



The effect of red label tea (RLT) on *Drosophila melanogaster*

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Abstract

In the current study Brooke Bond Red label Natural Care Tea (RLT) was used to observe the effect on *Drosophila melanogaster* and its life cycle. *Drosophila melanogaster* is generally known as fruit fly or vinegar fly. The flies were trapped and transferred into the culture bottle containing the rava-jaggery media. In addition to the natural goodness of tea, RLT contains many ayurvedic ingredients, which are known to have beneficial effects on immune health. The control and treated bottles containing different concentrations of tea extract 0.01g/ml, 0.02g/ml, 0.1g/ml were maintained for two generations. One male and one female fly were transferred to the control and RLT treated bottles. The second-generation progenies were observed. Some of the observations in our study included movement of the larvae, vertical climbing assay, and egg to adult viability, life span and survival rate of the flies, fertility assay and sex ratio. As the concentration of tea extract increased there was significant increase in the viability of eggs, life span and survival rate of the flies, the ability to move against the gravity and fertility of the flies. There was approximately equal number of male and female progeny flies produced in control whereas in the experimental bottles the female progenies were more than that of male flies. Hence, there was a significant effect of RLT on *Drosophila melanogaster*.

Keywords: RLT, *Drosophila melanogaster*, progenies, life span, fertility assay

Introduction

Drosophila melanogaster belongs to the phylum Arthropoda, order Diptera and family Drosophilidae. The species is generally known as fruit fly or vinegar fly. It is also known as the "Cinderella of Genetics" as it is used as a model organism right from the beginning of the 20th century.

At room temperature fruit flies can develop into adults within one or two weeks. At room temperature fruit flies can develop into adults within one or two weeks. The life cycle comprises with egg, 3 larval instars, and a pupal stages. Under the normal environmental conditions, hatching of eggs takes place after 12-15 hours at 25°C (Farzana K.P 2018) [3]. The larva after hatching from the egg undergoes two molts so that the larval period consists of three instars (Deepa P 2009) [2]. The food is taken by resulting larval forms and their growth takes place for nearly four days for pupation. The puparium undergoes a four day long metamorphosis and thus adults will emerge.

From the experimental approach it has been found that chemicals, radiations or any other spices and beverages have adverse effects on *Drosophila melanogaster*. They can alter the morphology, cytology and molecular nature of the organism. Methotrexate (MTX) has teratogenesis (Virginia K.W 2006) [8]. Flies reared on curcumin supplemented diet had significantly higher life span and the progenies of flies reared had a higher viability (Chandrasekhar K.T 2014). The gender specific effects of green tea on the life span of fruit flies and observed that green tea extended the life span of male flies only (Terry Lopex 2014).

In India the common beverages are coffee and tea. Tea may be consumed early in the day to heighten calm, alertness. The tea is available in different forms like ginger tea, masala tea, green tea, black tea and cinnamon tea etc. It contains L-theanine, theophylline, and bound caffeine. Tea has

properties of both the solution and a suspension. It is a solution of water soluble compounds that have been extracted from the tea leaves such as polyphenols and amino acids but in a suspension all the insoluble components are considered such as the cellulose in the tea leaves. Caffeine constitute about 3% of tea's dry weight. The astringency in tea can be attributed to the presence of polyphenols. These are the most abundant compounds in tea leaves making up to 30-40% of their composition. In our experiment we have used Brooke Bond Red Label Natural Care Tea, in addition to the natural goodness of black tea, RLT contains five ayurvedic ingredients and they are ginger, ashwagandha, tulsi, mulethi, cardamom which are known to have beneficial effects on immune health

Green tea is an excellent source of phenolic antioxidants including catechins and epigallocatechin 3-gallate. (Rice Evans C.A 1996) [5] A great deal of scientific interest has been focused on the beneficial health effects of green tea. Epidemiological surveys have provided evidence that heavy GT drinking populations like the Japanese have a lower risk of type 2 diabetes mellitus (Iso H.C 2006) [4] as also lower mortality rates of coronary heart disease. (Unno T 2005) [7] The current study aims at finding the effect of RLT on life cycle, morphological characters and motile behaviour of *Drosophila* flies.

Materials and Methods

***Drosophila* stock (control):** 30 gms of jaggery was added to 300ml of distilled water, allowed to melt, later 30 gms of rava was added for semi solid medium formation. Further 2 gms of agar agar and pinch of yeast powder was added. The *D. melanogaster* flies were trapped to enter the culture bottle and life cycle of flies were maintained for two generations. From the second generation flies one male and one female fly was transferred in the bottles containing

different concentration of RLT as well as for the untreated bottle.

Extraction of RLT: The different concentrations of RLT powder 0.5, 1.0, & 5.0 gm were weighed and soaked in 50ml of distilled water for 15 minutes and was filtered. The 2ml of the filtrate from each concentration were pipetted to the respective media prepared bottles. After cooling propionic acid was added to avoid fungal contamination. The vapours produced were wiped using cotton and the bottles were cotton plugged. The RLT composition was noted which included tea leaves, cardamom (1.5%), ginger (1.5%), mulethi (0.5%), ashwagandha (0.5%), tulsi (0.5%). Some of the observations in our study included movement of the larvae, egg to adult viability vertical climbing assay, life span and survival rate of the flies, fertility assay and sex ratio. These observations were compared for both control and RLT treated.

Results and Discussion

Motility of Third Instar Larvae: The mobility of 3rd instar larvae was compared in both control and RLT treated medium. The time taken for the movement of 1cm distance was recorded. In the normal media, the larvae took 38 seconds to travel where as it took 18.8, 25.5, and 28.8 seconds with 0.01, 0.02, and 0.1 concentration respectively for the movement. This indicated that as the RLT concentration increased the time taken for its mobility also increased. (Table No 01, Fig No 01)

Fertility Assay: Four virgin female and four virgin male flies from the respective holding bottles, were recombined into four independent progeny breeding cages and allowed to mate and age. The flies in these four bottles were maintained on standard rava-jaggery media and were given fresh food every alternative day. The identity and purity of these flies were strictly observed. On the designated age, eggs were collected on a laying plate from the respective holding bottles and dispensed into four bottles each, at a density of twenty five eggs per bottle with 15ml of standard experimental media which contained different concentrations of red label tea powder extract. All the four bottles were incubated at room temperature. The number of emerging flies from each of these bottles were recorded. The sex ratio of the flies were calculated there were approximately 30 females and 20 males were produced in control whereas in the experimental bottles the female progenies were significantly more ie 40 females 5 males were observed.

Vertical climbing assay

The ability to move against gravity and climb is suggested to indicate the level of physical fitness of test animals. Vertical climbing ability of the flies that emerged from different treatment bottles was assessed. Ten flies per treatment group were collected and transferred to the empty 0 to 15 cm graduated vial. The vial was gently tapped and placed in vertical position. The number of flies that crossed 10cm mark in 30seconds was counted. The data is expressed as the percentage of flies that crossed the 10cm mark. By this we conclude that, as the concentration of the tea extract increased the time taken by the flies to move against the gravity decreased. This proves that the flies became very active with the tea extract in their growth medium

Table 1: Motility of third instar larvae

Sl. No	Concentration of tea extract(g/ml)	Distance travelled by 3 rd instar-larvae (cm)	Time in seconds
1	Control	1	38.0
1	0.01	1	18.8
2	0.02	1	25.5
3	0.1	1	28.8

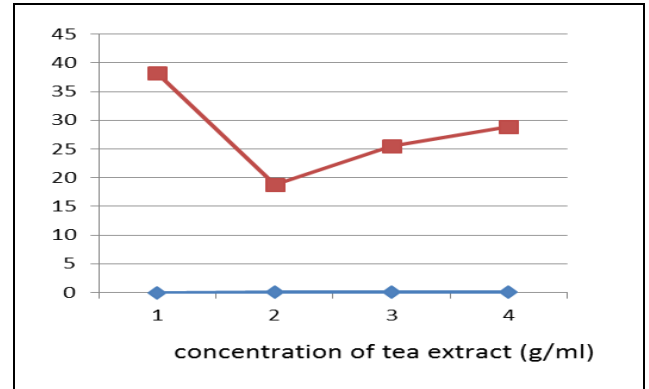


Fig 1: Motility of third instar larvae

Table 2: Vertical Climbing Ability

Sl. No	Concentration of tea extract (g/ml)	Distance travelled by flies(cm)	Time in seconds
1	control	5	7.2
2	0.01	5	5
3	0.02	5	5.6
4	0.1	5	5.8

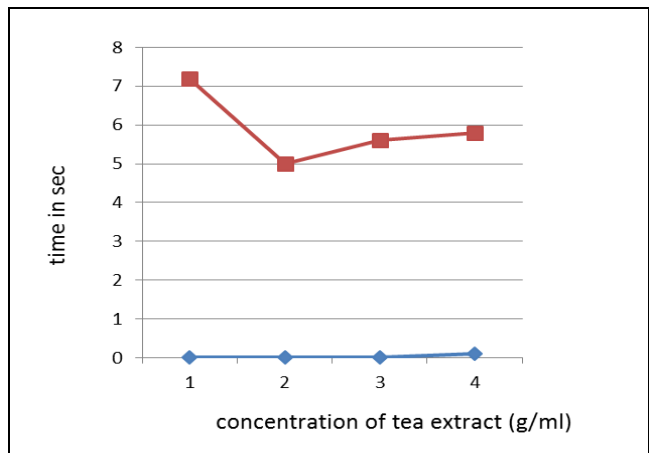


Fig 2: Vertical climbing assay

Longevity in life span and stages of life cycle

The life span and staged of life cycle were compared the result was very significant. The survival rates of both female and male flies were significantly influenced by tea supplementation compared to that of flies in control. The life span of flies in control was around 40 days, but that of the experimental flies were around 45 to 50 days. normally the formation of egg to third instar-larval stage takes 4days and from larval to pupal stage takes 4days at room temperature 28°C, but the flies grown in the experimental media took around 6 days for transformation for both egg to larval stage and also from larval to pupal stage. The age of the flies significantly influenced their fertility, in that

fertility of flies increased with declined in their age. However, supplementing the media with tea extract significantly increased the fertility of the flies.

Conclusion

From the experimental approach it has been found that chemicals, radiations or any other spices and beverages have adverse effects on *Drosophila melanogaster*. They can alter the morphology, cytology and molecular nature of the organism. It has been popularised as an excellent source of health modulating dietary antioxidant which is rich in flavonoids known as catechins, epicatechingallate. Tea may be consumed early in the day to heighten calm and alertness. In the present study the tea supplementation to *D. melanogaster* significantly increased the life span. The current lifestyle demands to identify and evaluate molecules that could potentially increase reproductive health span.

References

1. Chandrashekara KT, Sonam Popli, Shakarad MN. Curcumin enhances parental reproductive life span and progeny viability in *Drosophila melanogaster* American aging association. 2014; 36:9702.
2. Deepa Parvathi V, Akshaya Amritha S, Solomon Paul FD. Wonder animal model for genetic studies- *Drosophila melanogaster*- its life cycle and breeding methods- a review. Sri Ramachandra journal of medicine. 2009; 2:2.
3. Farzana KP. Model for recent advances in genetics and therapeutics, open access peer reviewed chapter, 2018, DOI:10:5772/67731
4. Iso HC, Date K, Wakai M, Fukui A, Tamakoshi E. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med. 2006; 144:554-62.
5. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol Med. 1996; 20:933-56.
6. Terry Lopez, Samuel E. Schriener, Mahtab Jafri. Green Tea polyphenols extend the lifespan of male *Drosophila melanogaster* while impairing reproductive fitness. Journal Of Medicinal Food. 2014; 17:12.
7. Unno T, Tago M, Suzuki Y, Nozawa A, Sagesaka YM, Kakuda T, *et al.* Effect of tea catechins on postprandial plasma lipid responses in human subjects. Br J Nutr. 2005; 93:543-7.
8. Virginia K, Walker Joslynn G, Affleck Katerina Neumann Lily Wong. The Effects of Methotrexate on *Drosophila* Development, Female Fecundity, and Gene Expression *Toxicological Sciences*. 2006; 89(2):95-503.