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Impact of rotenone on the climbing ability and survival of wild type *Drosophila melanogaster* (Oregon R^+)

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ABSTRACT

Neurodegeneration is the major problem among the old aged people and is of major concern as it is increasing the social burden. The second major neurodegenerative disease of major concern is Parkinson's disease. One of the major motor symptoms of Parkinson's disease is locomotory incompetence. Here in this study we are using different concentration of rotenone for the generation of the locomotory disability in the wild type *Drosophila melanogaster* (Oregon R^+) strain. With the different concentration of the rotenone we found that the flies are showing the locomotory disability along with the death of flies during the treatment period. In two higher concentrations, 5 ppm and 0.5 ppm of rotenone, the fly dies after 24 hours and 48 hours of treatment respectively thus leading to early mortality.

Keywords:

Parkinson's disease, Rotenone, Climbing, Viability, Oregon R^+, *Drosophila melanogaster*

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INTRODUCTION

A second major neurodegenerative disease is Parkinson's disease which is characterized by the presence of α-synuclein inclusions, called Lewy Bodies (LB) and discriminatory damage of dopaminergic neurons in the substantia nigra pars compacta (SNPC) [5]. The diagnostic feature of Parkinson's disease is motor impairment because of decline of striatal dopamine intensities. Motor diminishing includes tremor, stiffness, retards movement, and unsteadiness of posture [8]. Though, sustained intake of Levadopa leads to various motor dyskinesia's which weakens the benefits of therapy [12]. The procurement of operative treatment for Parkinson's disease is tough because of its complex mechanism which is due to many pathways simultaneously [7].

In present study rotenone is used for the generation of locomotory impairment as it inhibits complex I of mitochondria which is responsible for quantitative ATP depletion, early death and oxidative impairment [10]. The *Drosophila* is used here for the generation of neurodegenerative model as necessary phases of cell biology. Drosophila has been unspoiled during evolution in complex organism like humans. Nearly 75% of the loci related to disease in humans have, one *Drosophila* homolog, which shows higher degree of conservation in Drosophila [9]. In *Drosophila*, rotenone is responsible for locomotive difficulties and degeneration of dopaminergic neurons [3]. The Parkinson's disease model *Drosophila*, induced by rotenone, imitates the same response to L-dopa medication [2, 3]. Additionally, *Drosophila* is beneficial as it allows simple and rapid testing of therapeutics and genetic management [4].

MATERIAL AND METHODS

Drosophila stock and rotenone exposure: The stock of wild type *Drosophila melanogaster* (Oregon R^+) was kindly gifted from Dr. Anurag Sharma, NITTE University, Mangalore, India. The flies were fed on standard *Drosophila* diet according to protocol standarized by Singh et al.[11]. The Drosophila was exposed to different concentration of rotenone that is 5.0 ppm, 0.5 ppm, and 0.05 ppm in the *Drosophila* meal. The flies were exposed to the different concentration of rotenone for maximum of 120 hours (5 days). After the treatment the flies were checked for the survival and the surviving flies were checked for their locomotory impairment.

Locomotory ability:

Locomotor malfunctioning was judged by enumerating climbing ability in a negative geotactic assay [3]. Flies were given treatment for 24 hours in triplicate with ten flies in each vial. Flies were assayed in a transparent plastic column open at one end, from where flies were inserted and there after closed with cotton plug. The line was drawn at 10 cm from the base. Initially 10 flies were shifted to the plastic column and were allowed to facilitate, for 10

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min. Flies were then settled to the base by tapping the column. After 10 sec, the numbers of flies crossed the 10 cm mark and remaining was counted separately. Each vial was tested ten times and each group has three vials, one minute of relaxation given to flies between trials.

Surviving capacity:

Survival was recorded by scoring the number of live flies after every 24 hours. In this the flies were transferred to the normal food (no treatment) and food with different concentrations of rotenone. Initially the 10 flies were transferred to different concentration and the numbers of flies survived were counted after every 24 hours till 120 hours.

Statistical analysis:

All assays were performed three times independent in triplicates. Flies in each vial of the triplicates for locomotory assay were given 10 trials. Results were shown in Mean±SEM. Unpaired Student t-test for statistical significant evaluations between the groups. If the *P*-values are ≤ 0.05 then the result was considered statistically significant.

RESULTS

Locomotory assay:

The flies were assessed for their ability of crossing the mark in 10 sec, was scored. The flies were locomotory impaired with different levels at different concentration. This shows that the rotenone is generating the neurodegenerative model for the study of parkinson's disease. The treatment of rotenone for 120 hours impaired the locomotive power in 0.05 ppm rotenone treated flies showing improvised climbing ability to cross the mark. The flies fed on 5 ppm and 0.5 ppm of rotenone concentration could not be assayed for climbing ability after 120 hours as all the flies dies after 24 hours and 48 hours. The flies are showing less inability in 0.05 ppm concentration, in climbing as compared to the higher concentration of rotenone, the higher concentration showed more effect in 24 hours than the low concentration rotenone. And the flies died in the 5 ppm and 0.5ppm concentration. Only the flie that were viable and can be assayed for longer duration and are only with 0.05 ppm. Higher the concentration more is the effect on climbing inability and had early mortality. None of the flies treated on 5ppm reached the 10 cm mark, that means the effect is dose dependent. This assay is showing the climbing ability after 24 hours only (Figure 1). The flies of treatment on 0.05 ppm can be assayed for climbing ability after 120 hours as the 93% flies survived and have less climbing ability. An increase in the rotenone concentration the climbing inability is also increasing in dose dependent manner.

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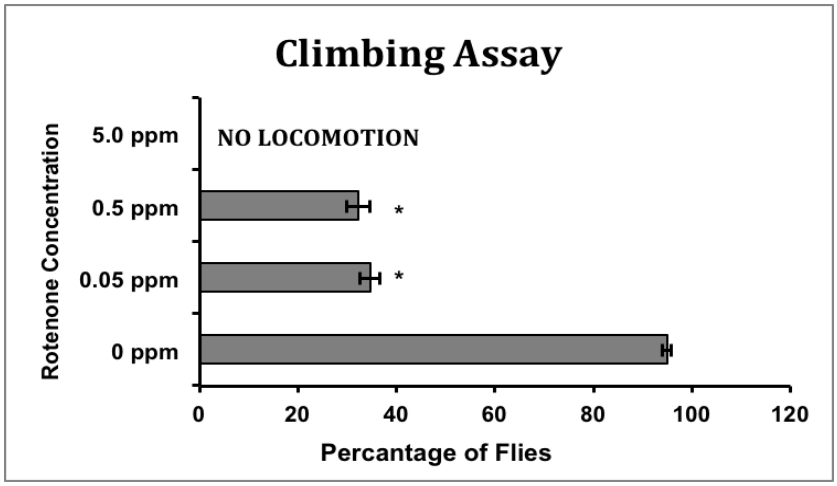

Figure 1: Climbing assay showing the ability in crossing the 10 cm mark in 10 sec in different concentrations of rotenone treatment after 24 hours. All observations are expressed in mean±SEM, n=10, and is performed 3 times. Significance ascribed **P*<0.05 as compared to control.

Surviving capacity:

The flies showed different surviving capacity in different concentrations of rotenone. The flies fed on normal food (no treatment) showed no mortality till 120 hours that is 100% viability. The flies fed on 0.05 ppm of rotenone displays only 93.30% survival and the rest two concentrations that is 5 ppm and 0.5 ppm of rotenone had only survival for 24 and 48 hours respectively (Table 1).

Table1:Effect of different concentrations of rotenone on the survival of wild type *Drosophila melanogaster* (Oregon R^+) at different time intervals

Rotenone concentration	**Percentage of flies survived**					
	0h (Start point)	24h	48h	72h	96h	120h
Normal food without rotenone	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

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Food with rotenone 5 ppm	100±0.00	33.3±0.33	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Food with rotenone 0.5 ppm	100±0.00	43.0±0.33	23.3±0.33	0.0±0.00	0.0±0.00	0.0±0.00
Food with rotenone 0.05 ppm	100.0±0.00	96.6±0.33	96.6±0.33	96.6±0.33	96.6±0.33	93.3±0.33

DISCUSSION

In this study, researchers have made the model for neurodegeneration modelling Parkinson's disease which had been evaluated by assessing their climbing disability. The different concentrations of rotenone lead to the death of the flies at different time intervals. The flies assessed for their locomotory ability confirmed the generation of the Parkinson's disease model. The 0.05 ppm concentration of rotenone in 50 ml food can be used for giving treatment of long duration as the flies showing the neurodegeneration symptoms and survival pattern. So, the different parameters can be assessed on this concentration without improvising the life of flies, and various changes can be seen at different time intervals on the same sample size. In the recent studies the different concentrations of rotenone were used which were higher than the present study but these concentrations were showing early mortality in the flies [1, 6] and the different assays were assessed on different number of flies. An increase in the dose of rotenone exhibited increased climbing disability in our study that may be compared to the previous research where the climbing inability increased with increase in rotenone concentration [3]. The survival of the flies decreased with the increase in rotenone concentration although the concentration used in present study is less than the recent studies [3]. There are many studies present which have used higher concentration of rotenone than the present study showing locomotive impairment and higher concentration leads to

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early death. Here in the present study we are using much less concentration of rotenone which is showing the locomotive impairment and also the no of flies survived decreased with the higher concentration of rotenone along with locomotive impairment.

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Conflict of interest: Authors declare no competing authorities.

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