the wings. The exposure to the herbicide paraquat leads to oxidative stress and other harmful effects in organisms. Further studies may reveal the detailed mechanism of such effects on the development and functioning of organisms and also to investigate possible protective factors. Our study establishes *D. melanogaster* as a useful model organism to study the harmful effects of herbicides like paraquat and its better management.

5. Author Contributions

Conceptualization, S.K.S. and J.A.; methodology, S.K.S. and T.; software, S.K.S. and S.G.; validation, S.K.S. and S.G.; formal analysis, S.K.S. and J.A.; investigation, S.K.S., T. and J.A.; resources, J.A.; data curation, S.K.S. and J.A.; writing-original draft preparation, S.K.S. and J.A.; writing-review and editing, S.K.S. and J.A.; visualization, S.K.S., S.G. and J.A.; supervision, J.A. All authors provided critical analysis and assisted to shape the manuscript. All authors have read and approved to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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