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# Impact of *Piper longum* extract on motor function in *Drosophila*: An investigation using the Negative geotaxis assay.

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## Abstract

This study investigates the impact of *Piper longum* on the climbing behavior of *Drosophila melanogaster*, focusing on different concentrations (10 mg/L and 20 mg/L) and age groups (5-day-old and 20-day-old). Using a climbing assay, we measured the motor function of male and female flies fed with *Piper longum* treated media compared to control media. The results indicated that *Piper longum* significantly enhances climbing activity in both male and female *Drosophila melanogaster*, with the effect varying by age and concentration. Specifically, 5-day-old flies showed the highest climbing activity with 20 mg/L *Piper longum*, whereas 20-day-old males exhibited the greatest improvement with the same concentration, and 20-day-old females responded better to the 10 mg/L concentration. These findings suggest that *Piper longum* can enhance motor function in *Drosophila melanogaster*, with differential responses based on sex, age, and treatment concentration.

***Key words:*** *Drosophila melanogaster*, *Piper longum*, climbing behavior, Motar function, Neuroenhancement

## Introduction

Organisms require energy for every task essential for survival and reproduction. Mobile organisms, in particular, depend on coordinated motor functions for activities such as finding food, shelter, mates, and evading predators. The central nervous system of *Drosophila melanogaster* allows for the study of various processes, including sensory functions (smell, taste, hearing, and vision) and motor activities (walking, flying), as well as learning and memory responses to phytochemicals. Drosophila is also pivotal in understanding molecular mechanisms and developing phytochemical-based medications for cancer and neurological diseases like Parkinson's and Alzheimer's, which affect locomotion. (Madabattula S T et al., 2015)

Piperine, a natural alkaloid found in *Piper nigrum* and *Piper longum*, has demonstrated potential in treating CNS disorders, including Parkinson's disease, depression, anxiety, and epilepsy. Its anti-inflammatory and antioxidative properties contribute to its anti-parkinsonian potential. Piperine exhibits neuroprotective effects by reducing oxidative stress, improving mitochondrial function, and inhibiting apoptosis. Additionally, it counteracts dopamine depletion and motor dysfunction. However, piperine's hydrophobic nature and poor water solubility limit its medicinal use, necessitating strategies to enhance its bioavailability to maximize therapeutic potential. (Srivastav et al.,2020)

Locomotion is a complex behavior influenced by the segregation of various interacting quantitative trait loci (QTLs) and is sensitive to environmental conditions. Understanding the genetic basis of locomotor activity is crucial for both human health and evolutionary biology. Despite significant research, our understanding of the genetic and environmental variance in locomotor activity remains limited.(Jordan, ., 2006)

*Drosophila melanogaster* serves as a model organism to investigate the molecular causes of numerous human illnesses, including cancer, cardiovascular diseases, and neurological conditions. Because of its short lifespan and access to potent genetic modification and genome-wide screening methods, *Drosophila* is a popular model organism for studying several aspects of neurodegenerative diseases in humans. (Boulea & Tricoire, 2015). Behavioral tests, such as the RING experiment, assess *Drosophila's* climbing abilities and larval mobility, providing insights into the influence of genetic mutations and environmental conditions on behavior. These assays are designed to be reliable, cost-effective, and sensitive enough to detect even minor variations in motor function.

The climbing assay, also known as negative geotaxis, measures the flies' natural inclination to climb. Proposed by Benzer in 1967, this assay utilizes the counter-current apparatus initially used for phototaxis studies. When tapped, adult flies exhibit an escape reflex by climbing towards the top of a vial. This reflex, studied for nearly thirty years, forms the basis of the Rapid Iterative Negative Geotaxis (RING) experiment, commonly known as the climbing assay. Researchers, such as Ganetzky and Flanagan (1978), and Gargano et al. (2005), have widely employed this technique to study motor output in *Drosophila.* (Mattew et al.,2019)

**Materials and Methods:**

**Materials and *Piper longum* [LP] Product Collection:** The *Piper longum* product was collected from Ayurveda College in Mysore.

**Establishments of stock**

The *Drosophila melanogaster* experimental stock was obtained from the University of Mysore's Manasagangotri *Drosophila* Stock Center. As one of the most widely used and well-understood model organisms, *Drosophila melanogaster* has a short and straightforward reproduction cycle lasting between 8 and 14 days, influenced by ambient temperature. The collected flies were distributed and cultured in several bottles containing wheat cream agar media (prepared by boiling 100 g of jaggery, 100 g of wheat powder, and 10 g of

agar agar in 1000 ml of double-distilled water, with 7.5 ml of propionic acid added). Twenty flies (10 males and 10 females) were placed in each culture bottle and kept in the dark for 12 hours at 22°C and 70% relative humidity. Virgin flies were isolated at the pupa stage and cultured in test media containing 10 mg/L and 20 mg/L of long pepper (*Piper longum*). Flies grown in normal wheat agar media served as controls. After five days in their respective media (control and test), the flies were used for climbing assay studies. The results from both the tested media and the control media were compared and discussed.

| **SL. No** | **Ingredients** | **Control media (1000ml)** | **Test media-1 (1000ml)** | **Test media-2 (1000ml)** |
|---|---|---|---|---|
| 1 | Wheat powder | 100g | 100g | 100g |
| 2 | Jaggery | 100g | 100g | 100g |
| 3 | Agar | 10g | 10g | 10g |
| 4 | Propanoic acid | 7.5ml | 7.5ml | 7.5ml |
| 5 | Long pepper powder | - | 10mg | 20mg |

Table 1**:** constituent of the media for treatment (group and control).

**Equipment**

- Timer/stop watch.
- Behaviour room with controlled temperature (~25 °C) and humidity (~60%)

**Protocol**

- Freshly eclosed flies of both sexes should be collected in vials with normal *Piper longum* media in batches of 10 and aged for 5 and 20 days.
- Transfer a batch of 10 non-anaesthetized flies to a glass cylinder by tapping the vial on a pounding pad, removing the cotton plug, inverting the flies, tapping firmly, and closing with a fresh cotton plug.
- Allow the flies to adjust to their new surroundings for 3-4 minutes.
- As soon as the stop watch is ready, gently tap the measuring cylinder three times with ten flies, making sure that all of the flies fall to the bottom of the cylinder, then turn on the stop watch.
- Count the number of flies that cross the 10 cm mark within 2 sec.
- Calculate the average climbing ability for the both sex of flies by repeating the experiment twice more with the same set of flies, allowing 2 minutes to pass between attempts. After the assay is completed for a male *Drosophila* and female *Drosophila* wipe the cylinder with dry tissue paper. When all of the experiments are finished, give the cylinder a quick wash with water and allow it to air dry. Be careful not to clean with ethanol, soap, or any other type of detergent. (Madabattula *et al*. 2015)

**Statistical analysis**

SPSS IBM version 29.0 was used to analyse the data that were collected. For the data collected for the climbing experiment, the following analyses were performed: mean, standard error, two-way ANOVA, and Tukey's Post-Hoc test. A mean ascending assay graph was created. The graph was examined.

**Results**

- **Fig. 1** Represent a *Piper longum* on climbing assay of 5days old male *Drosophila* fed with control and treated media. According to the data obtained the climbing assay was found high in *Drosophila* fed with 20mg *Piper longum* tested media compared to control. In between the concentration groups the climbing assay is found high in *Drosophila* fed with 20mg treated media which is significant with $P<0.05$, df=2 and F= 33.8
- **Fig. 2** Represent a *Piper longum* on climbing assay of 5days old female Drosophila fed with control and treated media. According the data obtained the climbing assay was found high in *Drosophila* fed with 20mg *Piper longum* treated media compared to control. in between the concentration groups the climbing assay is found high in *Drosophila* fed with 20mg treated media witch is significant with $P<0.05$, df=2 and F=14.162
- **Fig 3** represent a comparison graph on climbing assay of 5days old male and female *Drosophila* fed with control and treated media according to the data obtained the climbing assay was found high in male *Drosophila* fed with treated media compare to control. In between concentration groups climbing assay is high on 20mg *Piper longum* compared to 10mg long pepper which is insignificant with $P>0.05$, df= 2 and F=1.206
- **Fig. 4** Represent a *Piper longum* on climbing assay of 20 days old male Drosophila fed with control and treated media. According the data obtained the climbing assay was found high in *Drosophila* fed with 20mg piper longum treated media compared to control. in between the concentration groups the climbing assay is found high in drosophila fed with 20mg treated media which is significant with $P<0.05$, df=2 and F= 38.005.
- **Fig. 5** Represent a *Piper longum* on climbing assay of 20days old female *Drosophila* fed with control and treated media. According the data obtained the climbing assay was found high in *Drosophila* fed with 10mg *piper longum* treated media compared to control. in between the concentration groups the climbing assay is found high in *Drosophila* fed with 10mg treated media which is significant with $P<0.05$, df=2 and F= 7.121
- **Fig. 6**; represent a comparison graph on climbing assay of 20days old male and female *Drosophila* fed with control and treated media. Climbing assay was found high on treated media. in between the concentration groups the climbing assay is found high in Flies fed with 20mg treated media which is significant with $P<0.05$, df=2 and F= 38.005 .

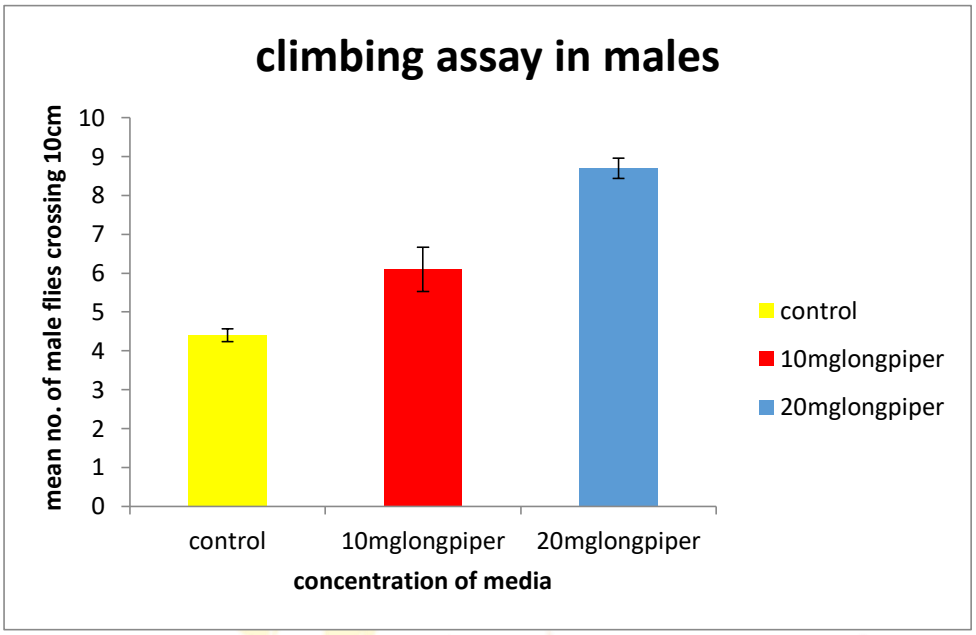


**FIG 1**; Effect of *piper longum* on climbing assay in 5days old male *Drosophila melanogaster*

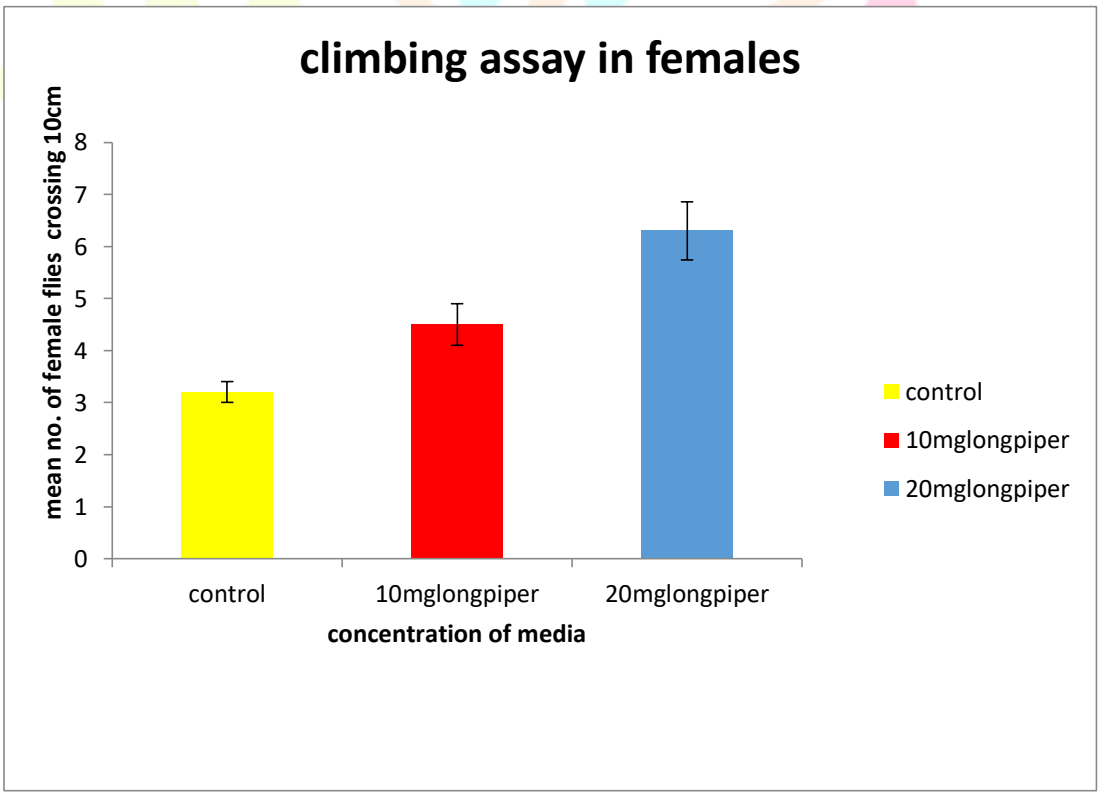


**FIG 2**: Effect of *Piper longum* on climbing in 5 days old female *Drosophila*

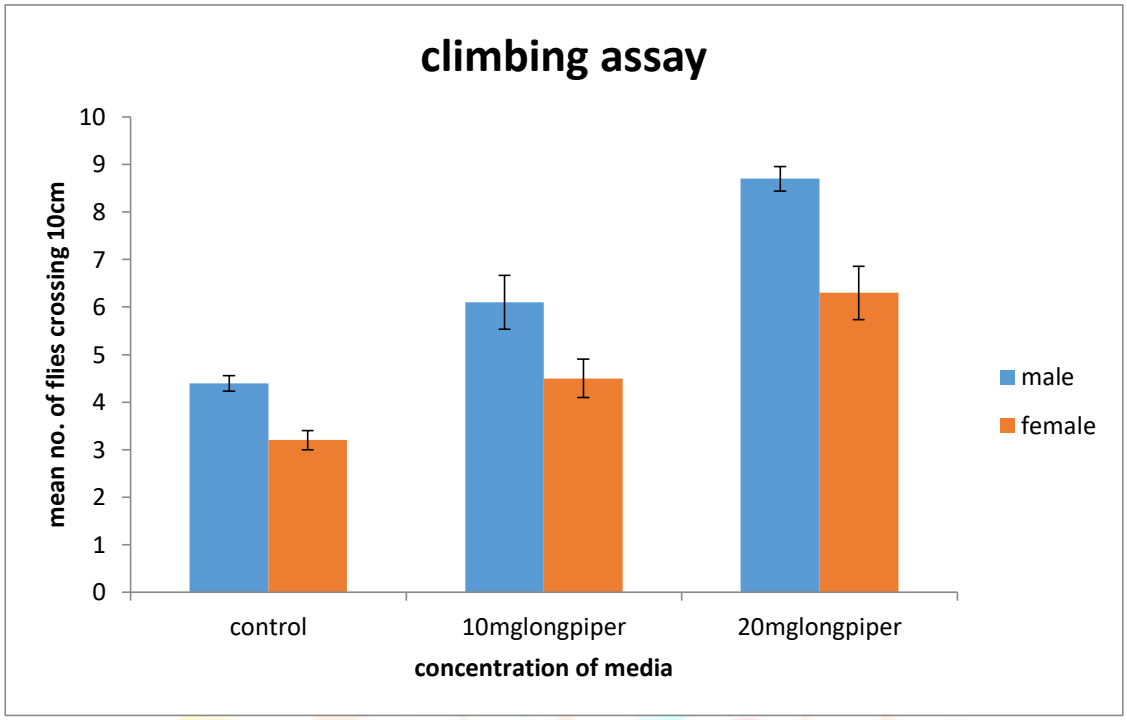


**FIG 3**: Effect of *Piper longum* on climbing assay in5 days old male and female *Drosophila*

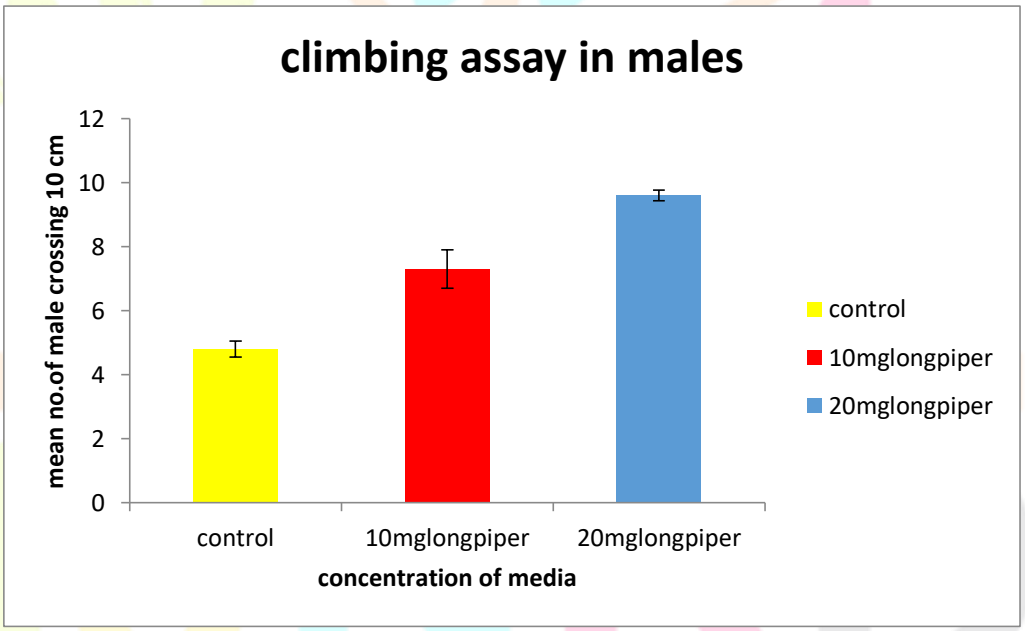


**FIG 4**; Effect of *piper longum* on climbing assay in 20 days old male *Drosophila melanogaster*

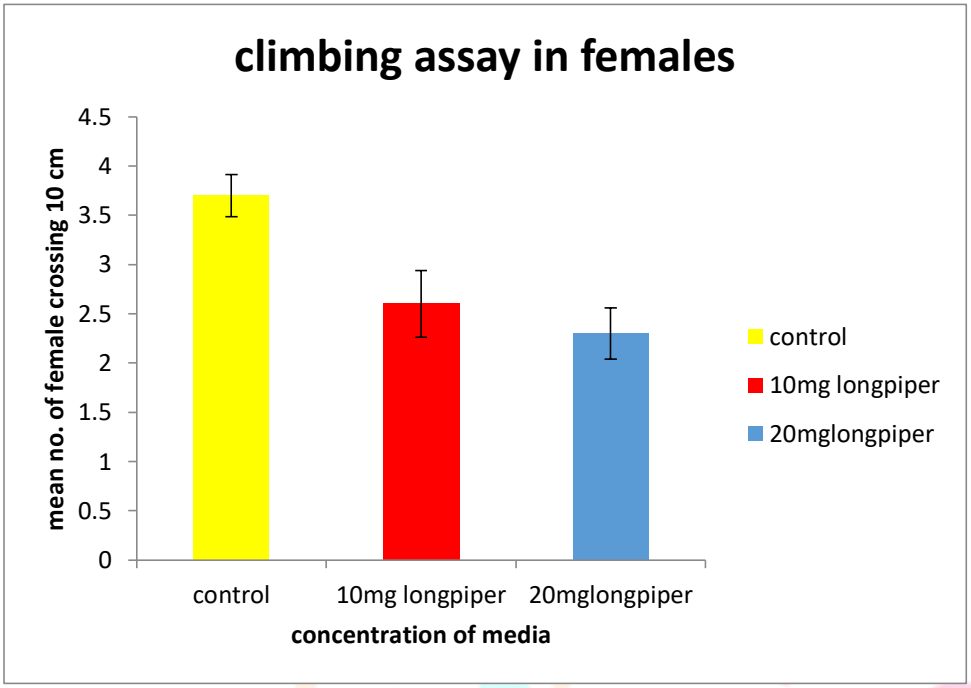


**FIG 5;** Effect of *piper longum* on climbing assay in 20 days old female *Drosophila melanogaster*

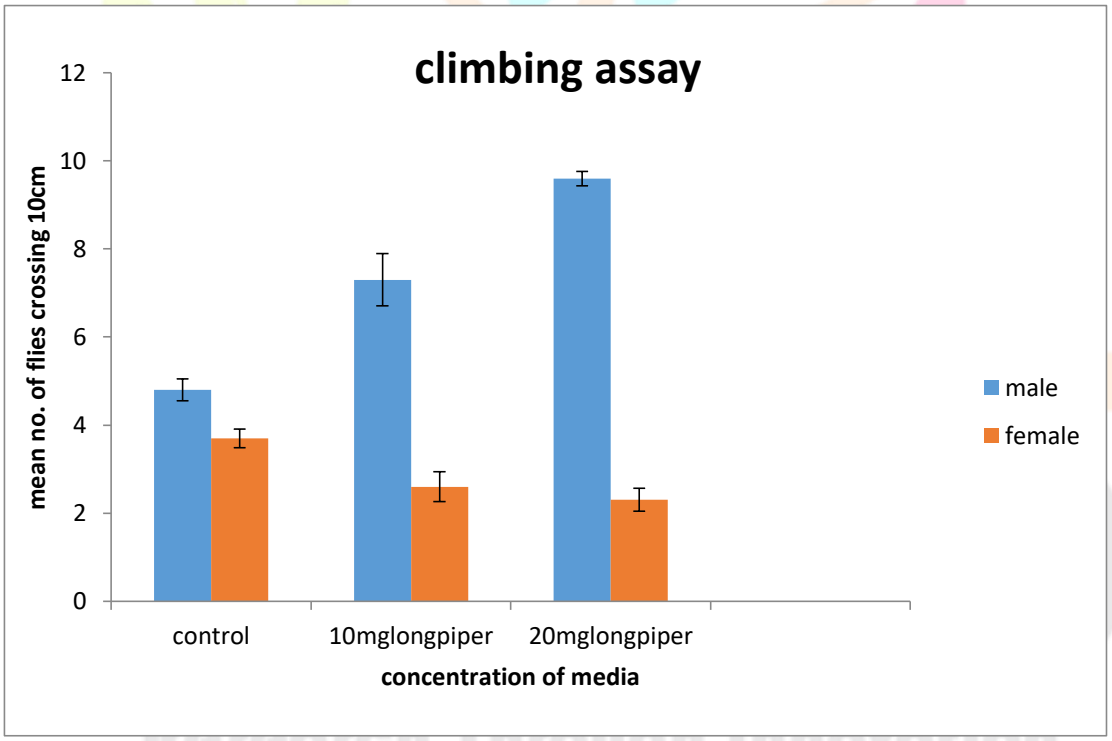


**FIG 6**; Effect of *piper longum* on climbing assay in 20 days old male and female *Drosophila melanogaster*

**Discussion:**

The climbing assay results for 5-day-old male *Drosophila melanogaster* fed with control and treated media indicate that flies fed with 20 mg *Piper longum* treated media exhibited significantly higher climbing activity compared to those fed with control media. Among the concentration groups, the climbing assay was highest in flies fed with 20 mg treated media, which was statistically significant ($P < 0.05$, $df = 2$, $F = 33.8$), whereas, 5-day-old female *Drosophila melanogaster*, the climbing assay also showed higher activity in flies fed with 20 mg

*Piper longum* treated media compared to control media. Similarly, within the concentration groups, the climbing assay was highest in flies fed with 20 mg treated media, with a statistically significant result ($P < 0.05$, $df = 2$, $F = 14.162$).

A comparative analysis of the climbing assay in 5-day-old male and female *Drosophila melanogaster* revealed higher climbing activity in males fed with treated media compared to control. Among the concentration groups, the climbing assay was higher in flies fed with 20 mg *Piper longum* compared to 10 mg, although the difference was not statistically significant ($P > 0.05$, $df = 2$, $F = 1.206$).

In 20-day-old male *Drosophila melanogaster*, the climbing assay results indicated significantly higher climbing activity in flies fed with 20 mg *Piper longum* treated media compared to control. Within the concentration groups, the highest climbing activity was observed in flies fed with 20 mg treated media, which was statistically significant ($P < 0.05$, $df = 2$, $F = 38.005$). For 20-day-old female *Drosophila melanogaster*, the climbing assay showed higher activity in flies fed with 10 mg *Piper longum* treated media compared to control. Among the concentration groups, the climbing assay was highest in flies fed with 10 mg treated media, with a statistically significant result ($P < 0.05$, $df = 2$, $F = 7.121$). A comparative analysis of the climbing assay in 20-day-old male and female *Drosophila melanogaste*r showed higher climbing activity in flies fed with treated media compared to control. Within the concentration groups, the highest climbing activity was observed in flies fed with 20 mghe results of the climbing assays indicate that *Piper longum* significantly enhances climbing activity in both male and female *Drosophila melanogaster*, with notable differences based on age and concentration of the treatment.

5-**Day-Old Flies**: Both male and female flies fed with 20 mg *Piper longum* exhibited higher climbing activity compared to control. The increase in climbing activity was more pronounced in males, although not statistically significant when compared to females.

**20-Day-Old Flies**: In older flies, males showed significantly higher climbing activity with 20 mg *Piper longum* compared to control. Females, on the other hand, exhibited the highest climbing activity with 10 mg *Piper longum*, suggesting a possible age and sex-specific response to the treatment.

**Concentration vs. Age**

- **5-Day-Old Flies**: Higher climbing activity was observed with 20 mg *Piper longum* in both sexes, indicating that younger flies benefit more from higher concentrations of *Piper longum*.
- **20-Day-Old Flies**: Older male flies showed significantly higher climbing activity with 20 mg *Piper longum*, whereas older female flies exhibited higher activity with 10 mg *Piper longum*. This suggests a differential concentration response based on age, with older females possibly benefiting from a lower concentration.

These findings suggest that *Piper longum* can improve motor function in *Drosophila melanogaster,* with varying effects depending on sex, age, and concentration. The observed age-specific responses highlight the need for

further research to understand the underlying mechanisms and optimize treatment protocols for different age groups.

**Conclusion**

The study demonstrates that *Piper longum* significantly improves climbing activity in *Drosophila melanogaster*, with variations observed based on sex, age, and concentration.

**Key Findings:**

- **5-Day-Old Flies**: Both male and female flies showed enhanced climbing activity with 20 mg/L *Piper longum*, with males exhibiting a more pronounced response.
- **20-Day-Old Flies**: Older males responded best to 20 mg/L *Piper longum*, while older females showed the highest climbing activity with 10 mg/L *Piper longum.*

**Implications:**

Age-Specific Responses**:** Younger flies benefit more uniformly from higher concentrations, whereas older flies exhibit a sex-specific response to different concentrations.

**Potential Applications**: These findings suggest that *Piper longum* could be used to enhance motor function in experimental models, with implications for studies on aging, neurodegenerative.

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