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Dietary Supplementation of *Ocimum tenuiflorum* Improves Life Span and Shows Anti- Aging Effects in Model *Drosophila melanogaster*

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KEYWORDS
Anti-aging, Drosophila melanogaster, Anti-oxidative, Ocimum tenuiflorum, Oxidative stress, ROS species

ABSTRACT:
Aging is a complex and progressive physiological phenomenon. It is a global challenge characterized by the gradual decline of physiological function and cell integrity, leading to a decline in an organism's health. Out of all the other factors, ROS accumulation and increased oxidative stress are the major factors that cause aging in organisms. This research aims to study the anti-aging effects and their associated mechanism of action of the herbal plant Ocimum tenuiflorum in the model organism Drosophila melanogaster. We studied the various fitness physiological parameters, stress parameters, and oxidative parameters. Our study found that the supplementation of the herbal drug significantly improved the longevity of both sexes in Drosophila melanogaster flies. Also, the plant improved the anti- oxidative enzyme levels, which were decreased in aged flies as compared to control flies. This study concludes that the O. tenuiflorum shows significantly positive effects on the improvement of health, life span, and anti- oxidative stress parameters in D. melanogaster flies.

1. Introduction

Aging is the gradual and complex decline of the organism's cells' functionality, which results in many degenerative activities in the body of an organism. These include a decline in the immune capacity, nervous system, metabolism, stress tolerance, reproductive capacity, and the behavior of an organism, and it finally results in the death of the subject (Lee & Min, 2019). The healthy life expectancy of an organism, including humans, has not increased that much compared to the average life span since aging is directly correlated with many serious diseases like cardiovascular diseases, neurodegenerative diseases, obesity, myopathies, diabetes, cancer, metabolic disorders, etc. (López-Otín et al., 2013; Crimmins, 2015). Model organisms from lower phyla like *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Bombyx mori,* which include mainly invertebrates, have been widely studied to analyze the mechanisms and results of aging (Chattopadhyay & Thirumurugan, 2018).

Dietary supplements with pharmacological properties are able to improve the health of organisms, prevent age- associated diseases, and slow down the aging process. The Lamiaceae family of plants comprises herbs and shrubs popularly known for their aromatic components and various pharmacological activities. *Ocimum tenuiflorum* L., synonym *Ocimum sanctum* Linn. (Commonly Holy Basil or Tulsi) is a well-known and prominent species of this family and is grown throughout the world for various purposes like medicinal, religious, food, ceremonial, essential oils, etc. *O. tenuiflorum* contains volatile oils, phenolic compounds, flavonoids, terpenoids, and various fatty acid derivatives. The plant has been reported for various pharmacological activities like anti-diabetic, anti-cancer, anti-microbial, anti-fecundity (Mousavi et al., 2020; Sharma et al., 2022; Kumar et al., 2022; Singh and Chaudhuri, 2018).

As a model organism to study the aging mechanisms, *Drosophila melanogaster,* commonly

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known as the fruit fly, become evident as a potential model organism to study aging and dementia (Akinyemi et al., 2018). This organism was one of the first known organisms to have a sequenced and complex genome. Its genome comprises approximately 180 Mb, 4 pairs of chromosomes, and 13,600 genes. The fruit fly has 75% disease associated genes that are homologous to humans. Besides that, *Drosophila* has certain peculiar features like a short life cycle, high fecundity rate, short life span (3 months), low maintenance cost, and four different life stages that make it a model organism to study aging. A few basic biological processes are similar in both humans and *Drosophila* like mechanism of glucose utilization and signaling pathways. These advantages make *Drosophila melanogaster* a perfect model for aging studies and related diseases (Piper & Partridge, 2018; Chow & Reiter, 2017; Gumeni & Trougakos, 2019).

In this study, *D. melanogaster* is used as an alternative and complementary model to study anti- aging and anti- oxidative effects of *O. tenuiflorum* during the natural aging phenomenon.

2. Materials and Methodology

2.1 Chemicals and Reagents

Chemicals and reagents used in this research work, such as agar powder, yeast, sodium benzoate, propionic acid, H_2O_2, paraquat, glucose, pyrogallol, phosphate buffer, EDTA, and others, are purchased from Sigma-Aldrich Pvt. Ltd. Company, USA. All the chemicals used are of analytical grade and commercially available.

2.2 Plant Collection, Identification, and preparation of Plant extract

Ocimum tenuiflorum was collected from the Herbal Garden, Maharshi Dayanand University, Rohtak, India. The identification of the plant was performed by a Botanist Associate Professor. Surender Yadav, Department of Botany, Maharshi Dayanand University, Rohtak, India. The plant leaves were collected and dried in the open air for weeks. Dried leaves were ground in a mortar pestle, and the powdered form was stored for further use. The 70% hydro-methanolic plant extract was prepared by a cold maceration process. 100 g of powdered leaves were dissolved in one litre of 70% hydro-methanolic solution prepared in distilled water for 48 h with continuous stirring, filtered using WHATMAN No. 1 filter paper, and concentrated in an oven. The concentrated *Ocimum tenuiflorum* leaf extract (OTE) was lyophilized (Allied Frost Lyophilizer- FD- 3) and stored for further use.

2.3 *Drosophila* stock and husbandry

Wild-type Oregon R species of *Drosophila melanogaster* flies collected from the Department of Biotechnology, UIET, Maharshi Dayanand University, Rohtak, Haryana, India. The flies were cultured on a basal diet prepared from cornmeal (72 g), yeast (40 g), sugar (64 g), and agar powder (15 g) in one litre of water. The sodium benzoate (1g) and propionic acid (3 mL) were used to control the fungal growth. Firstly, the lethal concentration of OTE extract was calculated by treating flies with different concentrations from 0.5 mg/mL to 10 mg/mL. The lethal concentration is calculated at which a 50% mortality rate of flies is observed within 24 h of treatment. The experimental groups 0.5 mg/mL, 1 mg/mL, 2 mg/mL, and 4 mg/mL plant extract diets were prepared. The flies were cultured in plastic vials with 5 mL basal food media (50 flies/ vial) in a BOD incubator (Macro Scientific Works PVT. LTD.) at 25 °C and 60% relative humidity in a controlled manner (80- 100 eggs/vial). The flies were transferred to a fresh vial every 3rd day for better growth.

2.4 Physiological assays

2.4.1 Fecundity assay

The fecundity assay was performed to observe the effect of diet on the egg-laying capacity of the flies. For this, virgin males and

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females from all groups (one pair/ vial; total 5 pairs) were allowed to mate for only 24 h by removing males after mating. The remaining female flies were then transferred to egg chambers (one female/ chamber), and eggs laid by them were counted daily for a consecutive 10 days by transferring flies to fresh chambers daily. The fecundity assay was also performed at 5-day, 25-day, and 45-day-old flies to study the aging-associated decline in fecundity rate of flies. The assay was performed in triplicate.

2.4.2 Lifespan analysis

The survival of flies was measured to study the natural mortality rate and total lifespan of the flies. The survival was measured by the standard method previously described by Yoon et al., 1990 with minor modifications. The newly emerged one-day-old flies (n=200, 20 flies/vial) were cultured on the control diet and the diet having different plant extract concentrations (0.5mg/mL, 1mg/mL, 2mg/mL, 4mg/mL). For survival analysis, dead flies were counted daily, and culture was transferred to fresh food vials every 3rd day to prevent fungal infection. All the other factors, other than natural death, which can cause mortality, were removed. The assay was replicated 3 times and was performed for both sexes separately.

The plant concentration showing the best result in the fecundity assay and lifespan analysis, i.e., 2 mg/ mL, was used for further experiments. All assays were performed on male and female sexes separately. Also, all the assays were performed in triplicate on 5-day, 25-day, and 45-day-old flies.

2.4.3 Negative Geotaxis assay

The assay was performed by placing 10 flies into a climbing assay vial measuring 37× 100 mm. The flies were allowed to get acclimated to room temperature for 10 mins. No external stress was given to the flies. The flies were gently tapped down once, and the total number of flies reaching the top of the vial in 30 s. was counted.

2.4.4 Body weight analysis

Flies were kept in pre-weighted Eppendorf tubes. A total of 10 flies were kept in each Eppendorf tube, and a total of 50 flies were measured (N=50). The body weight of flies was measured by subtracting the weight of the Eppendorf tube and taking an average of all the flies in the tube.

2.5 Stress resistance assays

2.5.1 Cold Shock Recovery Test (CCRT)

The flies cultured on both the control and plant-treated were transferred to empty Eppendorf tubes and left for 30 mins for adaptation (10 flies/ tube, N=50). The tubes with flies were transferred to an ice box filled with ice for 3 h. After 3 h, the tubes were taken out, and the total number of flies gaining full consciousness was counted (Colpo et al., 2017).

2.5.2 Heat Shock Recovery Test

The flies cultured on both control and plant-treated were transferred to empty Eppendorf tubes and left for 30 mins for adaptation (10 flies/ tube, N=50). The flies in tubes were transferred to a hot- air incubator and kept at 38°C for 60 mins. The total number of dead flies was counted every 5 minutes till death of the last fly (Chattopadhyay et al., 2017).

2.5.3 Starvation test

The flies of each group were transferred in vials (10 flies/vial, N=50) having filter paper soaked with 2% agarose, which provides moisture in the absence of any food media. Survival analysis is performed by counting dead flies every 2 h (Colpo et al., 2017).

2.6 In-vitro oxidative stress resistance assay

2.6.1 H_2O_2 stress resistance assay

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Hydroxyl radicals (●OH) serve as the major factor that causes oxidative stress in a living system. This assay was performed to study the resistance of wild-type strain flies against hydroxyl radicals produced by H_2O_2. The flies were fed on 2 mg/ mL OTE and control food. After feeding for 5 days, 25 days, and 45 days, the flies were starved in new empty vials for 4 h. After starving, the flies (50 flies) were kept in fresh new vials (10 flies/ vial) having filter paper soaked with 30% H_2O_2 diluted in 6% glucose solution. The number of dead flies was counted every 2 h until all the flies were dead. The assay was performed for both sexes separately (Wang et al, 2015).

2.6.2 Paraquat- resistance assay

PQ (1,10-dimethyl-4,40-bi-pyridinium dichloride; Pq2+) is an herbicide that can produce superoxide anion (O_2^-) in a living system. After feeding, the flies from all age groups were starved for four h before the experiment. The flies (50 flies) were transferred to fresh vials (10 flies/vial) having filter paper soaked with 20 mM paraquat in 6% glucose solution. The number of dead flies was counted every 2 h until all the flies were dead. The assay was performed for both sexes separately (Wang et al., 2015).

2.7 Biochemical analysis

2.7.1 Preparation of sample homogenate for assays

50 flies from control and OTE- treated groups (male and female separately) were anesthetized in ice and homogenized in potassium phosphate buffer (0.1M; pH- 7.4) and centrifuged at 10,000g for 10 min at 4°C in a refrigerated centrifuge (1mg/10µl). The supernatant was separated from the pellet and stored in labelled Eppendorf tubes at -20°C. The sample was used for the determination of Superoxide dismutase (SOD), Catalase activity, and total protein concentration. All the assays were performed in triplicate for each of the 3 replicates of control and OTE-treated flies.

2.7.2 Superoxide Dismutase

The enzymatic activity of SOD is measured by monitoring the auto-oxidation of the substrate pyrogallol. The assay mixture contains 1.5 mL Tris buffer (50 mM), 0.5 mL EDTA (1 mM), and 0.5 mL tissue homogenate. The reaction was initiated by the addition of 1mL pyrogallol (0.2 mM). The Volume of the total reaction mixture is 3 mL. The increased absorbance was monitored at 420 nm for 3 min. One unit of enzymatic activity (SOD activity) is described as the amount of enzyme required to cause 50% inhibition of substrate (pyrogallol) autoxidation. Results have been expressed as Units/mg protein of tissue homogenate (Marklund and Marklund, 1974).

2.7.3 Catalase (CAT)

Catalase (CAT; EC 1.11.1.6) activity was determined by monitoring the removal of H_2O_2. The reaction mixture contains 1800 µl of 50 mM phosphate buffer (pH 7.0), 180 µl of 300 mM H_2O_2, and 180 µl of sample (1:50 dilution). The absorbance was taken at 240 nm for 3 min (30s intervals) using a UV-Visible spectrophotometer (Thermoscientific Genesys 10S UV- Vis spectrophotometer). The catalase activity was expressed as µmol of H_2O_2 consumed/min/mg protein. (Aebi, 1974).

2.7.4 Total protein concentration

The concentration of protein was determined by the method of Lowry et al., 1951 with minor modifications. BSA was taken as standard.

2.8 Statistical analysis

The data were expressed as mean ± SEM. Statistical analysis was performed by using GraphPad Prism version 10.2.0. One-way ANOVA, Two-Way ANOVA, and Tukey's Multiple comparison test were conducted for significance analysis. Kaplan- Meier analysis and Log-rank test were conducted for survival analysis. The p-value < 0.05 was considered significantly different.

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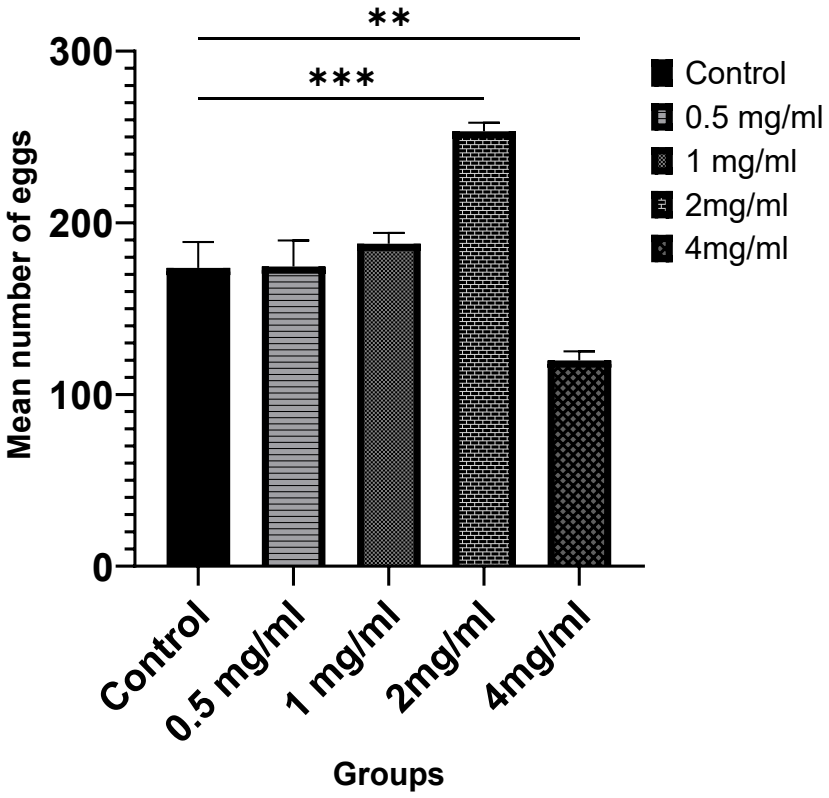
3. Results

3.1 Effect of plant extract on Fecundity

As shown in Figure 1. when *Drosophila melanogaster* flies were fed with an OTE-supplemented diet, the plant showed a hormetic effect on the fecundity of flies. The fecundity of flies improved when they were fed a concentration of 2mg/mL, compared to the control group. With an increase in the plant extract concentration in the diet, it is observed that the eggs laid by the flies decreased. As we can see, 0.5 mg/mL, 1 mg/mL, and 4mg/mL plant extract diet causes a decrease or no improvement in fecundity of *Drosophila* flies as compared to control flies. The plant extract 2 mg/mL increases the egg laying capacity up to 45.8% level (***p value < 0.0001), and an increase in concentration beyond this threshold, decreases the fecundity of flies. These findings suggest that 2mg/mL *Ocimum tenuiflorum* will be the optimal dose for improving fecundity and help in improving the life span of *D. melanogaster*.

To investigate the effect of 2 mg/mL OTE-diet on declined fecundity in aged flies, the fecundity is determined in 5-day-old, 25-day-old, and 45-day-old flies by mating virgin male and female flies (3 pairs) of the same age in all groups. After mating for 24 h, males were removed, and the number of eggs laid by the female after 24 h was counted. The elderliness caused by the aging phenomenon significantly decreases the fecundity of female flies. It is observed that the supplementation of 2 mg/mL OTE in the diet significantly improved the declining fecundity of flies. The highest and lowest fecundity rate is observed in 5-day and 45-day-old flies, respectively.



(a)

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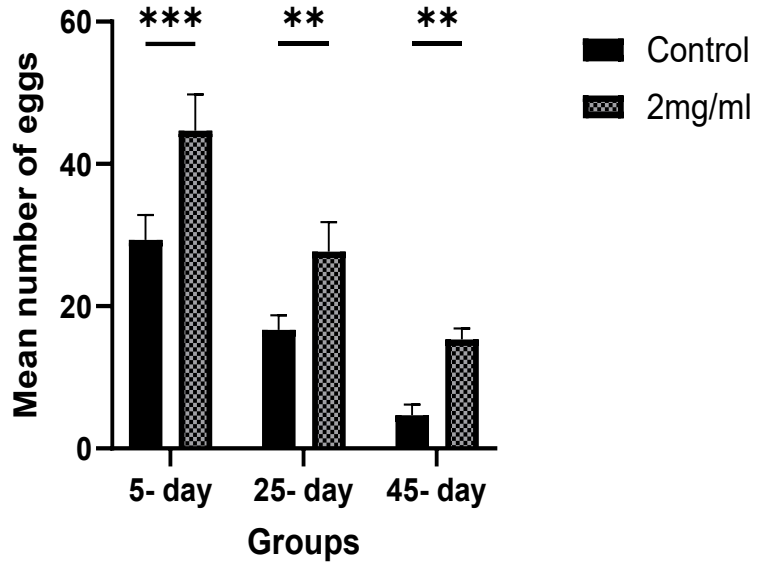

(b)

Figure 1. (a) Effect of different concentrations of plant extracts on the fecundity of newly emerged flies. (b) Showing the effect of 2mg/mL OTE-supplementation on the fecundity and development in different age groups of flies.

3.2 Effect of plant extract on Life Span, Negative geotaxis, and Body weight of flies

The effect of OTE on the life span extension of male and female fruit flies is shown in Figure 2. Effect of different OTE- plant extract concentrations on the life span of *D. melanogaster* flies (a) Male (b) Female . The maximum life span was 76 ± 3.2 days and 96 ± 2.6 days in female flies, while 73± 4.5 days and 87±3.1 days in male flies in control and 2 mg/mL OTE- treated groups, respectively. The 50% survival time is 58± 4.1 and 51± 3.7 days in 2 mg/mL OTE- treated female and male flies (45%, **p <0.007 and 34% increase, *p <0.03, respectively) as compared to 40± 5.3 days and 38± 2.6 days in control female and male flies, respectively. The mean lifespan in the control group was 37± 1.5 days (male), and 38.5± 2.7 days (female), while in the 2 mg/ mL OTE- treated group was 44± 3.2 days (male), 48.5± 2.8 days (female), respectively. The significant improvement in life span is observed in the 2 mg/mL OTE group as compared to the control group by the Kaplan-Meier test. No significant difference is found between any other OTE group and the control group.

The climbing ability of flies shows their physical health and activity. As shown in Figure 3. It is observed that with age, the physical performance of flies decreases. We observed that 2 mg/mL OTE supplementation in the diet improved the locomotor performance in 5-day-old, 25-day-old, and 45-day-old flies when compared with control flies. The locomotor activity of female flies increased by 20.7%, 22.7%, and 107% at 5 days, 25 days, and 45 days, respectively, as compared to the control group. Also, in male flies, climbing ability increases by 17.7%, 17.4%, and 158% at 5 days, 25 days, and 45 days, respectively.

To determine the underlying mechanisms of OTE in enhancing longevity of fruit flies, it is first determined whether OTE supplementation mimics calorie restriction (CR), which is the most

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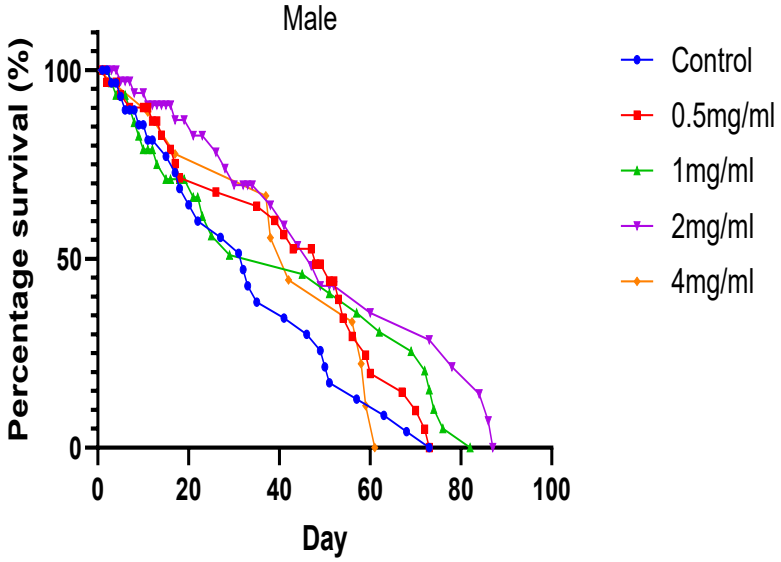
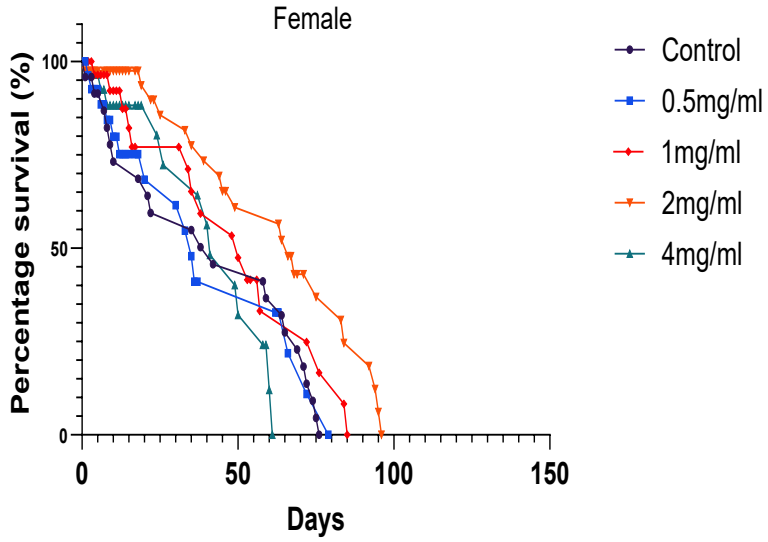
commonly known longevity-promoting mechanism. As shown in Figure 4, the mean weight of males and females at 5-day, 25-day, and 45-day is 0.65± 0.01, 0.746± 0.003, and 0.80± 0.005 mg; and 0.88± 0.003, 0.99± 0.006, and 1.28± 0.008 mg (female) respectively in the case of control groups. It can be observed from the figure that the OTE supplementation to flies does not cause any significant difference in the body weight of flies at any age. The mean weight of both male and female flies in case of 2 mg/mL OTE- supplemented group at 5-day, 25 day and 45 day was 0.67± 0.005, 0.76± 0.003 and 0.81± 0.003; and 0.92± 0.003, 1.05± 0.3 and 1.33± 0.01 mg respectively, which are almost similar to control groups depicting that the OTE supplementation not follow CR mechanism for enhancing the longevity of the flies.



(a)



(b)

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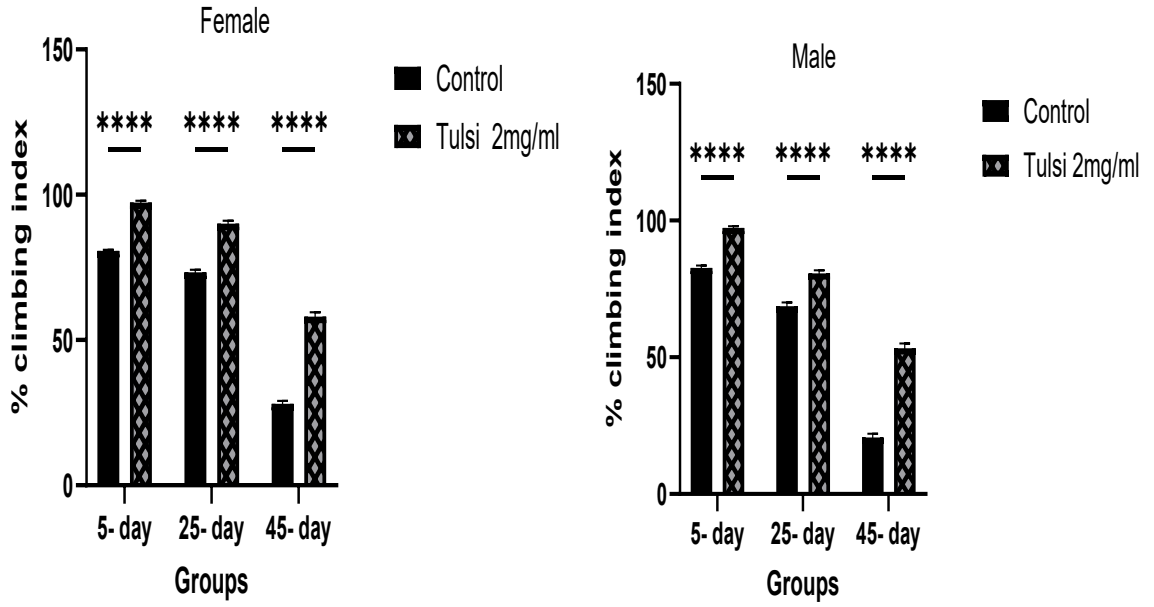
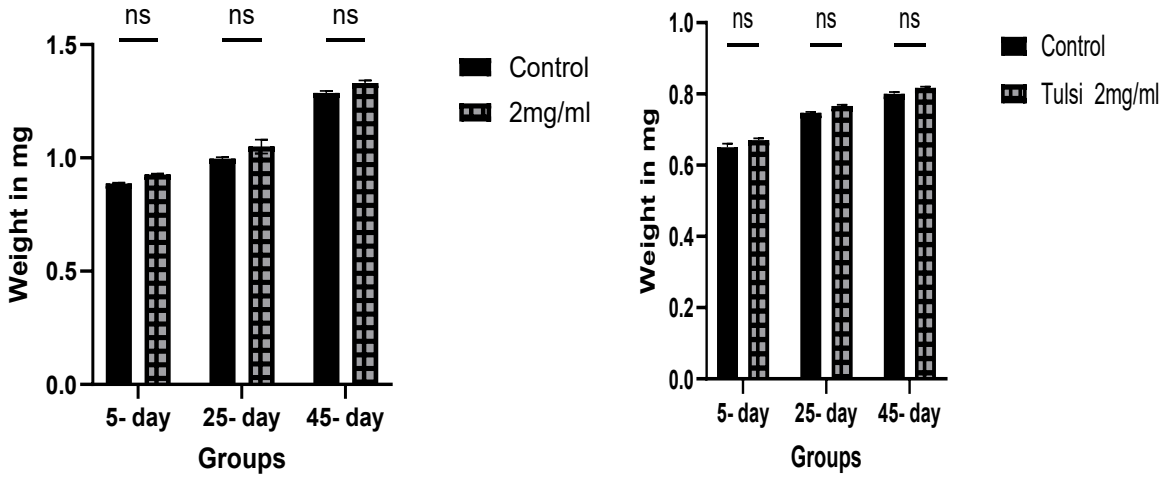
Figure 2. Effect of different OTE- plant extract concentrations on the life span of *D. melanogaster* flies (a) Male (b) Female



(A) Female (B) Male

Figure 3. Effect of 2mg/mL OTE on the climbing ability of male and female *D. melanogaster* in different age groups as compared to control flies



(A) Female (B) Male

Figure 4. Effect of 2mg/mL OTE on the body weight of male and female *D. melanogaster* in different age groups as compared to control flies

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3.3 Effect of plant extract on in vitro oxidative stress resistance

Table 1. As compared to the control group, OTE supplementation increased the survival time of flies during the intensive paraquat challenge test. The maximum survival time for male and female flies in the control group was 36± 2.3 h and 40± 3.1 h, whereas it increased to 48± 3.2 h (33% increase, **p <0.001) and 52± 1.6 h (30% increase, *p < 0.01) in 2 mg/mL. Similar results were observed during the H_2O_2 challenge test. The maximum survival times were 38± 2.8 and 34± 1.3 h in control female and male flies, and 46± 3.8 h (21% increase, *p< 0.01) and 40± 1.7 h (17% increase, *p< 0.03) in 2mg/mL OTE- treated female and male flies, respectively.

As shown in Figure 5 and

Table 1. The 50% survival time, maximum life span, and mean lifespan of flies of all groups (a) Female flies (b) Male flies.

Table 1 (a)

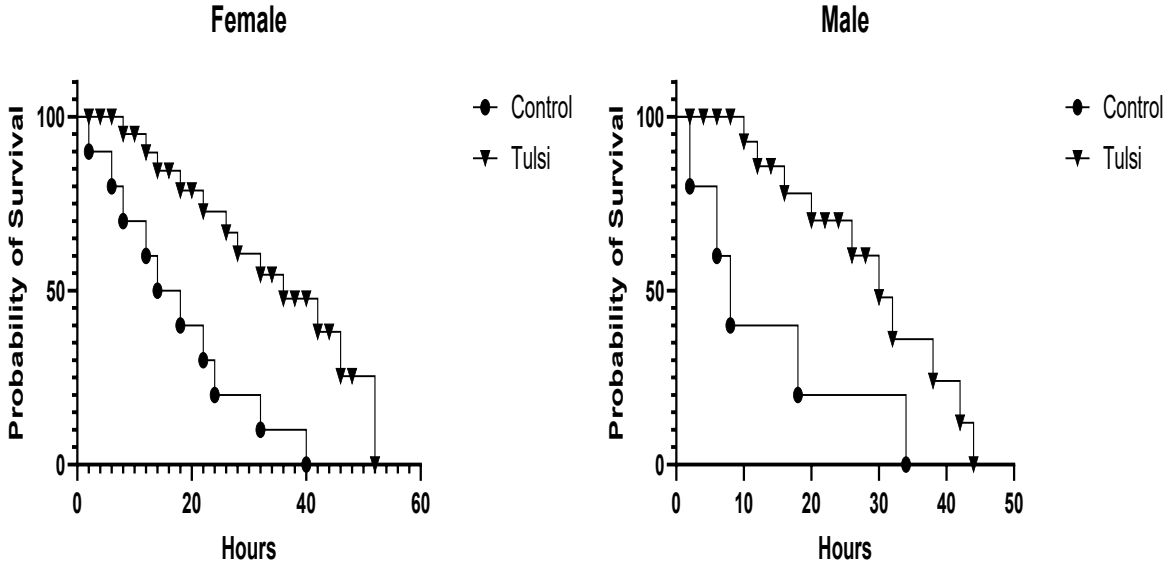
	Groups	50% survival time	Maximum life span	Mean life span
Paraquat- resistance assay	Control	23± 2.6 h	40± 3.1 h	20.5± 3.1 h
	2mg/mL	28± 2.9 h*	52± 1.6 h	26.5± 1.7 h
H_2O_2- resistance assay	Control	21± 1.3 h	38± 2.8 h	19.5± 1.2 h
	2mg/mL	27± 1.1 h*	46± 3.8 h	23.5± 2.4 h

*p< 0.01 and *p<0.01

Table 1 (b)

	Groups	50% survival time	Maximum life span	Mean life span
Paraquat- resistance assay	Control	22± 2.6 h	36± 2.3 h	18.5± 2.1 h
	2mg/mL	26± 1.4 h**	48± 3.2 h	24.5± 1.2 h
H_2O_2- resistance assay	Control	19± 0.8 h	34± 1.3 h	17.5± 1.9 h
	2mg/mL	23 ± 1.7 h*	40± 1.7 h	20.5± 1.1 h

**p<0.001 and *p< 0.03



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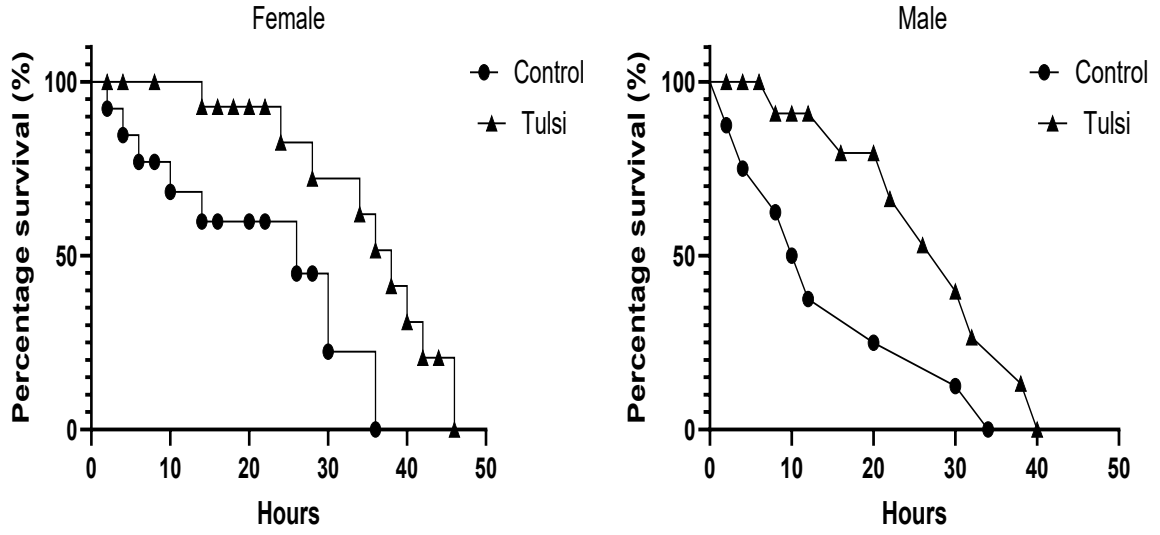
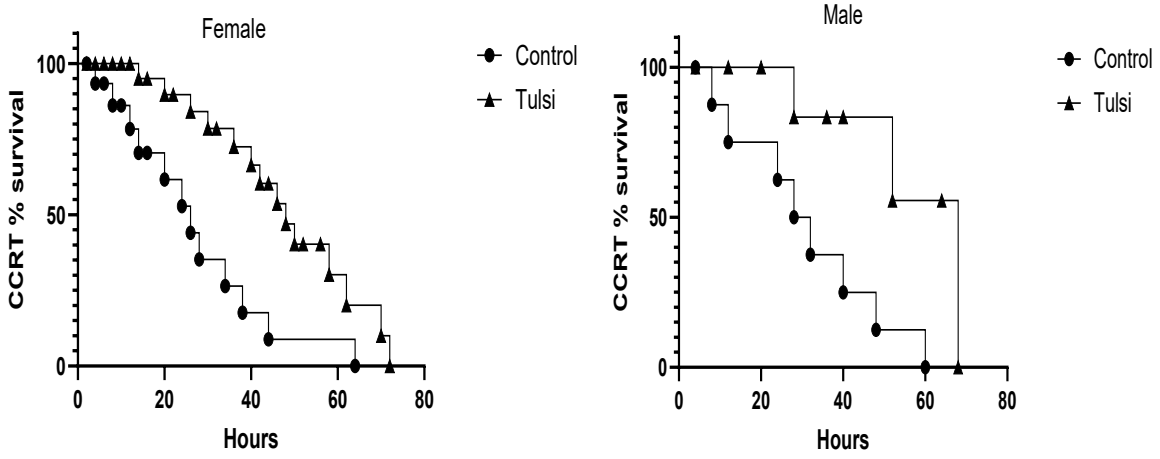


5(c) 5(d)

Figure 5. Depicting the effect of 2mg/mL OTE on paraquat [5 (a) and (b)] and H_2O_2 [5 (c) and (d)] on 5-day-old female (Left) and male (right) *D. melanogaster* as compared to control flies



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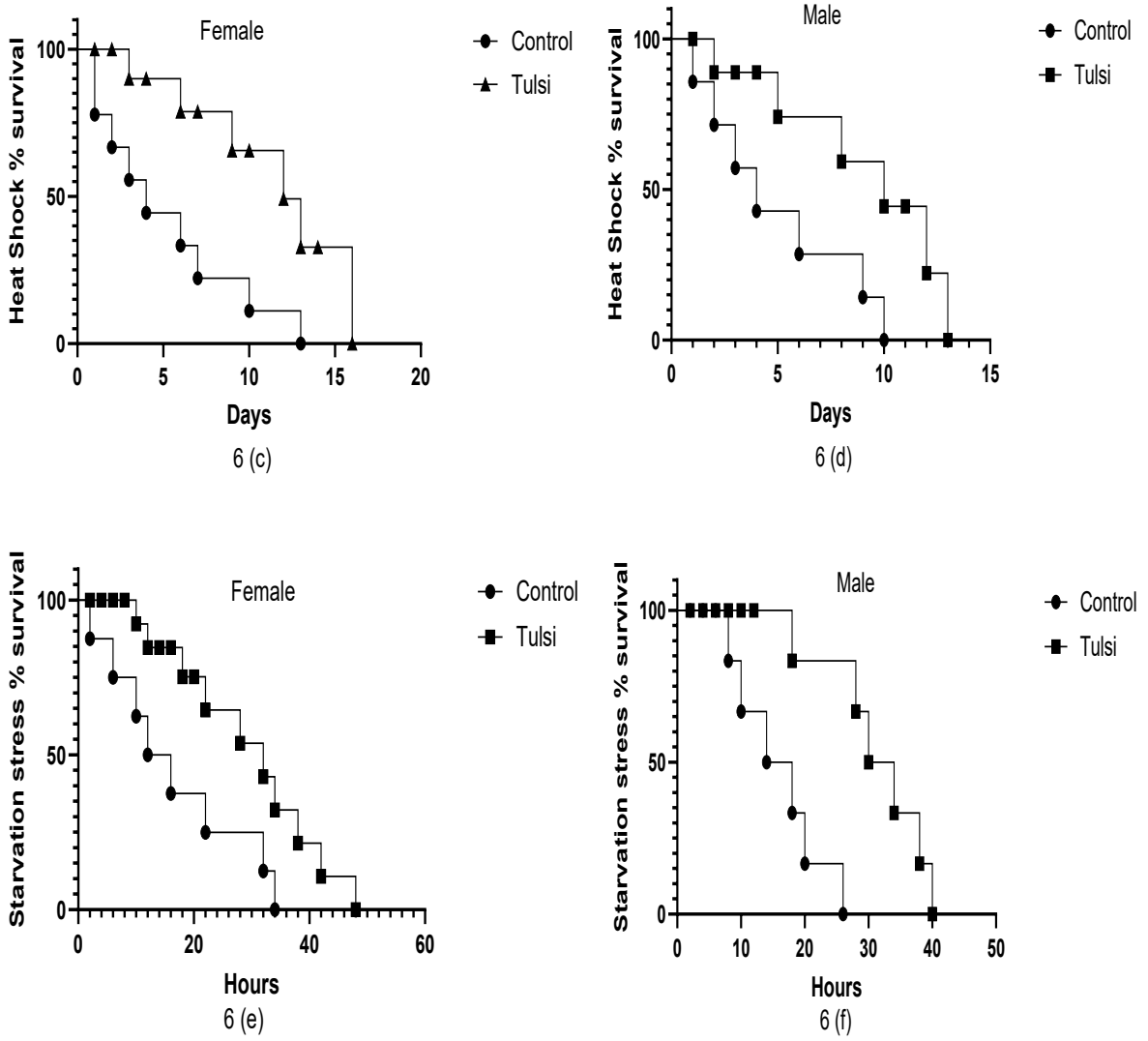

Figure 6. Effect of 2mg/mL OTE on CCRT [6 (a) and (b)], Heat shock [6 (c) and (d)], and Starvation test [6 (e) and (f)] on 5-day-old female (Left) and male (right) *D. melanogaster* as compared to control flies

Table 2. The 50% survival time, maximum lifespan, and mean lifespan of flies of all groups (a) Female flies (b) Male flies.

Table 2 (a)

	Groups	50% survival time	Maximum life span	Mean life span
CCRT	Control	39± 2.5 h	64± 2.1 h	31.25± 0.4 h
	2mg/mL	47± 2.6 h**	72± 2.3 h	35.15± 2.1 h
Heat shock assay	Control	7± 1.3 days	13± 2.1 days	7.7± 1.4 days
	2mg/mL	10± 1.6 days**	16± 3.1 days	8.5± 1.1 days
Starvation test	Control	23 1.4± h	38± 1.3 h	19.13± 1.6 h
	2mg/mL	30± 2.4 h*	48± 1.5 h	24.62± 2.4 h

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**p<0.002, **p<0.007 and *p<0.02

Table 2 (b)

	Groups	50% survival time	Maximum life span	Mean life span
CCRT	Control	37± 3.1 h	64± 2.3 h	32.16± 1.3 h
	2mg/mL	45± 2.7* h	72± 1.2 h	36.27± h
Heat shock assay	Control	5± 1.3 days	11± 1.7 days	6.8± 1.3 days
	2mg/mL	8± 0.4 days*	13± 0.5 days	7.4± 0.3 days
Starvation test	Control	21± 0.8 h	36± 1.2h	18.15± 1.4 h
	2mg/mL	26± 1.5 h**	40± 2.2 h	20.23± 1.9

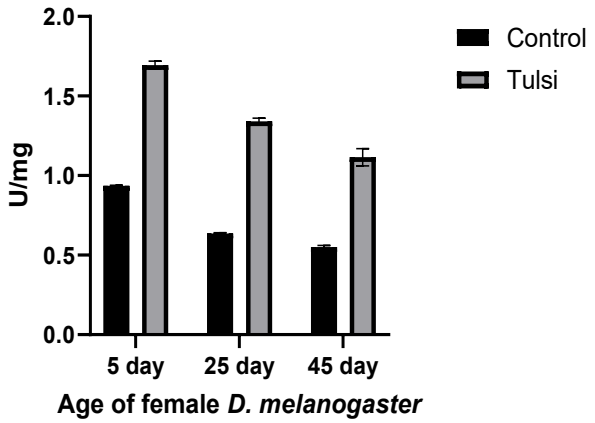
*p< 0.01, *p<0.02 and **p< 0.002

3.4 Effect of plant extract on stress tolerance of flies

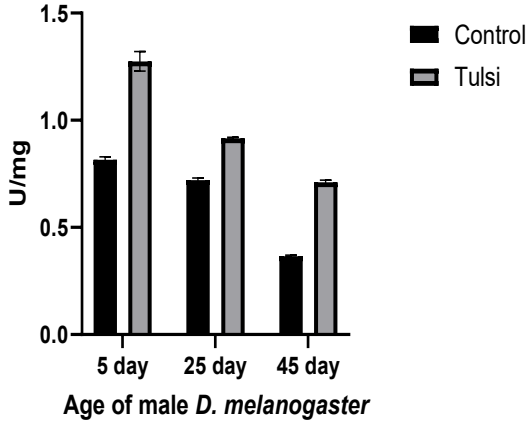
As shown in the Figure 6 and Table 2. OTE supplementation enhances the decreased stress tolerance of the flies with increasing age. With OTE treatment % survival rate of the flies following cold shock (*p< 0.01; **p < 0.002), heat shock (*p< 0.02; **p< 0.007), and starvation shock (**p value< 0.002; *p< 0.02) was significantly improved in both male and female flies, respectively, as compared to control group.

3.5 Effect of plant extract on biochemical assays

Due to an increase in the levels of ROS production and oxidative stress following natural aging phenomenon in flies, we decided to evaluate the selected anti- oxidative stress markers such as SOD, CAT, and total protein concentration. Supplementation of *O. tenuiflorum* concentration 2mg/mL to the flies significantly increased the SOD enzyme levels in both male and female flies at different age groups i.e. 5 day (**p value< 0.003), 25 day (*p value < 0.03) and 45 days (**p value< 0.007) when compared to control flies. As shown in Figure 7 similar results were found in case of CAT and total protein concentration. All the anti- oxidative parameters in experimental group show significant positive results as compared to control groups.



7(a)



7(b)

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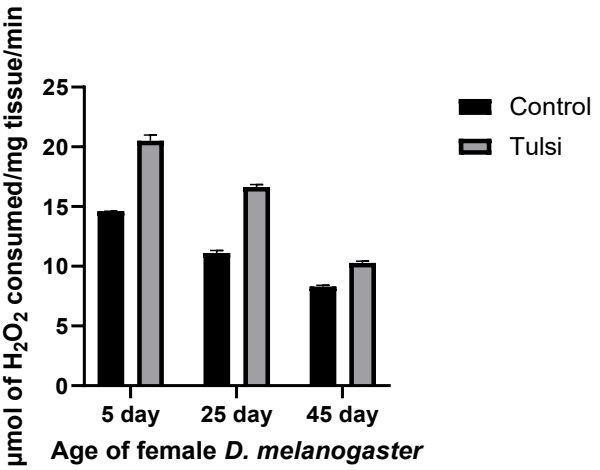
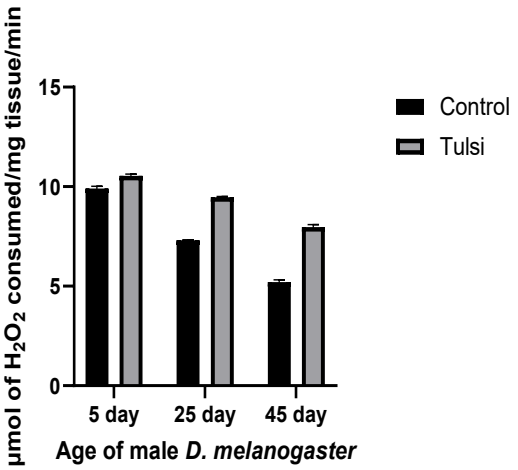
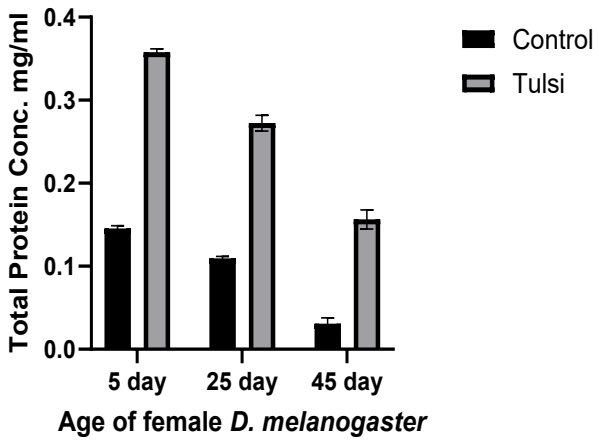


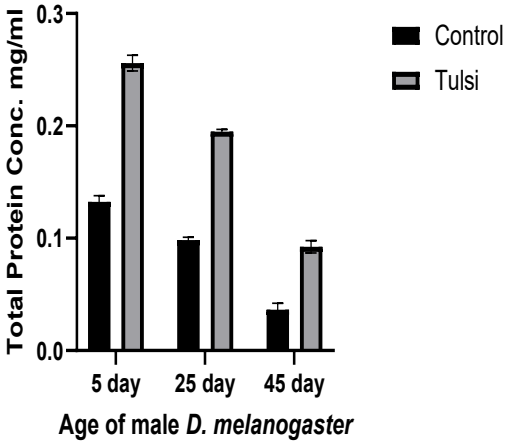
7(c)



7(d)



7(e)



7(f)

Figure 7, Depicting the effect of 2mg/mL OTE on SOD [7 (a) and (b)], CAT [7 (c) and (d)], and Total Protein [7 (e) and (f)] on 5-day-old female (Left) and male (right) *D. melanogaster* as compared to control flies

4. Discussion

Aging is a continuous progressive phenomenon characterized by a gradual decline in body functions. In the case of mammals, aging affects multiple organ systems of the body heterogeneously, leading to tissue deterioration and dysfunction. Aging mechanism is consequently associated with multiple health disorders, including cardiovascular problems, neurodegenerative disorders like dementia, Parkinson's disease, etc., osteoarthritis, type 2 diabetes mellitus, glaucoma, and cancer (McHugh and Gil, 2018). As explained earlier, increased oxidative stress is a major factor that contributes to aging and its associated problems. Oxidative stress theory, also known as free radical theory, states that age- associated disorders and functional losses are mainly caused by the increased levels of oxidative stress, resulting in damage to the macromolecules (DNA, RNA, Proteins and Lipids) by ROS species (Liguori et al., 2018). Nonetheless, there is a remarkable growing

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support that the aging phenomenon can be prevented or delayed to a significant extent by various pharmacological interventions like caloric restriction, dietary supplementation (Ros and Carrascosa, 2020; Gonzalez-Freire et al., 2020). Abundant research studies have been conducted in many vertebrate and non- vertebrate animal models, resulting in the detection of various compounds and medicinal plants like resveratrol, curcumin, caffeic acid, quercetin, metformin, etc., exhibiting anti- aging or longevity-promoting effects (Zia et al., 2021; Zhou et al., 2021; Ros and Carrascosa, 2020).

In this study, we used a medicinal plant *Ocimum tenuiflorum* (Synonym: *Ocimum sanctum*), known as Tulsi or Holy Basil, to study its anti- aging effects on model *Drosophila melanogaster*. The plant exhibits various pharmacological properties like anti- diabetic, anti-cancer; anti- inflammatory (Singh and Chaudhuri, 2018). Our study found that the OTE extends the life of both male and female *Drosophila* flies. Our results also show that the flies cultured on plant extract-supplemented diet show better resistance and improved life span against H_2O_2 and paraquat-induced oxidative stress as compared to control flies. Also, the locomotor activity of the flies increases in the treatment group. The life span of organisms is positively correlated with stress resistance (Deepashree et al., 2019). These results of our study provide strong support that the improved life span of flies may be due to the anti-oxidative properties of the plant. Similar results were also found in a previous study conducted by Suanarunsawat et al., depicting that the *Ocimum sanctum* leaf extract has strong free radical scavenging activity and shows anti- diabetic effects in diabetic mice (Suanarunsawat et al., 2016). Jiang et al., 2023 studied the anti- cancer properties of *Ocimum sanctum* mediated by Co nanoparticles and showed the free radical scavenging properties of the plant (Jiang et al., 2023). All the other pharmacological properties of the plant, like anti-inflammatory, anti- cancer, anti- diabetic all, are based upon its anti- oxidative properties.

For further analysis of the mechanism followed in life span extension by the OTE, we examined anti- oxidative enzymes level, including SOD and CAT. The whole-body homogenate of flies treated with 2mg/mL OTE in the diet showed increased levels of these enzymes. The decrease in anti- oxidative enzyme levels in the body is directly related to age (Ligouri et al., 2018). From our study, it is also observed that the flies show better resistance and improvement from Cold shock, Starvation, and heat shock. Our results showed that OTE can act as a protective factor that may help in limiting stress induced aging. Taken together, results from our study and previous studies show that *Ocimum tenuiflorum* can act as a potential anti-aging agent in *Drosophila* flies. Dietary supplementation of *O. tenuiflorum* leaves daily to organisms can increase their life span. However, further studies are required to understand the longevity-promoting mechanisms in mammalian models.

5. Conclusion

Summing up, our research study concludes that a lifelong supplementation of *O. tenuiflorum* leaf extract can improve the life span, decrease the stress induced mortality, and enhance the fitness parameters in *D. melanogaster* flies. The treatment flies show improvement in health and life span when exposed to in vitro oxidative stress, such as paraquat stress and H_2O_2- induced stress. Our results show that treatment with OTE improves the resistance to environmental stress, such as cold shock stress, heat shock stress, and starvation stress. Treatment flies show enhanced in vivo anti-oxidative enzyme levels. Based upon our findings, it can be concluded that OTE can act as a potent anti- aging agent to improve health span in *Drosophila melanogaster.* Also, it will be a topic of interest to identify the anti- aging compounds in the plant.

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7. Conflict of Interest

The authors declare that there are no known financial or personal relationship conflicts of interest that can affect the research work reported in this article in any way.

8. References

1. Aebi, H. (1974). Catalase. In *Methods of enzymatic analysis* (pp. 673-684). Academic press.
2. Akinyemi, A. J., Oboh, G., Ogunsuyi, O., Abolaji, A. O., & Udofia, A. (2018). Curcumin-supplemented diets improve antioxidant enzymes and alter acetylcholinesterase genes expression level in Drosophila melanogaster model. *Metabolic Brain Disease*, *33*(2), 369–375. https://doi.org/10.1007/s11011-017-0100-7
3. Adav, S. S., & Sze, S. K. (2020). Hypoxia-induced degenerative protein modifications associated with aging and age-associated disorders. *Aging and Disease*, *11*(2), 341–364. https://doi.org/10.14336/AD.2019.0604
4. Balasubramani, S.P., Mohan, J., Chatterjee, A., Patnaik, E., Kukkupuni, S.K., Nongthomba, U., Venkatasubramanian, P., 2014. Pomegranate juice enhances healthy lifespan in *Drosophila melanogaster*: an exploratory study. Front. Public Health 2, 245.
5. Chattopadhyay, D., & Thirumurugan, K. (2018). Longevity promoting efficacies of different plant extracts in lower model organisms. *Mechanisms of Ageing and Development*, *171*, 47–57. https://doi.org/10.1016/j.mad.2018.03.002
6. Chattopadhyay, D., Chitnis, A., Talekar, A., Mulay, P., Makkar, M., James, J., & Thirumurugan, K. (2017). Hormetic efficacy of rutin to promote longevity in Drosophila melanogaster. *Biogerontology*, *18*, 397-411.
7. Chow, C. Y., & Reiter, L. T. (2017). Etiology of Human Genetic Disease on the Fly. *Trends in Genetics*, *33*(6), 391–398. https://doi.org/10.1016/j.tig.2017.03.007
8. Colpo, A. C., Lima, M. E., Da Rosa, H. S., Leal, A. P., Colares, C. C., Zago, A. C., ... & Folmer, V. (2018). Ilex paraguariensis extracts extend the lifespan of Drosophila melanogaster fed a high-fat diet. *Brazilian Journal of Medical and Biological Research*, *51*(2), e6784.
9. Crimmins, E. M. (2015). Lifespan and healthspan: Past, present, and promise. *Gerontologist*, *55*(6), 901–911. https://doi.org/10.1093/geront/gnv130
10. Deepashree, S., Niveditha, S., Shivanandappa, T., Ramesh, S.R., 2019. Oxidative stress resistance as a factor in aging: evidence from an extended longevity phenotype of Drosophila melanogaster. Biogerontology. 20, 497–513.
11. Gonzalez-Freire, M., Diaz-Ruiz, A., Hauser, D., Martinez-Romero, J., Ferrucci, L., Bernier, M., de Cabo, R., 2020. The road ahead for health and lifespan interventions. Ageing Res. Rev. 59, 101037.
12. Gumeni, S., & Trougakos, I. P. (2019). Aging in drosophila melanogaster. In *Encyclopedia of Biomedical Gerontology*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-801238-3.11410-2
13. Kumar, R., Saha, P., Lokare, P., Datta, K., Selvakumar, P., & Chourasia, A. (2022). A systemic review of Ocimum sanctum (Tulsi): Morphological characteristics, phytoconstituents and therapeutic applications. *International Journal for Research in Applied Sciences and Biotechnology*, *9*(2), 221-226.
14. Lee, S. H., & Min, K. J. (2019). Drosophila melanogaster as a model system in the study of pharmacological interventions in aging. *Translational Medicine of Aging*, *3*, 98–103. https://doi.org/10.1016/j.tma.2019.09.004
15. Jiang, H., Sathiyavimal, S., Cai, L., Devanesan, S., Sayed, S. R., Jhanani, G. K., &

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3437-3452 | ISSN:2251-6727

Lin, J. (2023). Tulsi (Ocimum sanctum) mediated Co nanoparticles with their anti-inflammatory, anti-cancer, and methyl orange dye adsorption properties. *Environmental Research*, *236*, 116749.

16. Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., … Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *13*, 757–772. https://doi.org/10.2147/CIA.S158513
17. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & KrOTEmer, G. (2013). The hallmarks of aging. *Cell*, *153*(6), 1194-1217.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J biol Chem*, *193*(1), 265-275.
19. Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European journal of biochemistry*, *47*(3), 469-474.
20. McHugh, D., & Gil, J. (2018). Senescence and aging: Causes, consequences, and therapeutic avenues. *Journal of Cell Biology*, *217*(1), 65-77.
21. Mousavi, L., Salleh, R. M., & Murugaiyah, V. (2020). Antidiabetic and In Vitro Enzyme Inhibition Studies of Methanol Extract of Ocimum tenuiflorum Linn Leaves and Its Fractions. *Tropical life sciences research*, *31*(1), 141.
22. Piper, M. D. W., & Partridge, L. (2018). Drosophila as a model for ageing. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1864*(9), 2707–2717. https://doi.org/10.1016/j.bbadis.2017.09.016
23. Ros, M., & Carrascosa, J. M. (2020). Current nutritional and pharmacological anti-aging interventions. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1866*(3), 165612.
24. Sharma, A. D., Kaur, I., Angish, S., Thakur, A., Sania, S., & Singh, A. (2022). Comparative phytochemistry, antioxidant, antidiabetic, and anti-inflammatory activities of traditionally used Ocimum basilicum L. Ocimum gratissimum L., and Ocimum tenuiflorum L. *BioTechnologia*, *103*(2), 131.
25. Singh, D., & Chaudhuri, P. K. (2018). A review on phytochemical and pharmacological properties of Holy basil (Ocimum sanctum L.). *Industrial Crops and Products*, *118*, 367-382.
26. Suanarunsawat, T., Anantasomboon, G., & Piewbang, C. (2016). Anti-diabetic and anti-oxidative activity of fixed oil extracted from Ocimum sanctum L. leaves in diabetic rats. *Experimental and therapeutic medicine*, *11*(3), 832-840.
27. Wang, L., Li, Y. M., Lei, L., Liu, Y., Wang, X., Ma, K. Y., & Chen, Z. Y. (2015). Cranberry anthocyanin extract prolongs lifespan of fruit flies. *Experimental gerontology*, *69*, 189-195.
28. Zhou, D. D., Luo, M., Huang, S. Y., Saimaiti, A., Shang, A., Gan, R. Y., & Li, H. B. (2021). Effects and mechanisms of resveratrol on aging and age-related diseases. *Oxidative medicine and cellular longevity*, *2021*(1), 9932218.
29. Zia, A., Farkhondeh, T., Pourbagher-Shahri, A. M., & Samarghandian, S. (2021). The role of curcumin in aging and senescence: Molecular mechanisms. *Biomedicine & Pharmacotherapy*, *134*, 111119.
30. Yoon, J. S., Gagen, K. P., & Zhu, D. L. (1990). Longevity of 68 species of Drosophila.