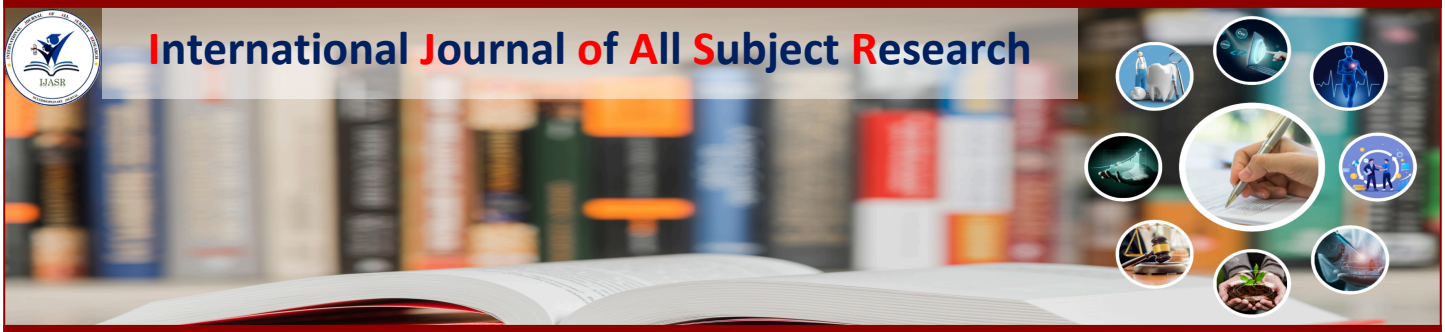
Impact Factor (SJIF): 5.158 E-ISSN: 2583-7206, P-ISSN: 3049-2432

International Journal of All Subject Research



Received: 28/April/2025 **IJASR:** 2025; 4(3):48-53 **Accepted:** 12/June/2025

Effect of Chronic Zinc Chloride Exposure on Climbing Ability in *Drosophila melanogaster* and Its Modulation by Vitamin C

[1]Mehek Naaz, [2]Shalini KS, [3]Yashaswini P, [4]Shree lakshmi K and [*5]Shakunthala V

[1, 2, 3, 4]MSc. Zoology, Department of Zoology, Manasagangotri, University of Mysore, *Drosophila* Stock Centre, Mysuru, Karnataka, India.

[*5]Professor, Department of Zoology, Manasagangotri, University of Mysore, Mysuru, Karnataka, India.

Abstract

The climbing assay, also known as the negative geotaxis assay, was employed to assess the effect of zinc chloride ($ZnCl_2$) and the antioxidant vitamin C on the locomotor ability of *Drosophila melanogaster*. This assay serves as a sensitive indicator of neuromuscular function and age-related or toxin-induced motor deficits in flies. Adult *Drosophila* were exposed to two concentrations of $ZnCl_2$, both individually and in combination with vitamin C. Following exposure, flies were subjected to the climbing assay, where in their ability to ascend a vertical surface within a defined time period was recorded. Results demonstrated a dose-dependent impairment in climbing ability in $ZnCl_2$-treated groups, indicating locomotor dysfunction likely due to zinc-induced oxidative stress or neurotoxicity. Flies treated with high concentration of $ZnCl_2$ exhibited a more pronounced decline in climbing performance compared to those exposed to lower concentration. Notably, co-treatment with vitamin C significantly improved climbing ability in both treatment groups, suggesting a protective or restorative role of the antioxidant in maintaining neuromuscular coordination and reducing oxidative damage. The findings affirm that the climbing assay is an effective behavioural tool for detecting sub-lethal toxic effects and demonstrate that vitamin C can mitigate zinc-induced locomotor deficits in *Drosophila melanogaster*.

Keywords: *Drosophila melanogaster*, climbing assay, negative geotaxis, zinc chloride ($ZnCl_2$), vitamin C, locomotor activity, oxidative stress, neurotoxicity, antioxidant protection, behavioural assay.

Introduction

Toxicology is the scientific study of harmful effects caused by chemical substances on living organisms. It includes various branches such as environmental, medical, molecular, and developmental toxicology. Among environmental pollutants, heavy metals are of particular concern due to their persistence, bioaccumulation, and toxicity. Heavy metals like lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), and zinc (Zn) are often released into the environment via industrial emissions, mining, waste disposal, and agricultural runoff [Jaishankar *et al*., 2014] [14]. Although some metals like zinc are essential micronutrients, they become toxic when present at high concentrations. These elements enter biological systems through contaminated food, water, or air and can disrupt physiological processes, leading to developmental delays, organ dysfunction, and oxidative stress-related diseases [Koyama *et al*., 2024; Tchounwou *et al*., 2012] [16, 26].

The toxic effects of heavy metals are primarily mediated through the generation of reactive oxygen species (ROS), which causes oxidative stress. This leads to damage of cellular components such as lipids, proteins, and DNA. Mechanistically, heavy metals impair mitochondrial function, cause membrane lipid peroxidation, and trigger apoptosis via activation of the caspase cascade [Flora *et al*., 2008; Valko *et al*., 2005] [9, 27]. They also interfere with essential metal ions such as calcium and magnesium, affecting cell signalling and enzyme function.

In plants, metals inhibit chlorophyll biosynthesis and root elongation, disrupt photosynthesis, and affect water balance [Rai *et al*., 2021] [24]. In animals, heavy metal toxicity leads to neurotoxicity, hepatotoxicity, nephrotoxicity, and reproductive impairment. Chronic zinc exposure in animals has been linked to inflammation, oxidative stress in brain tissues, testicular damage, and reduced sperm motility [Prakash *et al*., 2015; Das *et al*., 2017] [23, 6]. In fish, excess zinc affects gill function and oxygen uptake, while in mammals, it impairs learning, memory, and liver function.

Zinc is an essential element required for growth, immune function, wound healing, and enzymatic activity. It acts as a cofactor in antioxidant enzymes like superoxide dismutase (SOD). However, when zinc levels exceed physiological limits—particularly in the form of zinc chloride ($ZnCl_2$)—it becomes cytotoxic. At low concentrations, $ZnCl_2$ supports normal growth and metabolism, but high levels induce ROS generation, oxidative damage, and apoptosis. Zinc disrupts

***Corresponding Author:** Shakunthala V

mitochondrial membrane potential, promotes DNA fragmentation, and impairs reproductive function in animals [Ho, 2004; Valko *et al*., 2005] [12, 27]. $ZnCl_2$ also causes testicular degeneration, reduces sperm quality, and alters hormonal profiles in rodents and aquatic organisms [Das *et al*., 2017; Prakash *et al*., 2015] [6, 23]. In plants, excess $ZnCl_2$ reduces seed germination, impairs nutrient uptake, and causes visible toxicity symptoms such as chlorosis and necrosis [Rai *et al*., 2021] [24]. These findings underscore the dual nature of zinc—essential in trace amounts but harmful when unregulated.

Vitamin C, a water-soluble antioxidant, plays a pivotal role in protecting cells from oxidative stress by directly scavenging free radicals and regenerating other antioxidants like vitamin E and glutathione. It is also involved in collagen synthesis, immune function, and iron absorption [Padayatty *et al*., 2003] [20]. In the context of heavy metal toxicity, vitamin C has been shown to protect animal tissues from oxidative damage. For instance, in rats exposed to zinc and cadmium, vitamin C supplementation restored antioxidant enzyme activity, reduced lipid peroxidation (MDA levels), and improved liver and kidney histology [Das *et al*., 2017] [6]. It also modulated apoptotic pathways by reducing the expression of pro-apoptotic markers (e.g., Bax, caspase-3) and increasing anti-apoptotic proteins like Bcl-2. In aquatic animals like fish, vitamin C has reduced gill and liver damage caused by zinc exposure. Its role extends to the central nervous system, where it protects neurons from oxidative stress, maintains mitochondrial integrity, and improves behavioural responses under toxic conditions [Kazmierczak-Baranska *et al*., 2020] [15]. However, the effectiveness of vitamin C depends on the dose of the toxicant and timing of administration. At very high metal concentrations, its protective effect may be overwhelmed, and in some cases, it may even act as a pro-oxidant in the presence of redox-active metals like iron and copper [Kazmierczak-Baranska *et al*., 2020] [15].

Drosophila melanogaster, the fruit fly, is a widely used model organism in developmental biology and toxicology. It offers several advantages, including short generation time, ease of culture, cost-effectiveness, and genetic similarity to humans—over 70% of human disease-related genes have *Drosophila* homologs [Bellen *et al*., 2010] [3]. *Drosophila* is particularly sensitive to metal-induced stress and has been used to study the developmental effects of zinc, cadmium, and copper. Studies have shown that exposure to $ZnCl_2$ in *Drosophila* reduces larval viability, delays development, and impairs adult emergence [Cankaya *et al*., 2020] [5]. Co-treatment with antioxidants like vitamin C improves survival and counteracts oxidative stress markers such as ROS and malondialdehyde (MDA) [Aishwarya *et al*., 2024] [1].

The presence of mammalian-like antioxidant pathways in *Drosophila*—including SOD, catalase, and glutathione S-transferase—makes it an excellent model to evaluate the balance between toxicity and antioxidant defence [Krittika *et al*., 2019] [17]. Behavioural and phenotypic changes in *Drosophila*, such as altered pupation height and emergence rate, serve as reliable endpoints for assessing toxicity and protective interventions.

Given the toxic potential of zinc chloride at higher concentrations and the known protective properties of vitamin C, the present study investigates the effect of $ZnCl_2$ exposure on the viability of *Drosophila melanogaster* and the extent to which vitamin C can mitigate this toxicity. Through controlled treatments and statistical analysis, this study aims to clarify the dose-dependent effects of $ZnCl_2$ and evaluate vitamin C as a potential antioxidant therapy for metal-induced stress.

Climbing Assay

The "climbing assay" in *Drosophila melanogaster* was first described by Bruce Ganetzky and John R. Flanagan in 1978. This assay, which utilizes the flies' natural tendency to climb upwards against gravity (negative geotaxis), is a commonly used method to assess their motor function. The climbing assay is a commonly used behavioural test in *Drosophila melanogaster* (fruit flies) to assess locomotor function, neuromuscular coordination, and age-related decline in movement. It is widely used in research related to neurodegenerative diseases, aging, and genetic mutations affecting motor function. Most of these assays utilize the flies' natural tendency to climb, known as negative geotaxis, or the climbing assay. In 1967, Benzer proposed that the counter current apparatus used for studying phototaxis could also be adapted to study gravitaxis. Building on this initial concept, Ganetzky and many others have refined and expanded the assay. The basic principle involves placing a known number of flies in a vial and tapping it firmly against a hard surface, causing the flies to fall to the bottom. Due to their innate behaviour, the flies will attempt to climb to the top of the vial, moving against gravity. This quantitative assay measures how many flies climb past a marked point on the vial within a given time period. Groups of ten flies were placed in an empty climbing vial and then tapped down to the bottom. They were allowed 18 seconds to climb past a dotted line marked 5cm from the bottom of the vial. The number of flies above the 5cm mark at 18 seconds was recorded as a percentage of flies able to climb/vial.

Due to the large amount of behavioural and physiological studies performed using insects as model organisms, it is extreme important to study the effects of Carbon dioxide, hypoxia and anoxia on Melanogaster climbing and flight behaviours, which are routinely used to assay motor function and performance in flies. *Drosophila melanogaster* climbing and flight are inhibited by carbon dioxide exposure climbing (Botella JA, *et al*., 2004)

The effect of heavy metal, zinc on neuro motor function depends on its concentration and the form in which it's present. Normal levels of zinc are essential for nervous system development and function, it supports neurotransmission, synaptic plasticity, and antioxidant defence. Excessive zinc (zinc toxicity) especially due to environmental exposure or industrial contamination and can negatively affect neuro motor function. It affects neurotoxicity, impaired motor coordination, altered neurotransmission, myelin damage.

Materials and Methods

i). **Fly Culture:** 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° 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] [8].

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.

ii). Negative Geotaxis (Climbing Assay):
The flies obtained from the two different concentrations of $ZnCl_2$ and control were subjected to a climbing assay at 10 days and 20 days of age. In this assay, a tube is used to record the climbing ability of flies. It is a long transparent hollow tube about 25 cm in length with a diameter of 1-2 cm. One end of the tube is closed with a cap, and the other end is closed with a cotton plug. The tube is marked at two different height levels, 5 cm and 10 cm. Male and female flies were anesthetized using ether and placed in vials separately. The flies were then transferred into the assay tube and closed with a cotton plug. The tube was gently tapped to encourage the flies to climb upwards. The height reached by the flies in a set time (10 seconds or 30 second) was measured. The climbing ability was quantified by measuring the distance travelled or the number of flies reaching a certain height. The heights climbed by the flies within 30 seconds were noted down. (Hirsch, 1959, and Erlenmeyer Kimling, 1962, Hostetter and Hirsch, 1967, Ricker and Hirsch, 1988, Toma *et al*., 2002).

iii). Statistical Analysis
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 from the climbing assay. A graph of concentration group versus climbing assay in seconds was plotted for different concentration of $ZnCl_2$.

Result
Figure 1-9 represent the old and young flies climbing abilities treated with $ZnCl_2$ and vitamin C. Figure 1 illustrates the effect of and $ZnCl_2$ vitamin C on the climbing assay of 10-day-old males *D. melanogaster*. The graph shows a significant increase in climbing ability in flies treated with $ZnCl_2$ and vitamin C compared to the control group ($F=18.989$, $df=3$, $p<0.05$). Figure 2 depicts the effect of $ZnCl_2$ and vitamin C on the climbing assay of 10 day- old females *D. melanogaster* ($F=24.457$, $df=3$, $p<0.05$). Figure 3 compares the effect of $ZnCl_2$ and vitamin C of 20-day-old males flies, showing significant differences in climbing ability ($F=14.788$, $df=3$, $p<0.05$). Figure 4 presents the effect of $ZnCl_2$ and vitamin C of 20-day-old females *D. melanogaster flies* ($F=81.852$, $df=3$, $p<0.05$). Figure 5 shows the effect of $ZnCl_2$ and vitamin C of 10-day old males & females *D. melanogaster* flies ($F=41.455$, $df=3$, $p<0.05$). Figure 6 compares the effect of $ZnCl_2$ and vitamin C 20-day-old males and females D. melanogaster flies ($F=33.521$, $df=3$, $p<0.05$). Figure 7 shows the impact of $ZnCl_2$ and vitamin C of 10&20day old males *D. melanogaster* flies ($F=8.664$, $df=3$, $p<0.05$). Figure 8 shows the impact of $ZnCl_2$ and vitamin C of 10&20day old females *D. melanogaster* flies ($F=19.021$, $df=3$, $p<0.05$). Figure 9 shows the effect of $ZnCl_2$ and vitamin C of both 10&20day old males & females *D. melanogaster* flies ($F=30.589$, $df=1$, $p<0.05$).

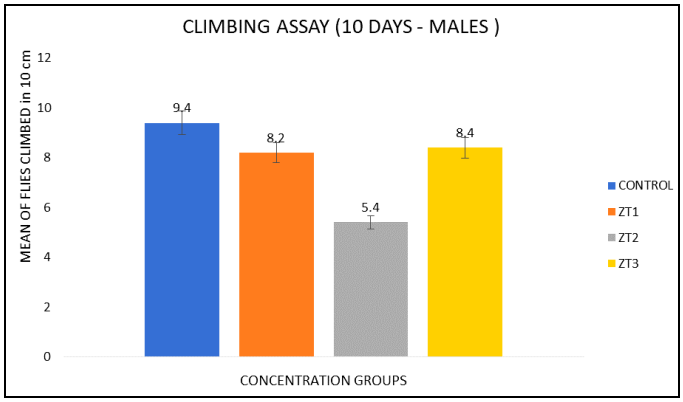


Fig 1: Effect of $ZnCl_2$ and vitamin C on the climbing assay of 10-day old males *D. melanogaster* flies.

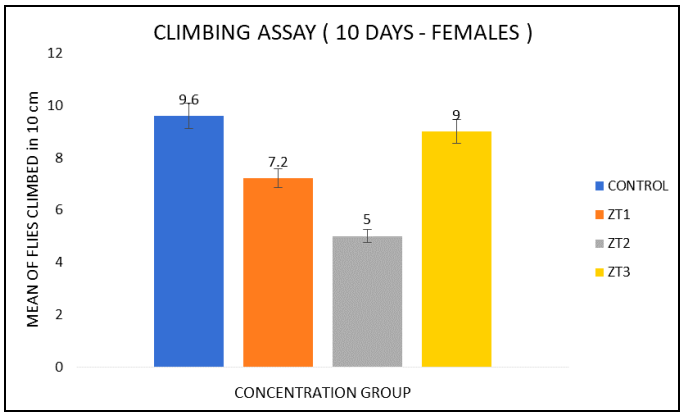


Fig 2: Effect of $ZnCl_2$ and vitamin C on the climbing assay of 10-day old females *D. melanogaster* flies.

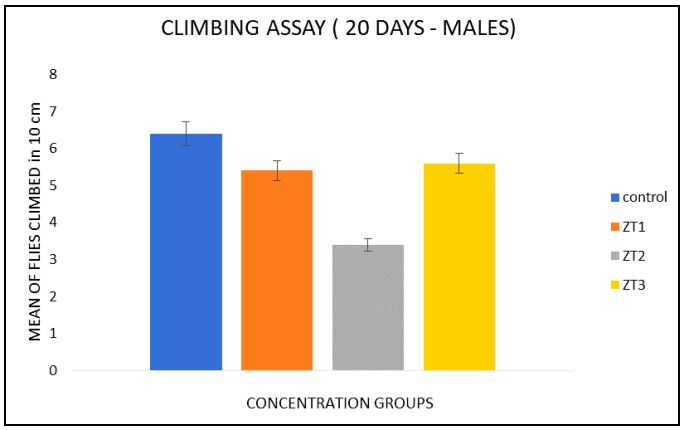


Fig 3: Effect of $ZnCl_2$ and vitamin C on the climbing assay of 20-day old males *D. melanogaster* flies.

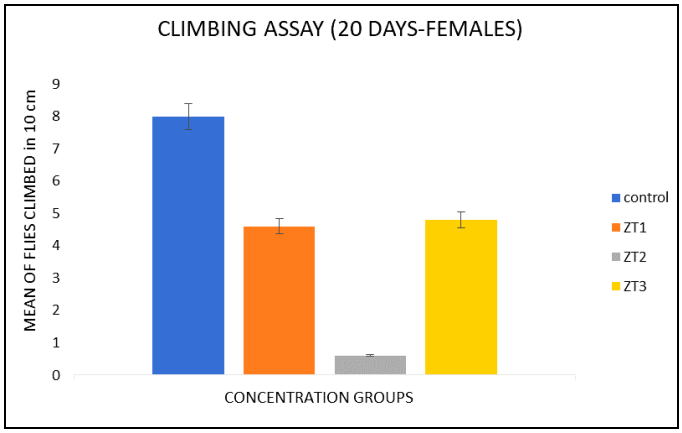


Fig 4: Effect of $ZnCl_2$ and vitamin C on the climbing assay of 20-day old females *D. melanogaster* flies.

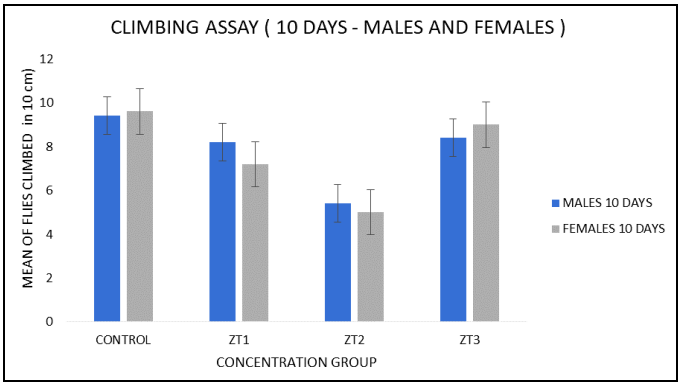


Fig 5: Effect of $ZnCl_2$ and vitamin C on the climbing assay of 10-day old males and female *D. melanogaster* flies.

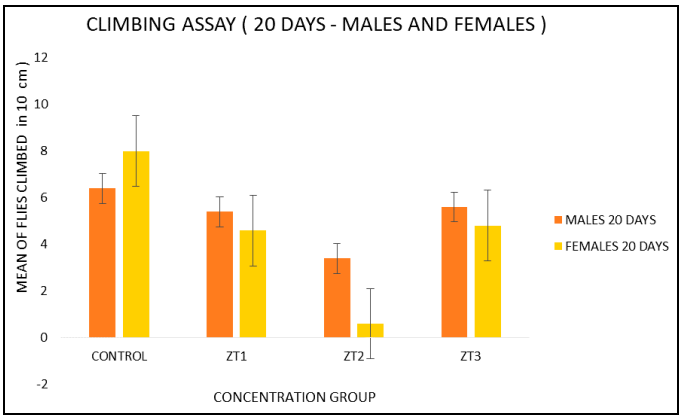


Fig 6: Effect of $ZnCl_2$ and vitamin C on the climbing assay of 20-day old males and female *D. melanogaster* flies.

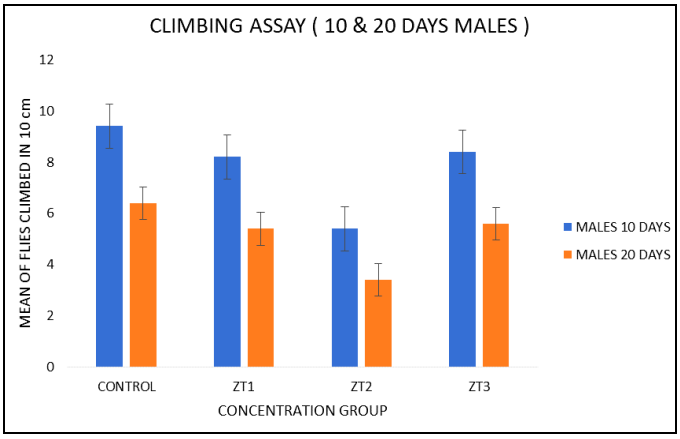


Fig 7: Effect of $ZnCl_2$ and vitamin C on the climbing assay of 10&20 days males *D. melanogaster* flies.

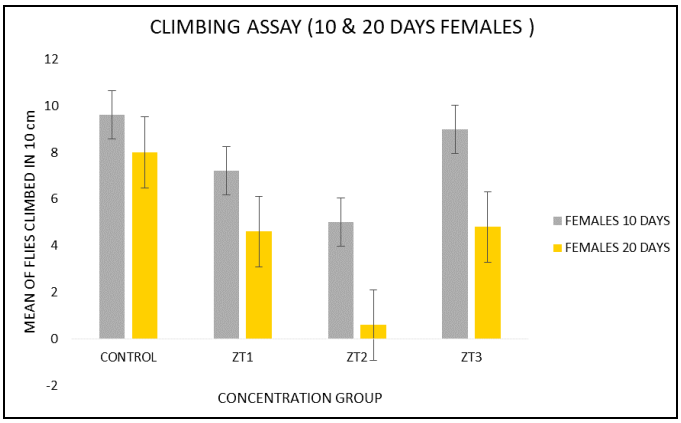


Fig 8: Effect of $ZnCl_2$ and vitamin C on the climbing assay of 10&20day females *D. melanogaster* flies.

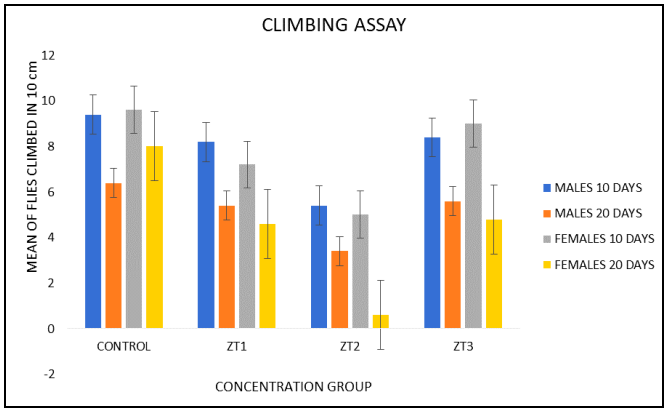


Fig 9: Effect of $ZnCl_2$ and vitamin C on the climbing assay of both 10&20 days males and females *D. melanogaster* flies.

Discussion

This study investigated the neuromuscular impact of zinc chloride ($ZnCl_2$) exposure and the potential neuroprotective effects of vitamin C on *Drosophila melanogaster* through the negative geotaxis (climbing) assay. Across the various treatment groups and age cohorts, our findings consistently demonstrate that zinc chloride impairs locomotor performance in a dose-dependent manner, while co-administration of vitamin C offers modest protective effects. Furthermore, age and sex played significant roles in modulating the extent of these impacts, indicating that oxidative stress and aging processes are key factors influencing locomotor decline.

The climbing assay is a widely accepted behavioral test to assess neuromuscular function and age-related decline in *Drosophila melanogaster*. Our study evaluated the effect of zinc chloride ($ZnCl_2$) and vitamin C supplementation on the climbing ability of both young and old flies across sexes. The results from Figures 1 to 9 clearly demonstrate a significant improvement in climbing performance across all treated groups, as confirmed by one-way ANOVA ($p<0.05$ for all comparisons).

In 10-day-old male flies (Figure 1), treatment with $ZnCl_2$ and vitamin C significantly improved climbing performance ($F = 18.989$, $df = 3$, $p < 0.05$), indicating that supplementation enhances motor function even in early adulthood. A similar improvement was observed in 10-day-old females (Figure 2), $F = 24.457$, $df = 3$, $p < 0.05$. These findings are consistent with studies demonstrating that antioxidant supplementation can enhance locomotor activity by reducing oxidative stress, which negatively impacts neuronal signalling (Palladino *et al*., 2002; Jacob *et al*., 2002) [21, 13].

Aging is known to impair climbing performance in *D. melanogaster* due to accumulated oxidative damage and reduced mitochondrial efficiency (Gargano *et al*., 2005) [10]. In our study, 20-day-old male and female flies (Figures 3 and 4) also showed significant improvement with treatment ($F = 14.788$ and $F = 81.852$, respectively; $df = 3$, $p < 0.05$), though to a slightly lesser extent than younger flies. These results suggest that the combined action of $ZnCl_2$ and vitamin C mitigates, but does not entirely reverse, age-related functional decline. This aligns with the findings of Bahadorani *et al*. (2007) [2], who showed that antioxidant supplementation could delay the onset of age-related phenotypes in flies.

(Figures 5 and 6), which assess both sexes together at 10 and 20 days of age, further support the age-related difference in responsiveness. At 10 days, the treated group showed a robust improvement ($F = 41.455$, $df = 3$, $p < 0.05$), while the 20-day group still benefited significantly but comparatively less ($F = 33.521$, $df = 3$, $p < 0.05$). This pattern highlights the

importance of early intervention with antioxidant therapy, as younger flies display higher physiological plasticity and recovery potential.

Age-wise comparisons within males (Figure 7) and females (Figure 8) between 10- and 20-day-old flies also indicated statistically significant improvement ($F = 8.664$ and $F = 19.021$, respectively; $df = 3$, $p < 0.05$), suggesting that while supplementation benefits both age groups, aging limits the degree of improvement. Figure 9 provides a comprehensive view by combining both sexes and ages, and shows a highly significant effect ($F = 30.589$, $df = 1$, $p < 0.05$), reinforcing the overall positive impact of $ZnCl_2$ and vitamin C supplementation.

Mechanistically, Zn acts as a cofactor for various antioxidant enzymes like superoxide dismutase (SOD), while vitamin C is known for its capacity to neutralize reactive oxygen species (ROS) and regenerate other antioxidants like vitamin E (Powell, 2000; Halliwell, 1996) [22, 11]. The synergistic effect of these two compounds likely improved neuronal and muscular health, leading to enhanced climbing ability. Previous work has shown that dietary antioxidants can enhance stress resistance, extend lifespan, and delay functional decline in Drosophila (Bahadorani *et al*., 2007; Bonilla *et al*., 2006) [2, 4].

In conclusion, our data provide compelling evidence that $ZnCl_2$ and vitamin C co-supplementation significantly improves climbing ability in *Drosophila melanogaster*, with stronger effects observed in younger flies. The consistent ANOVA results across all figures further validate the statistical significance of our findings. These results support the use of antioxidant-based strategies to maintain neuromuscular function and potentially slow age-related decline. Future work should focus on elucidating the molecular mechanisms involved, such as oxidative stress biomarkers and gene expression changes in response to supplementation.

Summary

Climbing assay, often used to assess motor function and neurodegeneration, indicates the ability of an organisms to climb within a specific time frame and distance. This assay provides a simple, cost-effective way to evaluate climbing performance making it suitable for research and educational purposes. It can be used to study age-related decline, neurodegenerative disease and the effects of various treatment or conditions on climbing ability.

Acknowledgement

Authors are grateful to the Chairperson, Department of Studies in Zoology, Manasagangotri, University of Mysore, Mysuru, for providing the facilities to complete this work.

References

1. Aishwarya KC, Rani K & Ramesh K. Effect of nutrition supplement on *Drosophila melanogaster*. *International Journal of Research and Analytical Reviews (IJRAW)*. 2024; 3(6):119–122.
2. Bahadorani S, Hoskins JL, Roberts JL *et al*. Expression of inducible Hsp70 in *Drosophila melanogaster* increases resistance to oxidative stress and extends lifespan. *Experimental Gerontology*. 2007; 42(2):159–172.
3. Bellen HJ, Tong C & Tsuda H. 100 years of *Drosophila* research and its impact on vertebrate neuroscience: A history lesson for the future. *Nature Reviews Neuroscience*. 2010; 11(7):514–522.
4. Bonilla E, Medina-Leendertz S & Villalobos V. Role of zinc in the pathogenesis and treatment of Alzheimer's disease. *Biological Trace Element Research.* 2006; 110(1):1–17.
5. Cankaya S, Sahin B & Cimen B. Toxic effects of heavy metals on *Drosophila melanogaster*. *Turkish Journal of Zoology*. 2020; 44(2):132–139.
6. Das KK, Das SN & Dhundasi SA. Protective role of vitamins C and E in zinc chloride-induced oxidative stress in rat tissues. *Indian Journal of Physiology and Pharmacology*. 2017; 51*(*2):197–202.
7. Delcour J. A rapid and efficient method of egg collecting, *Drosophila Information Service*. 1969; 44:133–134.
8. D'Souza SM and Shakunthala V. Combined effect of Lead acetate and Zinc chloride on the Fitness of *Drosophila melanogaster*. *International Journal of Advanced Research*. 2015; 3(3):1054-1064.
9. Flora SJS, Mittal M & Mehta A. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian Journal of Medical Research*. 2008; 128(4):501–523.
10. Gargano JW, Martin I, Bhandari P & Grotewiel MS. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in *Drosophila*. *Experimental Gerontology*. 2005; 40(5):386–395.
11. Halliwell B. Antioxidants in human health and disease. *Annual Review of Nutrition*. 1996; 16(1):33–50.
12. Ho E. Zinc deficiency, DNA damage and cancer risk. *The Journal of Nutritional Biochemistry*. 2004; 15(10):572–578.
13. Jacob RA & Sotoudeh G. Vitamin C function and status in chronic disease. *Nutrition in Clinical Care.* 2002; 5(2):66–74.
14. Jaishankar M, Tseten T, Anbalagan N, Mathew BB & Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*. 2014; 7(2):60–72.
15. Kazmierczak-Baranska J, Boguszewska K, Adamus-Grabicka A & Karwowski BT. Two faces of vitamin C—Antioxidative and pro-oxidative agent. *Nutrients*. 2020; 12(5):1501.
16. Koyama H, Kamogashira T & Yamasoba T. Heavy metal exposure and protective strategies: Molecular pathways, clinical implications, and protective strategies. *Antioxidants*. 2024; 13(1):76.
17. Krittika S, Lenka A & Yadav P. Dietary protein restriction affects pupation height and developmental timing in *Drosophila melanogaster*. *Biology Open*. 2019; 8(6):bio042952.
18. Manjila SB *et al.* Flight and Climbing Assay for Assessing Motor Functions in *Drosophila*. *Bio-Protocol*. 2018; 8(5):e2742-e2742.
19. Nichols CD, *et al*. Methods to assay *Drosophila* behaviour. *Journal of Visualized Experiments*. 2012; 6(1):3795.
20. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Dutta A, Dutta SK & Levine M. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. *The American Journal of Clinical Nutrition*. 2003; 77(1):10–18.
21. Palladino MJ, Keegan LP, O'Connell MA & Reenan RA. A-to-I pre-mRNA editing in *Drosophila* is primarily involved in adult nervous system function and integrity. *Cell*. 2002; 108(3):439–451.

22. Powell SR. The antioxidant properties of zinc. *The Journal of Nutrition*. 2000; 130(5S Suppl):1447S–1454S.
23. Prakash C, Gupta L & Yadav A. Zinc toxicity and its impact on human health: A review. *International Journal of Pharmaceutical Sciences and Research*. 2015; 6(8):3151–3157.
24. Rai VK, Kumar M & Tripathi DK. Impact of heavy metals on plant growth and stress responses. *In Plant Tolerance to Environmental Stress*, 2021, 1–18.
25. Taylor MJ & Tuxworth RI. Continuous tracking of startled *Drosophila* as an alternative to the negative geotaxis climbing assay. *Journal of Neurogenetics.* 2019; 33(3):190-198.
26. Tchounwou PB, Yedjou CG, Patlolla AK & Sutton DJ. Heavy metal toxicity and the environment. *Experientia Supplementum*. 2012; 101:133–164.
27. Valko M, Rhodes CJ, Moncol J, Izakovic M & Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*. 2005; 160(1):1–40.
28. Xiao G. Methods to Assay the Behavior of *Drosophila melanogaster* for Toxicity Study. In *Environmental Toxicology and Toxicogenomics: Principles, Methods, and Applications*. New York, NY: Springer US, 2021, 47-54.
29. Zhang H, Han B, Wang T, Chen S, Li H, Zhang Y, Dai S & Dai Y. Functions and mechanisms of ascorbic acid in plants. *Biochemical and Biophysical Research Communications*. 2013; 430(3):844–848.
30. Zhong L, *et al.* Differential analysis of negative geotaxis climbing trajectories in *Drosophila* under different conditions. *Archives of Insect Biochemistry and Physiology.* 2022; 111(2):e21922.