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Impact of Zinc Chloride and Vitamin C on Thermal and Nutritional Stress Resistance in *Drosophila melanogaster*: Assessment of Cold Resistance, Heat Resistance, and Starvation Resistance

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ABSTRACT:

Organisms are frequently exposed to a variety of environmental stressors that challenge their survival, growth, and reproduction. In this context, *Drosophila melanogaster* serves as a powerful model to study physiological responses to stress due to its genetic tractability and ecological relevance. This study focuses on three key stress resistance parameters—cold resistance, heat resistance, and starvation resistance—that reflect the organism's ability to endure thermal and nutritional stress. Cold and heat resistance are vital for maintaining homeostasis under temperature extremes, involving mechanisms such as heat shock protein expression, membrane stabilization, and ion regulation. Starvation resistance, on the other hand, involves metabolic adaptations that allow flies to survive prolonged periods without food by mobilizing energy reserves and reducing energy expenditure. These stress parameters are crucial indicators of fitness, adaptation, and evolutionary trade-offs. By assessing these traits in *Drosophila*, researchers can gain insights into the broader physiological and genetic strategies used by organisms to survive in fluctuating environments. The findings have significant implications for understanding stress biology, evolutionary adaptation, and the potential impact of environmental changes on insect populations.

Keywords: *Drosophila melanogaster*, stress resistance, cold tolerance, heat tolerance, starvation resistance, thermal stress, nutritional stress, environmental stressors, physiological adaptation, metabolic response.

INTRODUCTION:

Organisms in nature constantly face various environmental challenges that threaten their survival and reproduction. These challenges, known as stressors, can be physical, chemical, or biological factors that disrupt normal physiological functions. To cope with such stressors, organisms have evolved mechanisms to resist or tolerate adverse conditions, which are often measured as stress resistance parameters. Among these, thermal stress resistance—including cold and heat tolerance—and starvation resistance are widely studied in the model organism *Drosophila melanogaster* due to its genetic tractability and ecological relevance. Stressors are defined as external factors that impose physiological strain on an organism. In the context of *Drosophila*, common stressors include extreme temperatures, limited food availability, oxidative stress, and exposure to toxins or heavy metals (Feder & Hofmann, 1999). Understanding how *Drosophila* responds to such stressors helps reveal fundamental biological processes underlying stress tolerance and adaptation.

Thermal Stress Resistance:

Thermal stress is a major environmental factor influencing insect survival, distribution, and fitness. Temperature extremes can damage cellular structures, disrupt enzymatic functions, and impair physiological processes. To withstand these challenges, *Drosophila melanogaster* exhibits cold resistance and heat resistance mechanisms.

Cold resistance allows flies to survive and recover from low-temperature exposure that can induce chill coma—a reversible state of paralysis caused by cold-induced ion imbalance and membrane dysfunction (Overgaard & MacMillan, 2017). Assessment of cold resistance commonly involves exposing flies to low temperatures (0 – 4 ° C) for defined periods and measuring recovery time or survival rates after returning to normal conditions (MacMillan et al., 2012). Flies with better cold resistance have adaptations such as enhanced membrane fluidity, efficient ion regulation, and accumulation of cryoprotectants that stabilize proteins and membranes.

Heat resistance reflects the ability to tolerate high temperatures, which can cause protein denaturation and oxidative damage. Flies are typically exposed to temperatures between 37 ° C and 39 ° C, and survival or knockdown time during heat exposure is recorded (Feder & Hofmann, 1999). Heat shock

proteins (HSPs), molecular chaperones that assist in protein folding and repair, play a crucial role in heat tolerance. Thermal resistance traits are often correlated with other physiological parameters, indicating a complex network of stress response pathways (King & MacRae, 2015).
While thermal stress pertains to physical extremes, starvation resistance is a form of nutritional stress tolerance reflecting an organism's ability to survive prolonged food deprivation. In natural settings, food availability fluctuates, and survival during famine depends on metabolic adjustments and energy conservation strategies.

In *Drosophila melanogaster*, starvation resistance involves mobilization of stored energy reserves, primarily lipids and carbohydrates, to sustain vital functions during periods without food (Rion & Kawecki, 2007). Flies with higher starvation resistance exhibit larger fat stores, reduced metabolic rates, and altered behaviours such as decreased locomotion to minimize energy expenditure (Hulbert & Else, 2000). Genetic studies have identified pathways regulating energy homeostasis, including insulin signalling and nutrient-sensing mechanisms, which modulate starvation tolerance (Flatt, 2011). Starvation resistance is also intertwined with life history traits; increased starvation tolerance may trade off with reproduction or developmental rates, illustrating evolutionary compromises in resource allocation (Mair et al., 2003). Experimental evolution studies have demonstrated that selection for starvation resistance can lead to correlated changes in stress tolerance to other factors, such as cold or oxidative stress, highlighting shared physiological mechanisms (Rion & Kawecki, 2007).

Studying stress resistance parameters in *Drosophila melanogaster* offers insights into how organisms adapt to fluctuating environments and manage trade-offs between survival and reproduction. These parameters are also crucial for understanding the effects of environmental pollutants, climate change, and ecological pressures on insect populations. The genetic tools available in *Drosophila* enable dissection of molecular pathways underlying stress tolerance, which can inform broader biological concepts and applications in agriculture and pest management.
In conclusion, thermal stress resistance (cold and heat resistance) and starvation resistance represent key physiological traits that allow *Drosophila melanogaster* to cope with adverse environmental conditions. Research on these parameters enhances our understanding of stress biology, evolutionary adaptation, and ecological resilience.

MATERIALS AND METHODS:

ESTABLISHMENT OF STOCK: Wild type *Drosophila melanogaster* - Oregon K strain (OK) was obtained from *Drosophila* Stock Centre, University of Mysore. Flies were grown and aged in culture bottles/vials on wheat cream agar media (100 Sooji, 100 g jaggery, 10 g agar and 7.5 ml propionic acid in 1 L distilled water) with regular sub-culturing and maintained for all experiments at 24 ^{o}C with 60-70 % relative humidity and ambient lighting condition with a sprinkle of live Baker's yeast. All collection of virgins, adult flies were performed under brief anaesthesia. Dose administration was achieved via larval feeding for all treatments [D'Souza, & Shakunthala, 2015].

Diet preparation	
Control	100 Sooji, 100 g jaggery, 10 g agar and 7.5 ml propionic acid in 1 L distilled water
ZT1	250 ml of control media containing 0.17 g (5 mM) of heavy metal, $ZnCl_2$
ZT2	250 ml of control media containing 0.23 g (7 mM) of heavy metal, $ZnCl_2$.
ZT3	250 ml of control media containing 0.23 g (7 mM) of heavy metal, $ZnCl_2$ and 0.05 g of Antioxidant, Vitamin C.

HEAT RESISTANCE: Heat resistance experiment of control and different concentration of $ZnCl_2$ and Vitamin C treated mated male and female flies were used to study heat resistance. Ten flies (control and treated flies), in vials were kept in incubator at 40 ^{o}C. The flies were observed for every 15 minutes of interval until the death of each fly. The heat resistance was observed in minutes. A total of five replicates were run each of control, ZT1 (5 mM), ZT2 (7 mM), and ZT3 (7 mM and 0.05 g of Vitamin C), treated media. Separate experiment was run for both males and females.

COLD RESISTANCE: Five days old mated flies were cultured from wheat cream agar media, ZT1 (5 mM), ZT2 (7 mM), and ZT3 (7 mM and 0.05 g of Vitamin C), in order to study cold resistance. Fifty flies (mated male/mated female) were observed by moving them to empty vials, in five replicas each of which held ten flies. These vials were stored in a refrigerator at a constant frigid temperature of 4 ° C, and each fly's resistance to the cold was measured every 2 hours until it died. Five duplicates, each containing ten flies, were conducted for the control and different concentration of $ZnCl_2$ and Vitamin C.

STARVATION RESISTANCE IN MATED FLIES: After pupation, the adult flies were allowed to grow in respective media for 5-days. Then these 5-day old mated flies were taken for starvation resistance test. They were placed in 1% non-nutritive agar media. The duration of hours the flies were able to survive without food was recorded by observing the vials at 2 hours interval till all the flies had perished. A total of 5 replicates of 10 flies each were run separately for each group. Males and females were considered separately for this experiment. The data is subjected to statistics.

STATISTICAL ANALYSIS:

- **Heat resistance**: The data obtained were analysed using IBM SPSS version 29.0. Mean, standard error, one way ANOVA, and Tukey's Post-Hoc test were carried out for the data obtained for heat resistance. A graph of concentration v/s heat resistance in minutes was plotted for different concentration of $ZnCl_2$ and Vitamin C.
- **Cold resistance**: The data obtained were analysed using IBM SPSS version 29.0. Mean, standard error, one way ANOVA, and Tukey's Post-Hoc test were carried out for the data obtained for cold resistance. A graph of concentration v/s cold resistance in hours was plotted for different concentration of $ZnCl_2$ and Vitamin C.
- **Starvation resistance**: The data obtained were analysed using IBM SPSS version 29.0. Mean, standard error, one way ANOVA, and Tukey's Post-Hoc test were carried out for the data obtained for heat resistance. A graph of concentration v/s starvation resistance in hours was plotted for different concentration of $ZnCl_2$ and Vitamin C.

RESULT:

HEAT RESISTANCE:

The present study assessed the impact of zinc chloride ($ZnCl_2$) and vitamin C on the heat resistance of *Drosophila melanogaster* by measuring the survival time of adult flies exposed to 40 °C. The results revealed that male flies (**Figure 1),** in the control group exhibited the highest mean survival time, whereas a significant reduction in heat resistance was observed in both ZT1 (5 mM $ZnCl_2$) and ZT2 (7 mM $ZnCl_2$) treatment groups. Among these, ZT2 showed the lowest resistance, indicating a dose-dependent negative effect of zinc chloride. However, in the ZT3 group (7 mM $ZnCl_2$ + vitamin C), male flies demonstrated a partial recovery in survival, suggesting a protective role of vitamin C. One-way ANOVA revealed a significant effect of treatment on male heat resistance ($F = 8.345$; df = 3; $p < 0.05$), and Tukey's Post-Hoc test showed that ZT2 was significantly lower than control ($p < 0.05$), while ZT3 was significantly higher than ZT2 but not significantly different from control. In females (**Figure 2)**, a similar trend was observed. Control flies showed the highest survival time, with a noticeable decrease in ZT1 and a further significant reduction in ZT2. However, ZT3 females exhibited a strong increase in heat resistance, nearly reaching or surpassing control values. ANOVA for females showed a highly significant difference among groups ($F = 17.373$; df = 3; $p < 0.05$), and post hoc comparisons confirmed that ZT2 was significantly lower than all other groups ($p < 0.01$), and ZT3 was significantly higher than both ZT1 and ZT2 ($p < 0.05$). When comparing sexes across treatments (**Figure 3**), it was evident that females consistently showed greater heat resistance than males, especially in the ZT3 group, suggesting a sex-specific physiological response. In ZT2, the difference between males and females was statistically significant ($p < 0.05$), and females in ZT3 had the highest overall survival time. These findings indicate that while zinc chloride reduces heat tolerance in *Drosophila*, the inclusion of vitamin C can mitigate its effects—more effectively in females than in males.

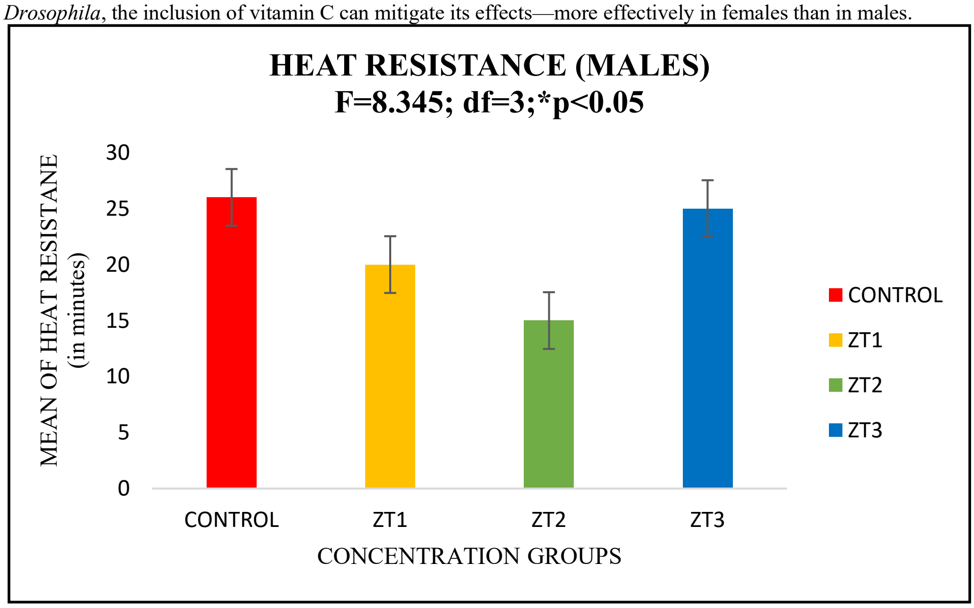

Figure 1: Effect of $ZnCl_2$ and vitamin C on heat resistance males *D. melanogaster*.

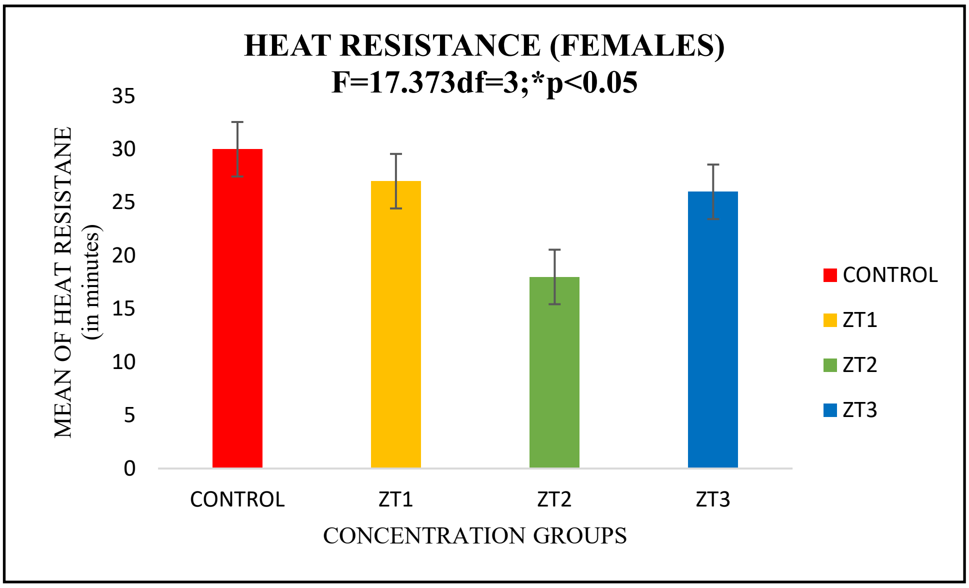

Figure 2: Effect of $ZnCl_2$ and vitamin C on heat resistance females *D. melanogaster*.

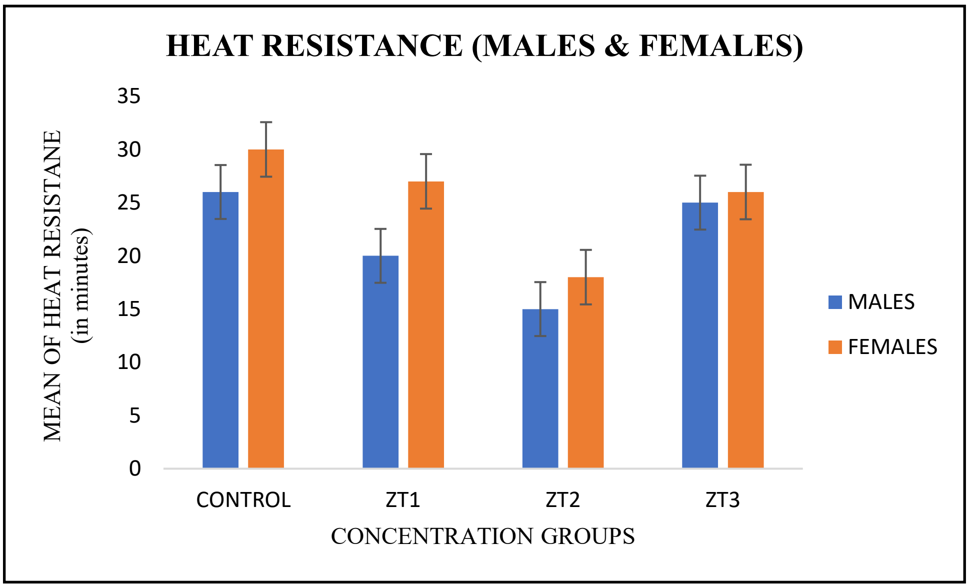

Figure 3: Effect of $ZnCl_2$ and vitamin C on heat resistance in both males and females *D. melanogaster*.

COLD RESISTANCE:

The effect of zinc chloride ($ZnCl_2$) and vitamin C on cold resistance in *Drosophila melanogaster* was evaluated, and notable differences were observed across treatment groups and between sexes. In male flies (**Figure 4**), the control group exhibited the highest resistance to cold-induced stress, as indicated by faster recovery times. A progressive decline in cold resistance was evident in the ZT1 and ZT2 groups, suggesting a concentration-dependent inhibitory effect of $ZnCl_2$. However, in the ZT3 group, where vitamin C was co-administered with $ZnCl_2$, cold resistance improved moderately, indicating a partial protective effect. Female flies followed a similar pattern (**Figure 5**), but they consistently showed higher cold tolerance than males across all groups. This trend was further confirmed when the data were combined for both sexes (**Figure 6**), with ZT3 again demonstrating better cold resistance compared to ZT1 and ZT2. A direct comparison between males and females (Figure 4) revealed that the sex-based differences were most pronounced in the control and ZT3 groups. Statistical analysis supported these observations: one-way ANOVA showed highly significant differences among treatment groups in males ($F = 27.556$, $p < 0.001$), females ($F = 22.067$, $p < 0.001$), and in the combined data ($F = 53.278$, $p < 0.001$). Two-way ANOVA further confirmed a significant effect of treatment ($p = 0.000$), a near-significant effect of sex ($p = 0.058$), and a non-significant interaction between sex and treatment ($p = 0.956$). Overall, the findings indicate that $ZnCl_2$ exposure reduces cold resistance in *D. melanogaster*, while the inclusion of vitamin C offers measurable protective benefits. The results also suggest sex-specific physiological responses, with females demonstrating greater resistance under stress conditions.

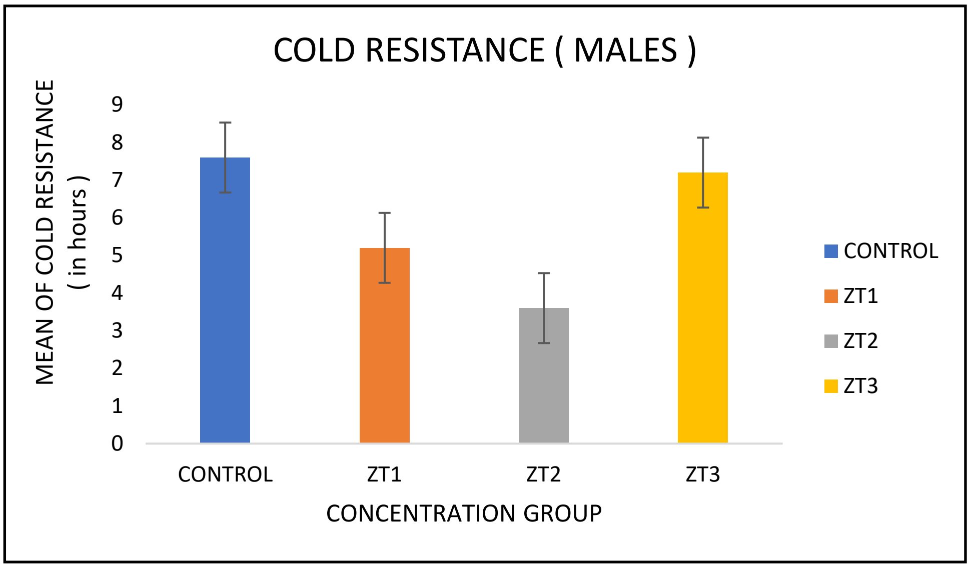

Figure 4: Effect of $ZnCl_2$ and Vitamin C on cold resistance in males of *Drosophila melanogaster*

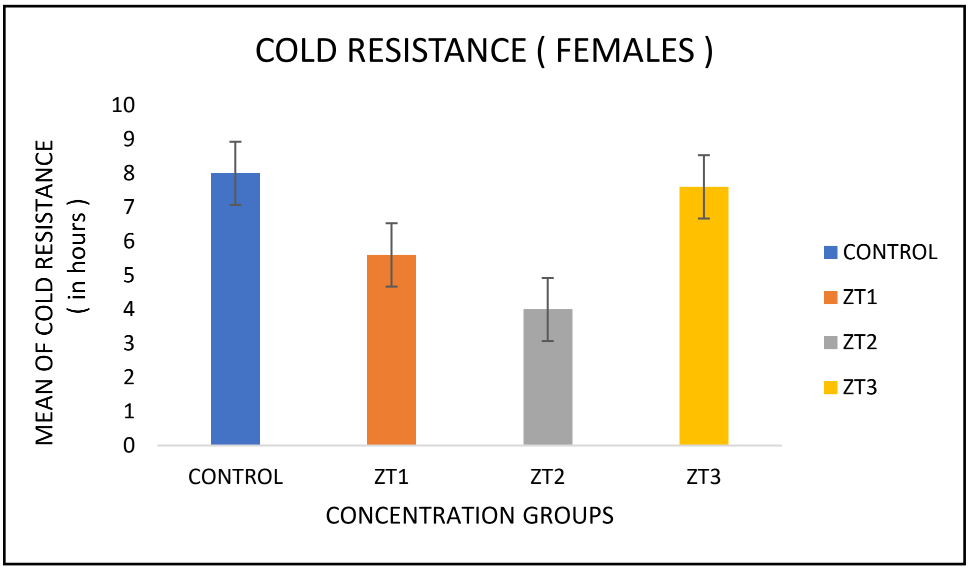

Figure 5: Effect of $ZnCl_2$ and Vitamin C on cold resistance in females of *Drosophila melanogaster*

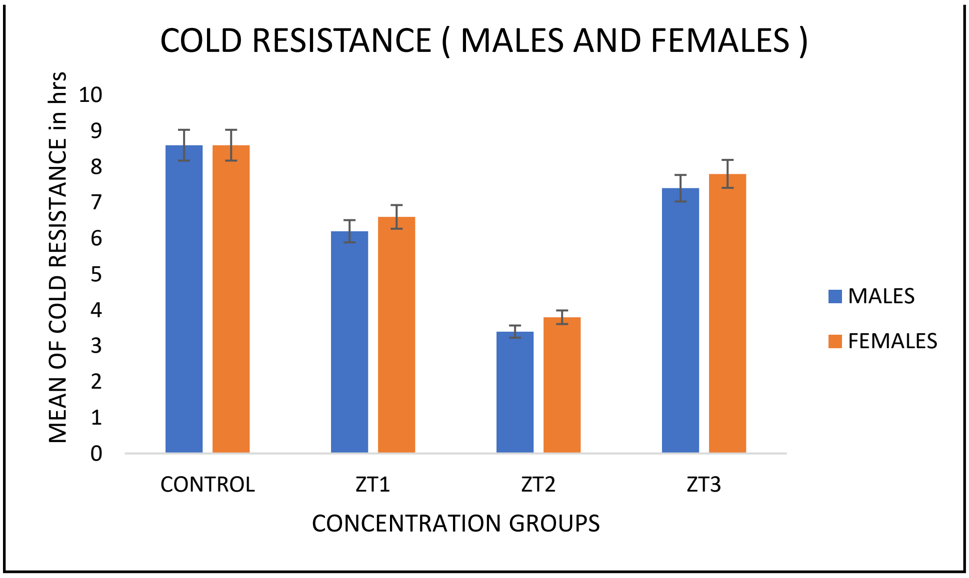

Figure 6: Effect of $ZnCl_2$ and Vitamin C on cold resistance in both males and females of *Drosophila melanogaster*

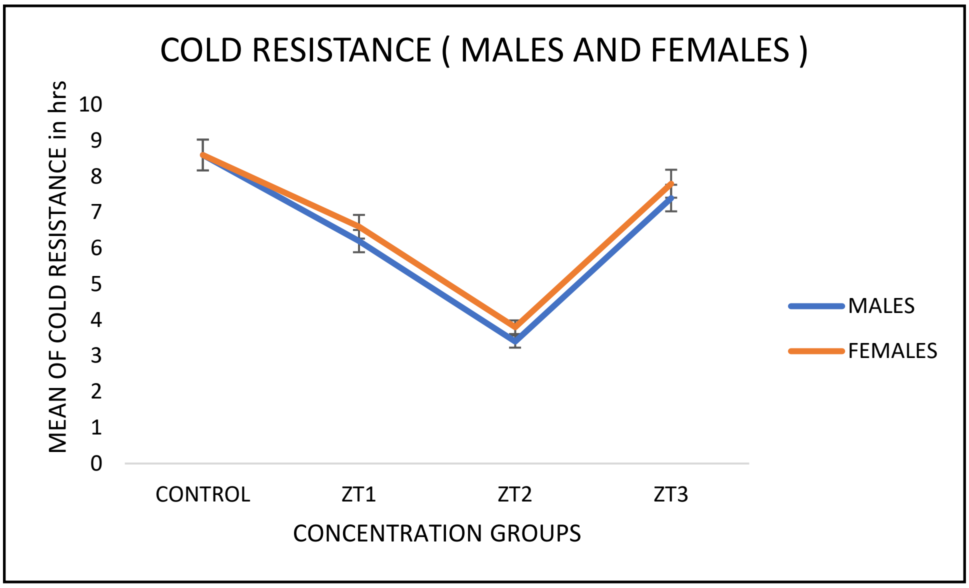

Figure 7: Effect of $ZnCl_2$ and Vitamin C on cold resistance in both males and females of *Drosophila melanogaster*

STARVATION RESISTANCE:

The effect of $ZnCl_2$ and Vitamin C on starvation resistance in *Drosophila melanogaster* was assessed by analysing survival in both mated males and females across four treatment groups: control, ZT1, ZT2, and ZT3. In males (**Figure 8**), starvation resistance was lower in ZT1 and ZT2 groups compared to the control, while a significant increase was observed in the ZT3 group, with a mean survival time of 72.2 hours—surpassing the control group mean of 67.4 hours. Similarly, in females (**Figure 9**), the starvation resistance decreased in ZT1 (58.8 hours) and ZT2 (53.2 hours), but showed a marked increase in ZT3 (71.2 hours), exceeding the control value of 70 hours. The data suggest that the highest dose (ZT3) provided a protective effect against starvation stress in both sexes.

Pie charts (**Figure 10 & Figure 11**) further visualized this pattern, where ZT3 consistently showed the largest proportion of survival in both males and females (71.2%), while ZT2 had the lowest survival. Line graphs (**Figure 12 & Figure 13**) depicted a U-shaped response curve in both sexes, indicating decreased starvation resistance at intermediate concentrations (ZT1 and ZT2), followed by a significant recovery at the highest concentration (ZT3). When comparing the sexes directly (**Figure 14 & Figure 15**), females consistently exhibited slightly higher starvation resistance than males across all treatments.

One-way ANOVA revealed that the differences among treatment groups were statistically significant for males ($F = 111.917$, $p < 0.001$), females ($F = 112.889$, $p < 0.001$), and when both sexes were combined ($F = 126.713$, $p < 0.001$). Two-way ANOVA further supported these findings, showing a significant effect of treatment group ($F = 170.858$, $p = 0.001$) and sex ($F = 21.077$, $p = 0.019$), while the interaction between group and sex was not significant ($F = 1.308$, $p = 0.289$), indicating that the treatment effects were consistent across both sexes.
Tukey's post-hoc test confirmed significant differences among all groups. ZT3 had the highest mean starvation resistance (72.2), followed by the control (67.4), ZT1 (57.6), and ZT2 (51.4), with all pairwise comparisons yielding p-values < 0.001. These findings strongly suggest that the combination of $ZnCl_2$ and Vitamin C at higher concentrations (ZT3) enhances starvation resistance in *Drosophila melanogaster*, while lower concentrations may be less effective or even mildly suppressive.

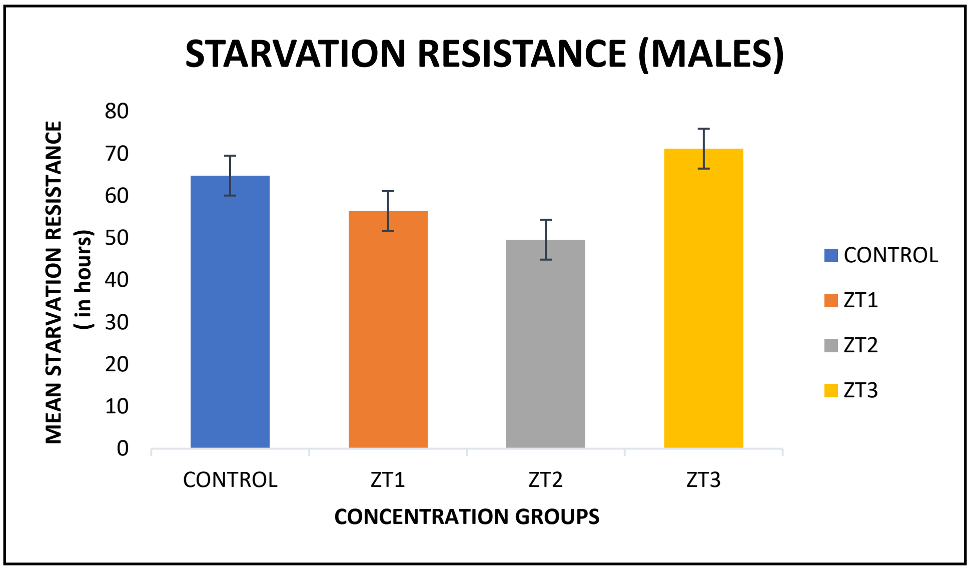

Figure 8: Effect of $ZnCl_2$ and Vitamin C on starvation resistance in males of *Drosophila melanogaster*

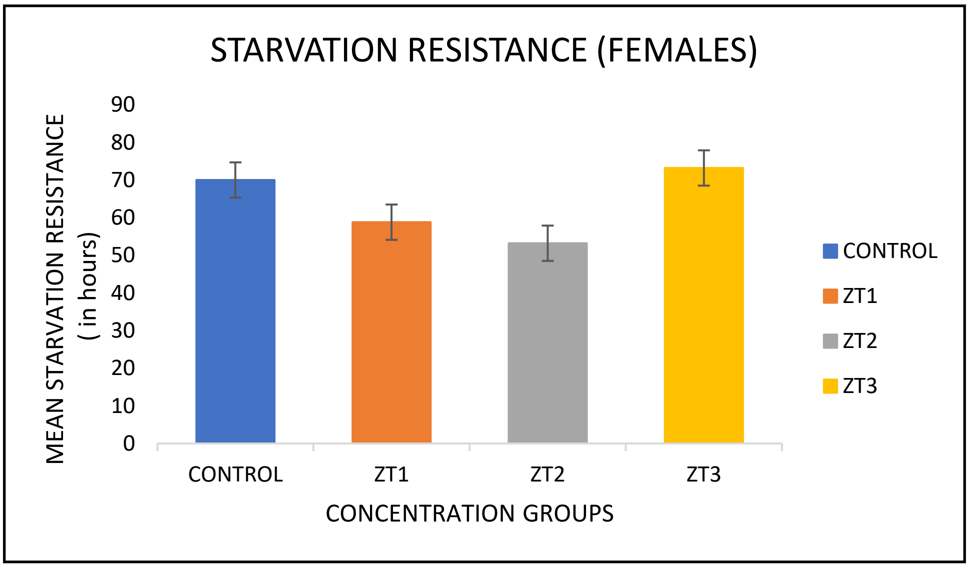

Figure 9: Effect of $ZnCl_2$ and Vitamin C on starvation resistance in males of *Drosophila melanogaster*

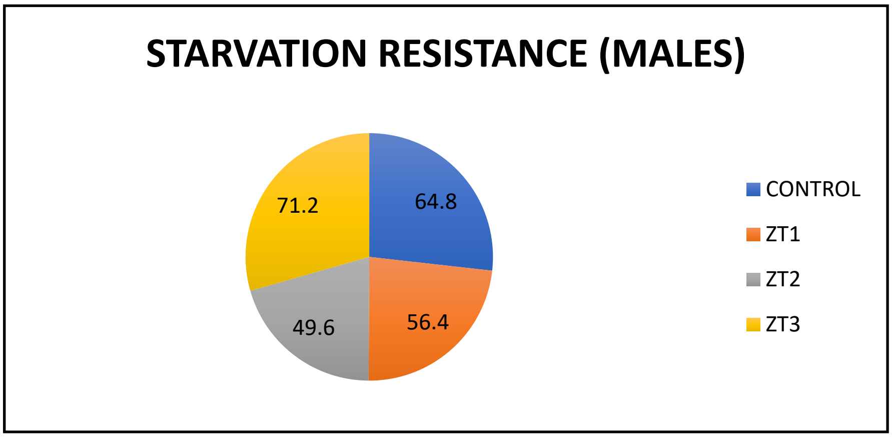

Figure 10: Effect of $ZnCl_2$ and Vitamin C on starvation resistance in males of *Drosophila melanogaster*

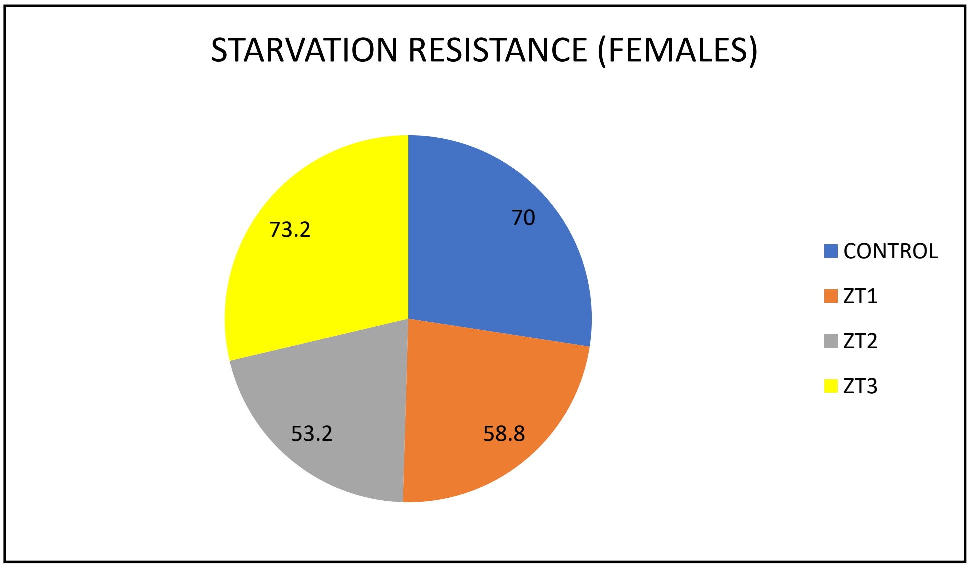

Figure 11: Effect of $ZnCl_2$ and Vitamin C on starvation resistance in females of Drosophila melanogaster

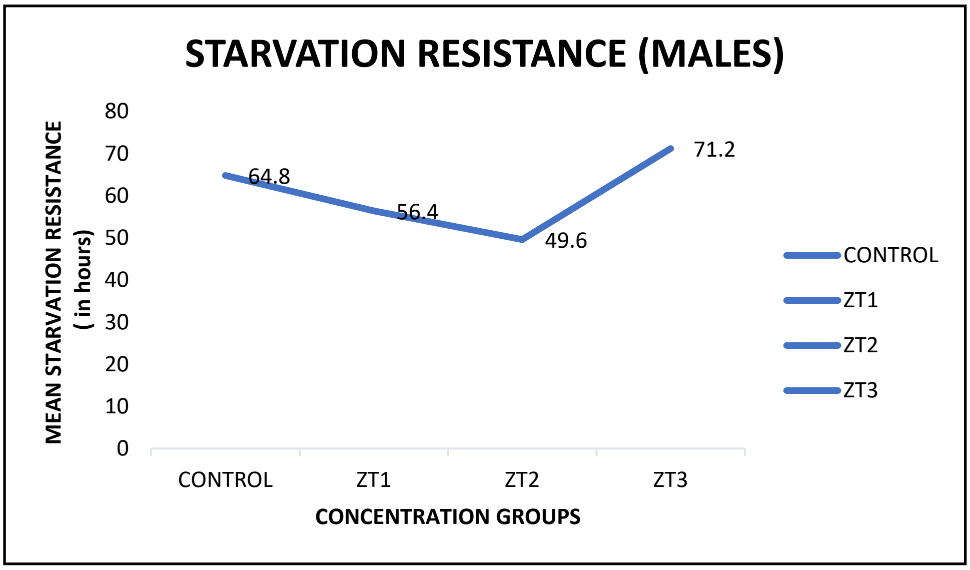

Figure 12: Effect of $ZnCl_2$ and Vitamin C on starvation resistance in males of *Drosophila melanogaster*

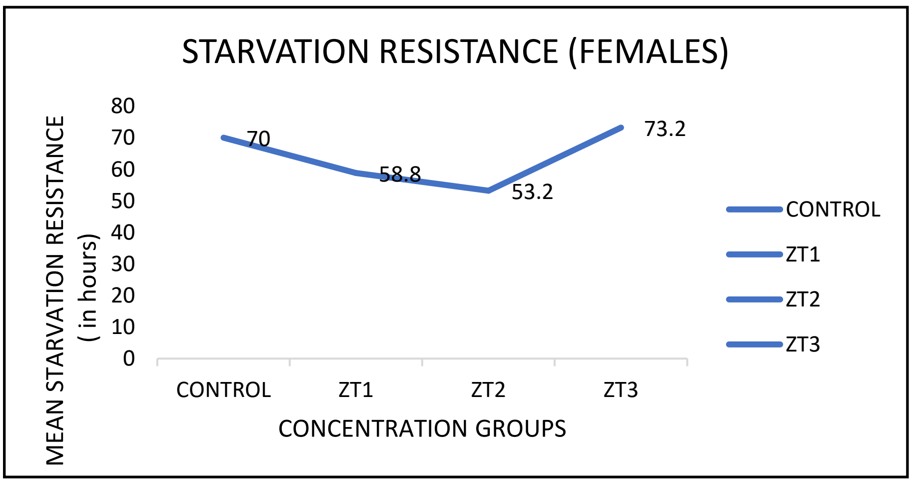

Figure 13: Effect of ZnCl2 and Vitamin C on starvation resistance in females of Drosophila melanogaster

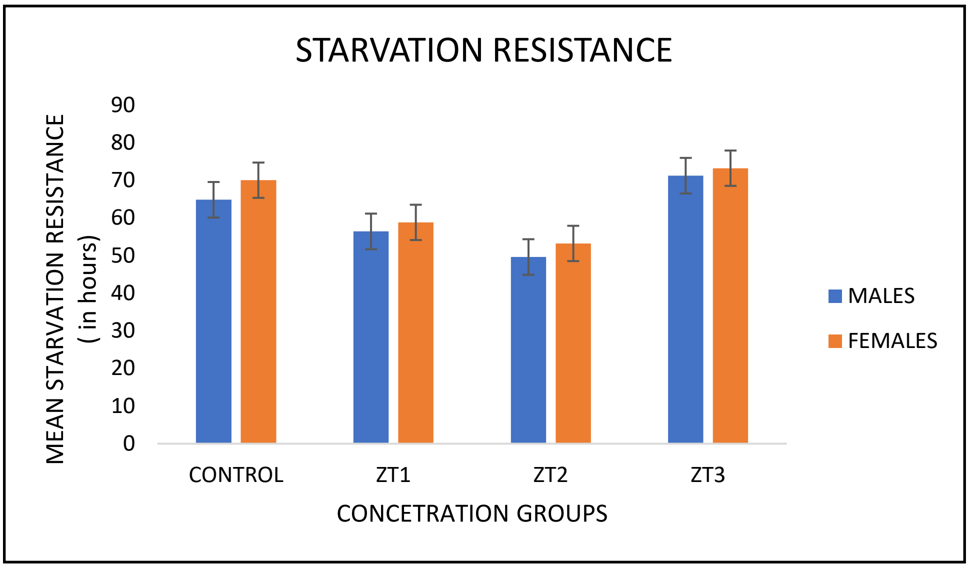

Figure 14: Effect of $ZnCl_2$ and Vitamin C on starvation resistance in both males and females of *Drosophila melanogaster*

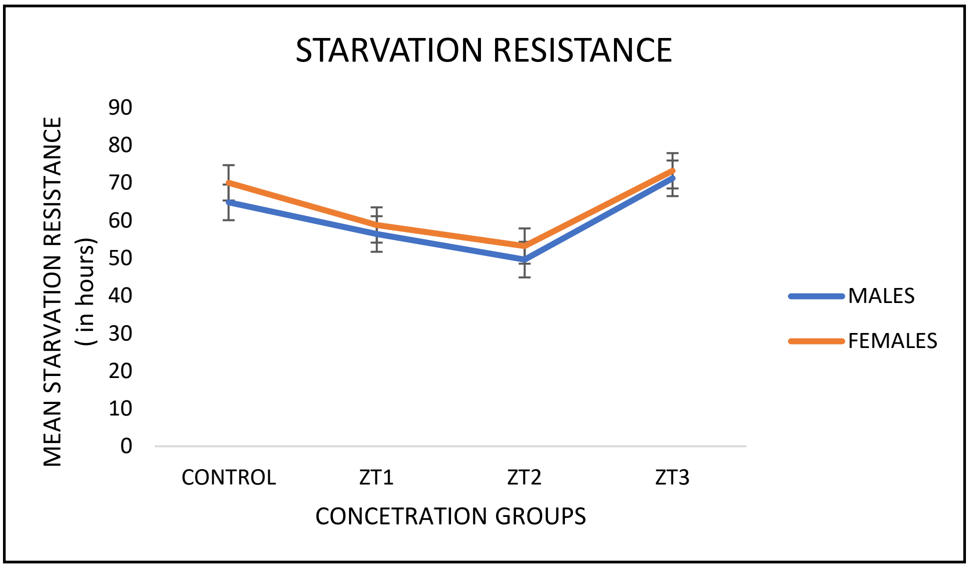

Figure 15: Effect of $ZnCl_2$ and Vitamin C on starvation resistance in both males and females of *Drosophila melanogaster*

DISCUSSIOIN:

HEAT RESISTANCE: The present study evaluated the effect of zinc chloride ($ZnCl_2$) and vitamin C on the heat resistance of *Drosophila melanogaster*, revealing significant variations across treatment groups and between sexes. Zinc exposure led to a concentration-dependent reduction in heat resistance in both male and female flies, with the 7 mM $ZnCl_2$ group (ZT2) showing the lowest survival times. These results align with previous findings indicating that heavy metals like zinc disrupt cellular redox balance, impair mitochondrial function, and induce oxidative stress, thereby lowering an organism's capacity to survive under environmental challenges such as heat (Sanders et al., 2009; Wang & Rainbow, 2008).

Interestingly, the group treated with both $ZnCl_2$ and vitamin C (ZT3) exhibited improved thermal stress tolerance compared to $ZnCl_2$-only groups, highlighting the protective role of vitamin C. As a well-known antioxidant, vitamin C is capable of neutralizing reactive oxygen species (ROS) and reducing oxidative damage to proteins, lipids, and DNA (Naidu, 2003). The improved survival of flies in the ZT3 group suggests that vitamin C may restore redox homeostasis and stabilize cellular structures disrupted by zinc toxicity, thereby enhancing heat resistance. This is consistent with reports that antioxidants improve thermotolerance by upregulating heat shock proteins and scavenging ROS (Wang et al., 2012).

Furthermore, a distinct sexual dimorphism in heat resistance was observed. Female flies consistently exhibited greater thermal tolerance than males across all treatment groups, particularly in the ZT3 group. This difference may be attributed to variations in physiological stress responses between sexes, including hormonal regulation, metabolic rate, and fat storage, all of which influence resilience to environmental stressors (Duneau & Lazzaro, 2018;

Sisodia & Singh, 2010). Previous studies in *Drosophila ananassae* also demonstrated sex-specific differences in heat and oxidative stress resistance, with females often showing superior adaptive responses (Sisodia & Singh, 2012).

The findings from this assay reinforce the idea that dietary antioxidants can serve as important modulators of stress resistance in *Drosophila*, with potential applications in understanding environmental toxicology and stress biology. The partial recovery observed in ZT3 groups suggests that the negative effects of zinc can be alleviated, but not entirely reversed, by antioxidant supplementation. Thus, future studies could explore the use of other antioxidants, such as vitamin E or plant polyphenols, in combination with zinc, and also assess gene expression changes in stress-responsive pathways like Hsp70 and Sod.

COLD RESISTANCE: The cold resistance assay conducted in this study demonstrated that exposure to zinc chloride ($ZnCl_2$) significantly reduced the ability of *Drosophila melanogaster* to withstand low-temperature stress, with a clear concentration-dependent pattern. The inclusion of vitamin C in the ZT3 group partially reversed the cold sensitivity induced by $ZnCl_2$, suggesting a protective role of antioxidant supplementation. Notably, female flies consistently showed higher cold resistance than males across all treatment groups, indicating sex-dependent physiological differences in cold stress tolerance. Heavy metals like $ZnCl_2$, although essential at trace levels, become toxic at higher concentrations due to their ability to disrupt cellular processes. In *Drosophila*, excess zinc is known to interfere with enzyme function, induce reactive oxygen species (ROS), damage mitochondrial activity, and disrupt membrane stability (Andrews, 2001; Bettedi et al., 2011). Cold stress itself can lead to ROS production, and the combination of cold exposure and zinc toxicity may cause synergistic oxidative damage, thereby exacerbating the impairment of cold recovery.

In this study, the ZT1 and ZT2 groups exhibited progressively poorer cold resistance with increasing $ZnCl_2$ concentration. This trend is consistent with earlier studies where high zinc levels reduced stress tolerance, developmental rates, and survival in flies and other insect models (Maloney et al., 2003). Zinc ions can inhibit the activity of enzymes like superoxide dismutase (SOD) and catalase if the redox balance is perturbed, which may explain the slower recovery and higher cold sensitivity observed.

Vitamin C (ascorbic acid), administered in the ZT3 group, improved cold resistance in both sexes compared to $ZnCl_2$-only treatments. Vitamin C is a potent water-soluble antioxidant that neutralizes ROS, regenerates other antioxidants like vitamin E, and stabilizes cellular membranes during environmental stress (Foyer & Noctor, 2005). In cold-stressed flies, ascorbic acid likely prevented oxidative damage to proteins and lipids, reducing the delay in recovery time and improving survival. Although the protection was not complete, the result indicates that antioxidant supplementation can partially mitigate metal-induced stress damage. This aligns with previous work showing that dietary antioxidants improve cold resistance in *Drosophila* by enhancing mitochondrial function and preserving ATP levels (Colinet et al., 2010; Wang et al., 2020).

Sex-specific differences observed in this study are also noteworthy. Females showed consistently faster recovery and better cold resistance across all treatments. This might be attributed to higher lipid reserves in females, which act as energy stores during stress recovery, or to sex-specific differences in the expression of stress-related genes, including cold-shock proteins and antioxidant enzymes (Marshall & Sinclair, 2010). Females may also possess more robust antioxidant defenses, leading to reduced ROS accumulation under stress.

Statistical analysis confirmed the observed trends. One-way ANOVA showed highly significant differences among treatment groups in both males and females, and two-way ANOVA revealed a significant treatment effect and a near-significant sex effect. The lack of significant interaction suggests that while sex and treatment independently affect cold resistance, their interaction does not significantly alter the overall pattern of response.

In conclusion, this study reinforces the finding that $ZnCl_2$ reduces cold resistance in *D. melanogaster*, likely through oxidative stress and cellular disruption. Vitamin C shows promise in mitigating some of the damage induced by zinc toxicity, though not entirely. The observed sex differences in cold resistance highlight the need for further investigations into sex-specific physiological adaptations to environmental stress. Future research could include molecular studies on the expression of antioxidant genes (e.g., sod, cat, hsp70) and cold-shock proteins to uncover the underlying mechanisms of protection and resistance.

STARVATION RESISTANCE: The present study investigated the effect of zinc chloride ($ZnCl_2$) combined with vitamin C on the starvation resistance of *Drosophila melanogaster*. The data clearly indicate that this supplementation impacts starvation tolerance significantly, with variations observed based on both concentration and sex of the flies. Results showed a clear dose-dependent effect, with the highest starvation resistance observed at the ZT3 concentration. In contrast, lower concentrations (ZT1 and ZT2) showed reduced starvation tolerance compared to the control group. These findings are statistically significant, as confirmed by one-way ANOVA for both males ($F = 111.917$, $p < 0.001$) and females ($F = 112.889$, $p < 0.001$), and supported by Tukey's post hoc test, indicating distinct group differences.

The improved starvation resistance at ZT3 may be attributed to the antioxidant and metabolic regulatory roles of vitamin C and Zn^{2+} ions. Zinc plays a critical role as a cofactor in numerous enzymatic reactions involved in stress responses, lipid metabolism, and cell signalling (Harshman & Schmid, 1998). Additionally, it helps maintain cellular membrane integrity under oxidative stress conditions. Vitamin C, a known antioxidant, scavenges free radicals and reduces lipid peroxidation during nutrient deprivation (Chippindale et al., 1996). Together, these compounds may reduce cellular damage during starvation, enhance energy conservation, and promote survival. At lower concentrations (ZT1 and ZT2), however, starvation resistance decreased compared to control. This may be due to suboptimal dosing, where cellular stress pathways are not sufficiently activated, or even mildly disrupted. It is also known that Zinc, at improper levels, can generate pro-oxidant effects, potentially leading to metabolic imbalance and reduced stress tolerance (Brown et al., 2019).

Moreover, the observed sex-based differences, with females showing higher starvation resistance than males across all treatment groups, align with existing literature. Female *Drosophila* typically possess greater lipid reserves and more efficient metabolic adaptations for energy storage and utilization, making them more resilient to food deprivation (Rion & Kawecki, 2007). Our two-way ANOVA confirmed a significant main effect of sex ($F = 21.077$, $p = 0.019$), though no interaction between sex and treatment was found, indicating that both sexes responded similarly across treatment levels, but females maintained a consistently higher resistance.

This experiment also supports previous findings that lipid content and metabolic flexibility are closely associated with starvation tolerance. According to Chippindale et al. (1996), increased lipid storage is a key factor underlying survival during food scarcity. The positive correlation between fat accumulation and SR has been documented in several studies involving selection lines of *Drosophila* (Jan et al., 2021; Folguera et al., 2008). Taken together, the study confirms that ZT3 (the highest tested dose of $ZnCl_2$ and Vitamin C) effectively enhances starvation resistance in *Drosophila melanogaster*, likely by improving antioxidant capacity, lipid metabolism, and stress tolerance. However, the reduction in SR at intermediate doses suggests that careful dosage optimization is necessary to avoid adverse effects. Future studies should explore the underlying molecular pathways, such as expression of antioxidant enzymes (e.g., SOD, catalase), lipid metabolism genes, and oxidative stress markers to better understand the mechanism of action. These findings have potential applications in the development of nutritional supplements that can enhance stress resilience in both model organisms and potentially higher animals.

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