Standardisation of Ld-50: Toxicity Study Using Paraquat Dichloride in *Drosophila Melanogaster* for Various Life History Traits

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ABSTRACT

Paraquat (1,10-dimethyl-4,40-dipyridinium chloride),is an effective herbicide used for crop protection. Several cases of acute paraquat induced poisoning are reported across globe. Toxicity mechanism involves production of harmful Reactive oxygen species (ROS) which causes systemic damage. In present study we tried to study the harmful effect of paraquat dosage on the survival of Drosophila melanogaster.

Keywords: Agriculture, Green Revolution, Paraquat, ROS and toxicity,

1. INTRODUCTION

Agriculture plays a crucial role in shaping the economy of the country. Green revolution was accompanied by extensive usage of pesticides and herbicides to meet the food grain demand of ever increasing human and live-stock populations. Paraquat dichloride is one such organochlorine herbicide registered to control weeds and grasses in many agricultural and non-agricultural areas (WHO 1984). In acute toxicity studies using laboratory animals, paraquat has been shown to be highly toxic [1,2] by the inhalation route and has been placed in Toxicity Category I (the highest of four levels) for acute inhalation effects. This herbicide can even prove to be fatal/ lethal eliciting further various physiological and behavioural effects [3,4], clearly suggesting that it poses a danger to extant life forms present on earth. Insects have been used as excellent model system to ascertain the health hazards of environmental pollutants. The present study was undertaken to investigate the dose dependent toxic effects of paraquat on lifespan in *Drosophila melanogaster*.

2. MATERIALS AND METHODS

Three *D. melanogaster* populations were used in the present study. All the three populations were maintained on three week egg-to-egg discrete generation cycle at standard laboratory conditions of 25 ± 1 °C temperature, 70 ± 5% RH and 24:0 L:D cycle (SLC) on standard banana-jaggery media (SM) [5]. Forty vials each population with approximately 50 eggs per 6ml media were incubated at

SLC till the emergence of all adults. All the adults from 40 vials per population were transferred to breeding population cage mass transfers were done 12th day and was provided with fresh food plate on which a smear of yeast paste was applied. Three days later eggs were collected these flies. At the time of initiating this study, the flies had 9 and half days of pre-adult (from egg till emergence of adult) duration, and an average lifespan of 30 days under SLC.

3. ASSAY FLIES

Parental flies for generating assay flies were derived from running cultures. These flies were termed as Standardized flies. Flies emerging from the eggs collected from standardized flies were separated at 4 h interval according to gender using light CO_2 anaesthesia and maintained as virgins in pre labelled holding vials containing 4ml SM until further use Paraquat stress tolerance assay.

4. SURVIVAL ASSAY

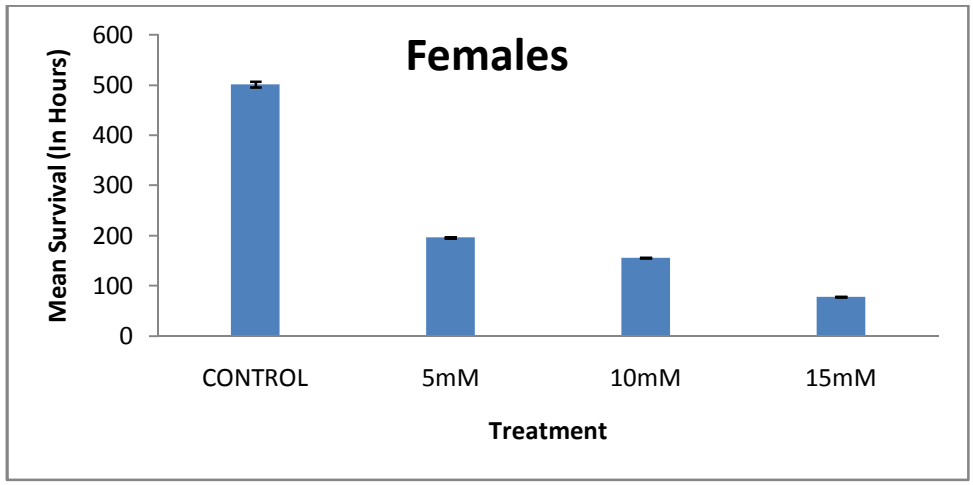
The survival assays were carried out using 3 concentrations of Paraquat namely, 5mM, 10 mM and 15mM. Both male and female flies were given the treatment. Prior to treatment, flies were transferred to pre-labelled vials containing Whatmann filter paper discs of 23 mm diameter saturated with distilled water (DW) for 4 h so as to empty their gut. At the end of 4 h, flies were transferred to pre-labelled vials containing cotton swab moistened with 500µl solution consisting 5% sucrose and appropriate concentration of paraquat, overlaid with 2 Whatmann filter paper discs saturated with the same solution mix. Sucrose was chosen as an external source of energy. Triplicate vials with eight flies each were maintained per population per gender per treatment. Mortality was recorded at two hour intervals, till the death of last fly. Filter paper discs and cotton swab were changed every alternate day so as to prevent desiccation. Controls were maintained in parallel in which only 5%sucrose was added with no chemical and observations were taken at an interval of 24 hours as mortality rate was too less in control.

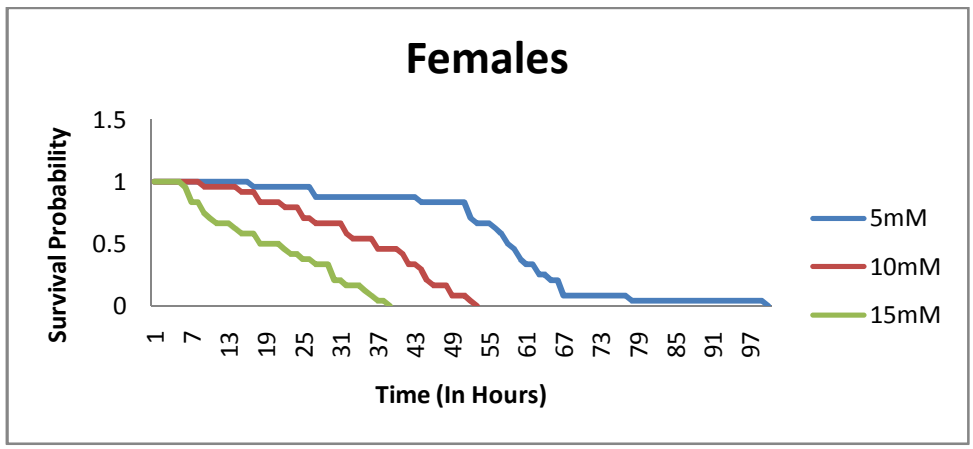
5. STATISTICAL ANALYSIS

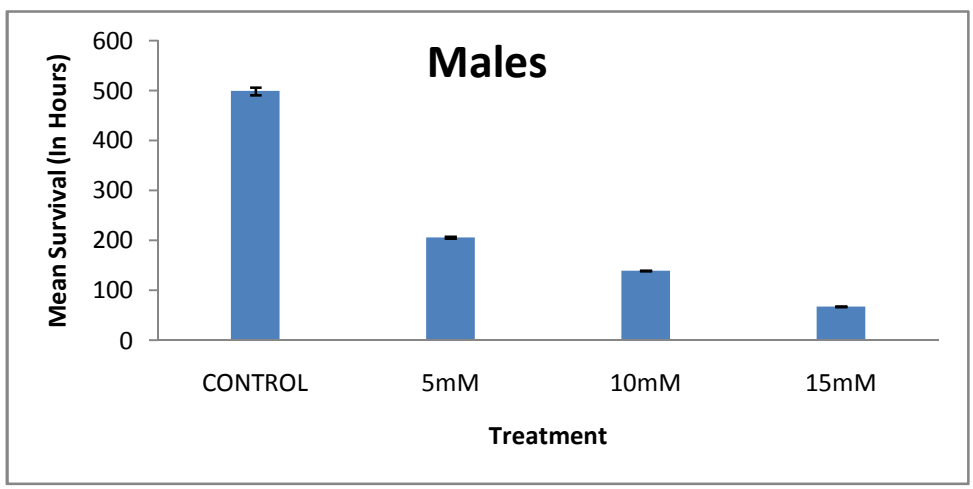
The effect of different treatment concentrations was analyzed by Univariate analysis of variance model. One way Analysis of variance (ANOVA) for different factor was done by SPSS 16.0.

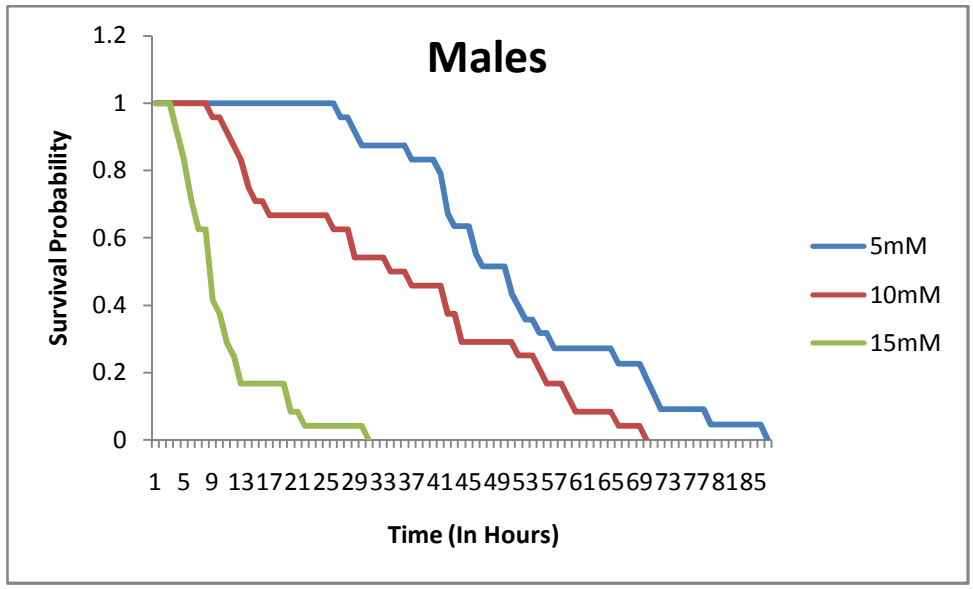
6. RESULTS

There was significant effect of treatment ($F_{3,6}$ = 668.25 p =0.000) on both the sexes and the optimum dosage for further life history assays was chosen to be 5mM, as it gave approximately 50% of mortality in 48 hours. The said dose is sufficient enough to induce in vivo changes. Further, there was concentration dependant mortality with 15mM being the most harmful in both females and males. Although both male and female responded in a similar manner, males were seen to be more susceptible than female at given concentrations.


Females
Mean Survival (In Hours)
600
500
400
300
200
100
0
CONTROL
5mM
10mM
15mM
Treatment


Females
Survival Probability
1.5
1
0.5
0
1
7
13
19
25
31
37
43
49
55
61
67
73
79
85
91
97
Time (In Hours)
5mM
10mM
15mM


Males
Mean Survival (In Hours)
600
500
400
300
200
100
0
CONTROL
5mM
10mM
15mM
Treatment



7. DISCUSSION

Paraquat is deterimental for the survival of flies, is a consequence of generation of ROS which results in damage[6]. The level of damage caused seen to be increased with increase in dosage. For better understanding, paraquat induced oxidative stress at optimum dose further needs to be worked upon.

8. ACKNOWLEDGEMENTS

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