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Effect of the *Terminalia chebula*, one of the Constituent of Triphala on Climbing Ability of *Drosophila melanogaster*

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Abstract

This study investigates the effect of *Terminalia chebula* on the climbing behavior of *Drosophila melanogaster*. The climbing assay, a measure of motor function, was conducted on both 5-day-old and 20-day-old male and female flies raised on control and treated media. The treated groups received either 250 mg or 300 mg of *Terminalia chebula*. The results showed a significant improvement in climbing ability in flies treated with *Terminalia chebula* compared to the control group. Notably, the 300 mg treatment group exhibited the highest climbing performance across all age and sex groups, with males outperforming females in both control and treated conditions. Age-related differences were evident, with younger flies (5 days old) displaying superior climbing ability compared to older flies (20 days old). These findings suggest that *Terminalia chebula* enhances motor function and may mitigate age-related declines in Drosophila melanogaster. The study highlights the potential of *Terminalia chebula* as an antioxidant, neuroprotective, anti-inflammatory, and metabolic-enhancing effects, warranting further research to elucidate the underlying mechanisms and its applicability to other species.

Keywords: *Drosophila melanogaster, Terminalia chebula,* climbing ability

Introduction

Climbing, also known as negative geotaxis, is the natural tendency of fruit flies to move against gravity when agitated. This behavior is innate to *Drosophila melanogaster*. Motor control requires the central nervous system to integrate numerous sensory inputs and transform them into specific movements involving motor neurons and muscles (Manjila *et al*., 2018) [17]. Climbing ability data can be used to assess motor function in *Drosophila* (Fauzi *et al.,* 2020) [7]. Climbing behavior exhibits plasticity, meaning it can be influenced by environmental factors such as light intensity, humidity, temperature, and the presence of conspecifics or competitors. Understanding climbing behavior provides insights into their ecological roles, adaptation strategies, and interactions within their habitats (D'souza *et al*., 2015) [6].

There has been great interest in using this simple behaviour to acquire insights into changes in brain function related with ageing, the effect of medications, mutant genes, and human neurological and neurodegenerative illnesses (Ali *et al.,* 2011) [4]. Locomotion is vital for an organism. In *Drosophila melanogaster*, it includes activities such as walking, flying, and climbing (Willenbrink *et al.,* 2016) [27]. Important aspects of climbing behavior in fruit flies include the ability to investigate their environment, discover food sources, and navigate complex terrains such as plants. Flies can ascend to avoid predators or poor conditions on the ground, using vertical surfaces or vegetation as a refuge (Fauzi *et al*., 2020) [7]. Climbing can aid in mate-seeking and courtship displays, particularly in species where males may ascend to higher vantage points to lure females or defend territory. Flies also climb to control their body temperature by moving to colder or warmer locations based on their physiological needs. Sensory organs allow them to recognize appropriate climbing surfaces and navigate properly (Agarwal *et al.*, 2015) [2]. The present study aims to understand the effect of *Terminalia chebula* (one of the constituents of Triphala churna in Ayurvedic medicine) on the motor and climbing abilities of *Drosophila melanogaster*.

Terminalia chebula Retz. (TC) is always at the top of the list in "Ayurvedic Materia Medica". It is a well-known Chinese herbal remedy that contains numerous chemical components with diverse pharmacological effects. It is also used in many Tibetan medicine prescriptions to treat various diseases, hence it is nicknamed "King of Tibetan Medicine." TC is renowned for its strong antibacterial (Kuchma and O'Toole, 2000) [12], neuroprotective (Sadeghnia *et al*., 2017) [24], antioxidant (Kim *et al*., 2012) [11], and anticancer properties (Chen *et al*., 2015) [5], antidiabetic (Kannan *et al.,* 2012) [10], and immunity-enhancing effects (Aher *et al.,* 2010) [3], as well as its role in preventing atherosclerosis (Lee *et al.,* 2015) [13]. Modern pharmacological research has extensively revealed that tannins such as chebulinic acid, chebulagic acid, ellagic acid, and corilagin, along with phenolic acids such as gallic acid, protocatechuic acid, and ethyl gallate, are the key bioactive

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components contributing to the pharmacological effects of TC (Walia and Arora, 2013; Reddy *et al.,* 1994) [26, 23].

For example, tannins like chebulinic acid protect the stomach by inhibiting the activity of the H+K+-ATP (proton pump) enzyme, suggesting that it could be an effective treatment to reduce the incidence of gastric ulcers (Chebulinic acid at 40 mg/kg has a good anti-gastric ulcer effect) (Mishra *et al.,* 2013). Chebulagic acid inhibits ERK, JNK, p38, Akt, and NF-KB signaling pathways, reduces MDR-1 through COX-2, and has a synergistic effect with azamycin-induced human hepatoma cell toxicity (HepG2). At a 50 µM concentration, chebulagic acid increased the sensitivity of HepG2 cells to Dox-induced cytotoxicity (Achari *et al*., 2011) [1]. Furthermore, phenolic acids (gallic acid, protocatechuic acid, and ethyl gallate) are active substances isolated from TC that have a wide range of pharmacological effects, including antioxidant and anti-inflammatory properties (Hazra *et al*., 2010) [9]. It is worth noting that the chemicals in TC have significant medical value and warrant further investigation and development. Chebulic acid, gallic acid, protocatechuic acid, corilagin, chebulagic acid, chebulinic acid, 1, 2, 3, 4, 6-O-pentagalloylglucose, ellagic acid, and ethyl gallate are the major bioactive components that contribute to the pharmacological actions of TC. (Li *et al*., 2018) [15]. However, their pharmacokinetics in *D. melanogaster* must be thoroughly researched to establish a theoretical foundation for the safe clinical use of TC.

Research Gaps

A detailed assessment of the available literature reveals a dearth of comprehensive investigations on the effects of *Terminalia chebula on Drosophila melanogaster*, highlighting the importance of addressing this gap. The current research focuses mostly on rats and lacks direct comparative data with *Drosophila melanogaster*, making it impossible to draw significant conclusions about its potential behavioral effects across species.

***Drosophila* Culturing and Establishment of Stock:**

Maintaining *Drosophila* in the laboratory requires an appropriate food medium in suitable containers. Conventionally, glass "milk bottles" (250 ml or 500 ml capacity) and glass vials (2.5 cm diameter and ~7.5 cm long) have been used for culturing. In recent decades, reusable and autoclavable semi-transparent plastic bottles and vials have become more popular. These containers are plugged with non-absorbent cotton (reusable a few times after sterilization) or disposable synthetic foam plugs. To avoid culture contamination, flies must be transferred regularly to fresh bottles or vials with food to keep them healthy. Younger larvae eat actively and mostly remain inside the food until they prepare for pupation. However, overcrowding forces even the first and second instar larvae to crawl out of the food. Starting a new culture with about 10-20 flies in vials and fewer than 50 flies in bottles avoids overcrowding and maintains a healthy culture. Flies reared at 24°C/25°C must be transferred to fresh food within 25-30 days when cultured in bottles and 15-20 days in vials. A proper record of fly stock transfers should be maintained. *Drosophila melanogaster* lines were reared at 25°C with a 12-hour light/dark cycle.

The most common diet used to culture *Drosophila* in the laboratory is the Semolina-Jaggery Diet. Different concentrations of *Terminalia chebula* were used for treatment groups alongside this diet. Stocks are usually maintained in vials at 18°C with four to five generation cycles before transfer. Because fly stocks can only be maintained by live culturing, it is crucial to keep two to four different cultures for each individual stock, with alternate generations separated by 1-2 weeks if possible.

Table 1: Ingredients used to prepare media for *Drosophila.*

Ingredients for Media Preparation				
Sl. No.	**Ingredients**	**Normal Media (1000 ml)**	***T. chebula* 250mg (T1) (1000 ml)**	***T. chebula* 300 mg (T2) (1000 ml)**
1	Semolina	100g	100g	100g
2	Jaggery	100g	100g	100g
3	Agar	10g	10g	10g
4	Propanoic acid	7.5ml	7.5ml	7.5ml
5	*Terminalia chebula*	-	250mg	300mg

Behavioral Parameter

Negative Geotaxis (Climbing Assay)

i). Flies were introduced from different food vials, one by one, into the negative geotaxis tube using a small funnel to prevent the escape of any flies. The geotaxis tube was tapped down on a foam pad, and the open end of the tube was plugged with cotton. The tube was made to stand vertically, and the timer was immediately started.

ii). The number of flies climbing up the tube and reaching the marked ring at a 10 cm height within 10 seconds were recorded.

iii). The observation was repeated by tapping down the flies. A minimum of ten observations (trials) for each tube were made, and the average mean of flies that climbed to the 10 cm height within 10 seconds was calculated. Ten replicates were taken for each group (control, T1, T2).

iv). The above steps were carried out for flies in each of the control and treatment groups (T1, T2) (Madabattula *et al.,* 2015) [16].

Statistical Analysis

Mean values of the climbing assay in control and treated groups T1 and T2 were expressed as Mean ± Standard Error (Mean ± SE). Data were analyzed using one-way and two-way analysis of variance (ANOVA) in SPSS software, followed by Tukey's HSD test to determine statistical differences between the means of control and treated groups. A significance level of $p < 0.05$ was considered significant.

Graphs and Figures
Climbing Assay

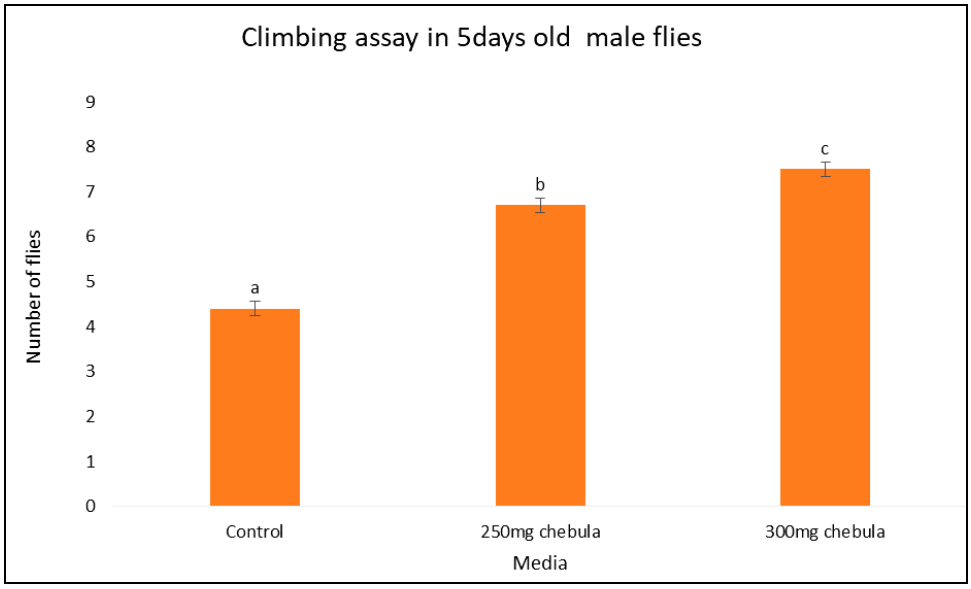

Fig 1: Effect of *Terminalia chebula* on climbing behaviour in 5days old males of *D. melanogaster*

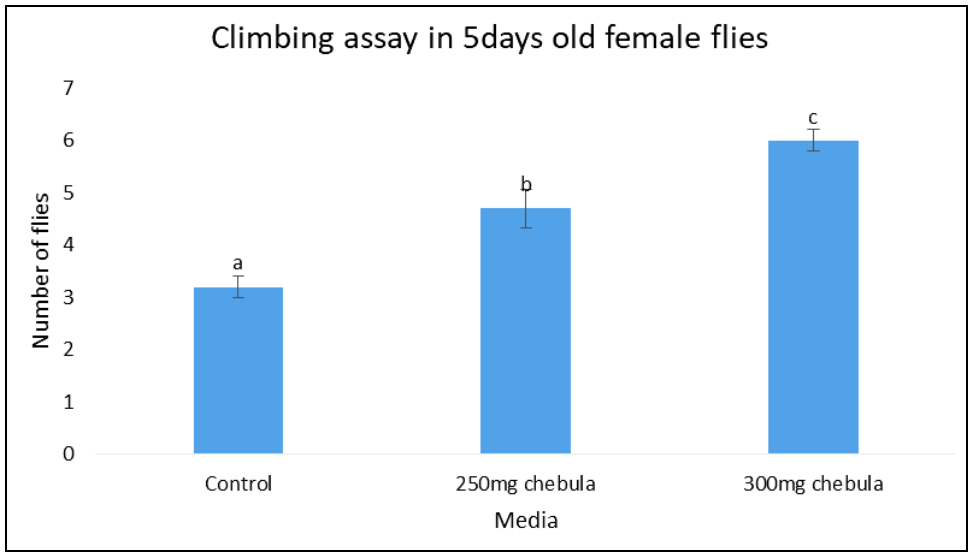

Fig 2: Effect of *Terminalia chebula* on climbing behaviour in 5 days old females of *D. melanogaster*

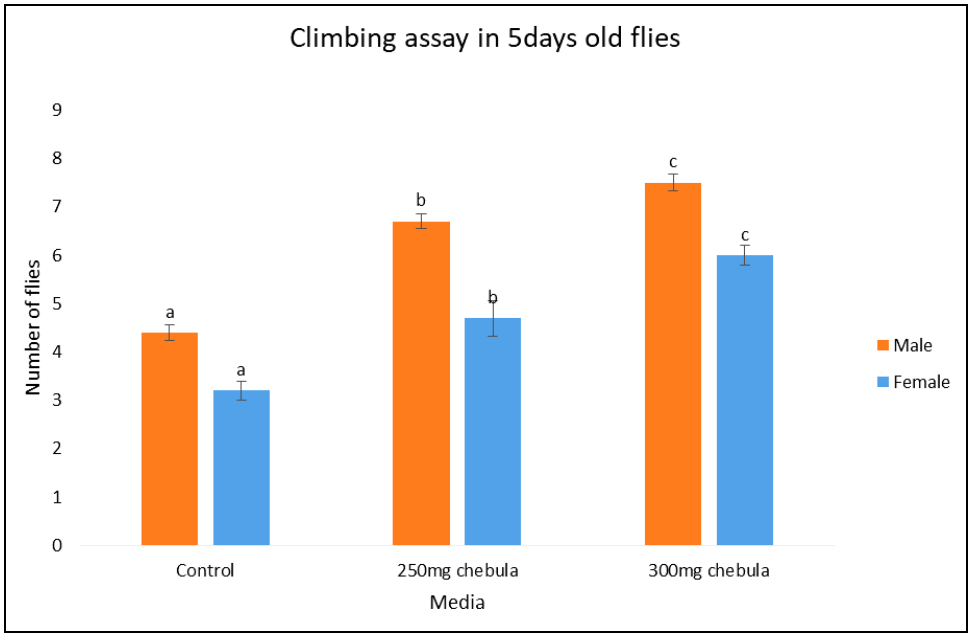

Fig 3: Effect of *Terminalia chebula* on climbing behaviour in 5 days old males and females of *D. melanogaster*

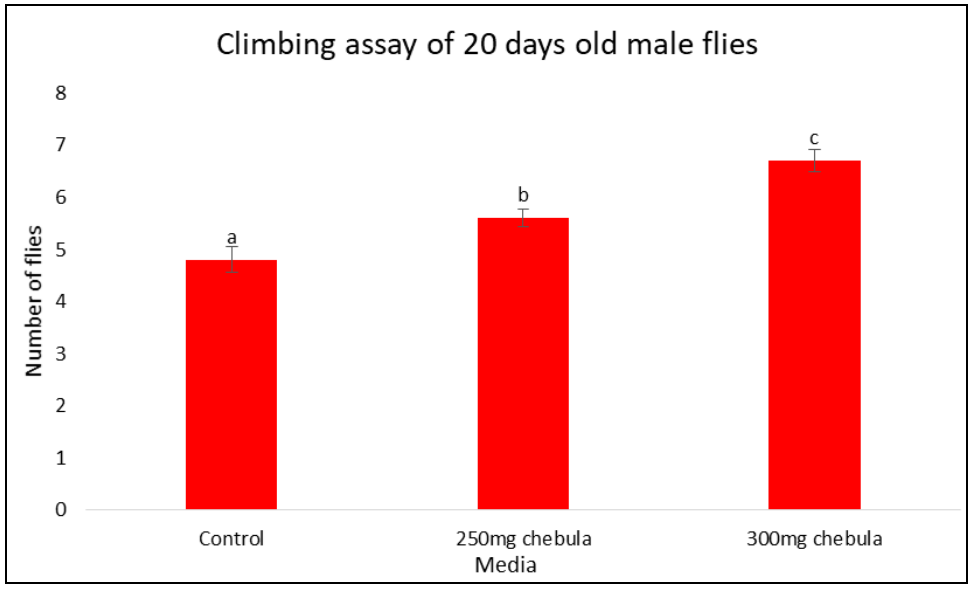

Fig 4: Effect of *Terminalia chebula* on climbing behaviour in 20days old males of *D. melanogaster*

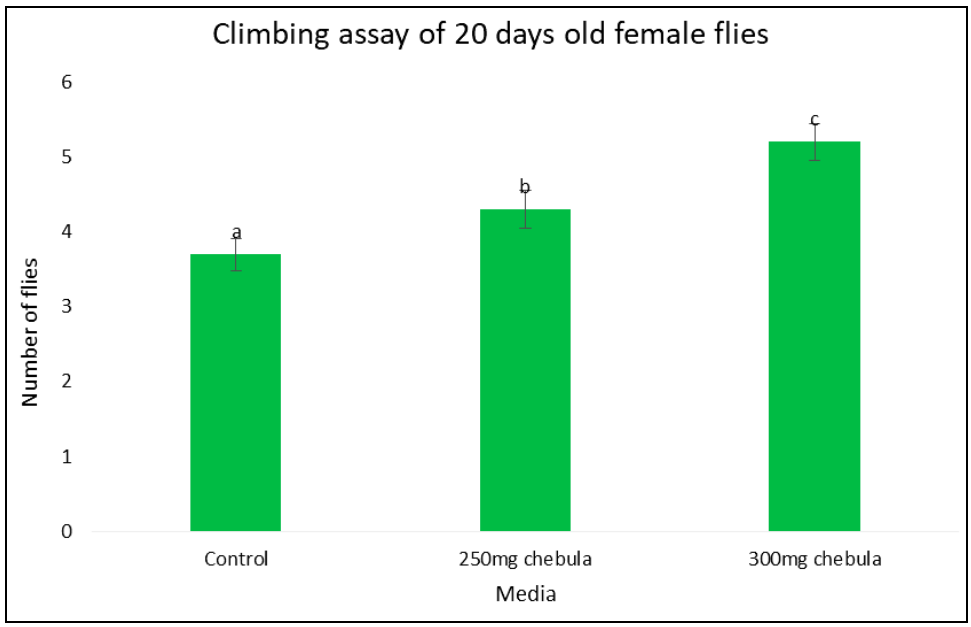

Fig 5: Effect of *Terminalia chebula* on climbing behaviour in 20days old females of *D. melanogaster*

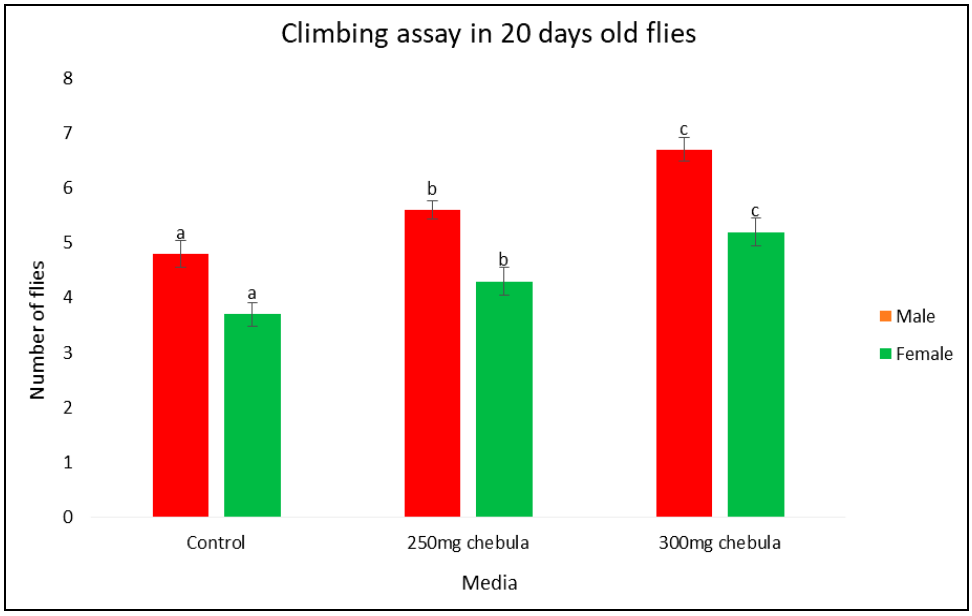

Fig 6: Effect of *Terminalia chebula* on climbing behaviour in 20days old males and females of *D. melanogaster*

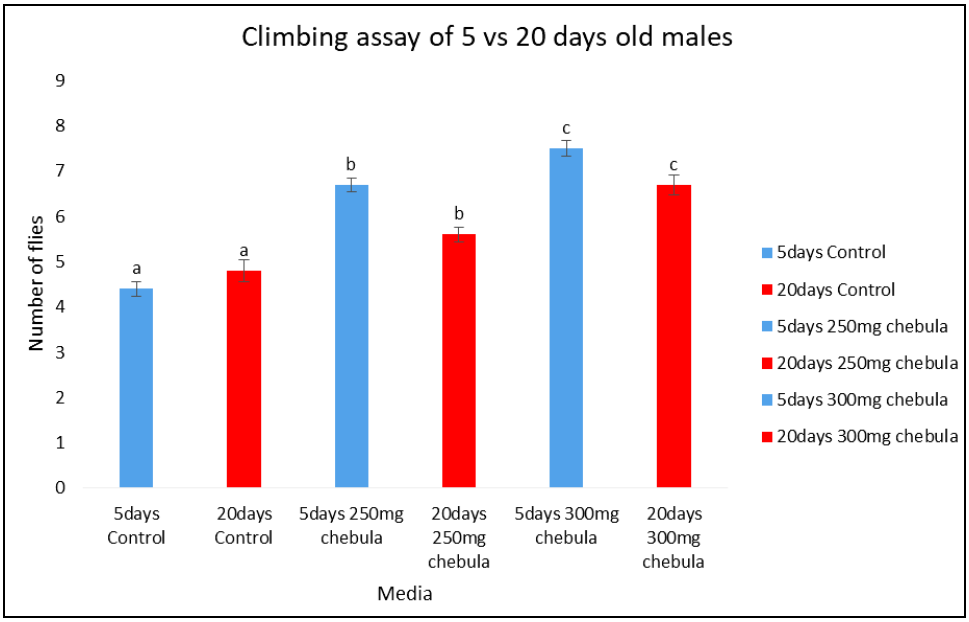

Fig 7: Effect of *Terminalia chebula* on climbing behaviour in 5 vs 20days old males of *D. melanogaster*

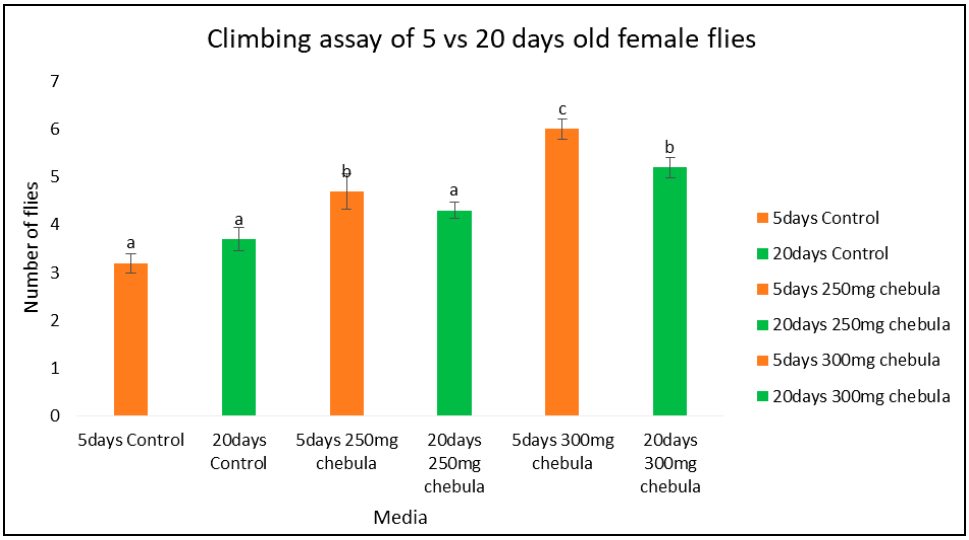

Fig 8: Effect of *Terminalia chebula* on climbing behaviour in 5 vs 20days old females of *D. melanogaster*

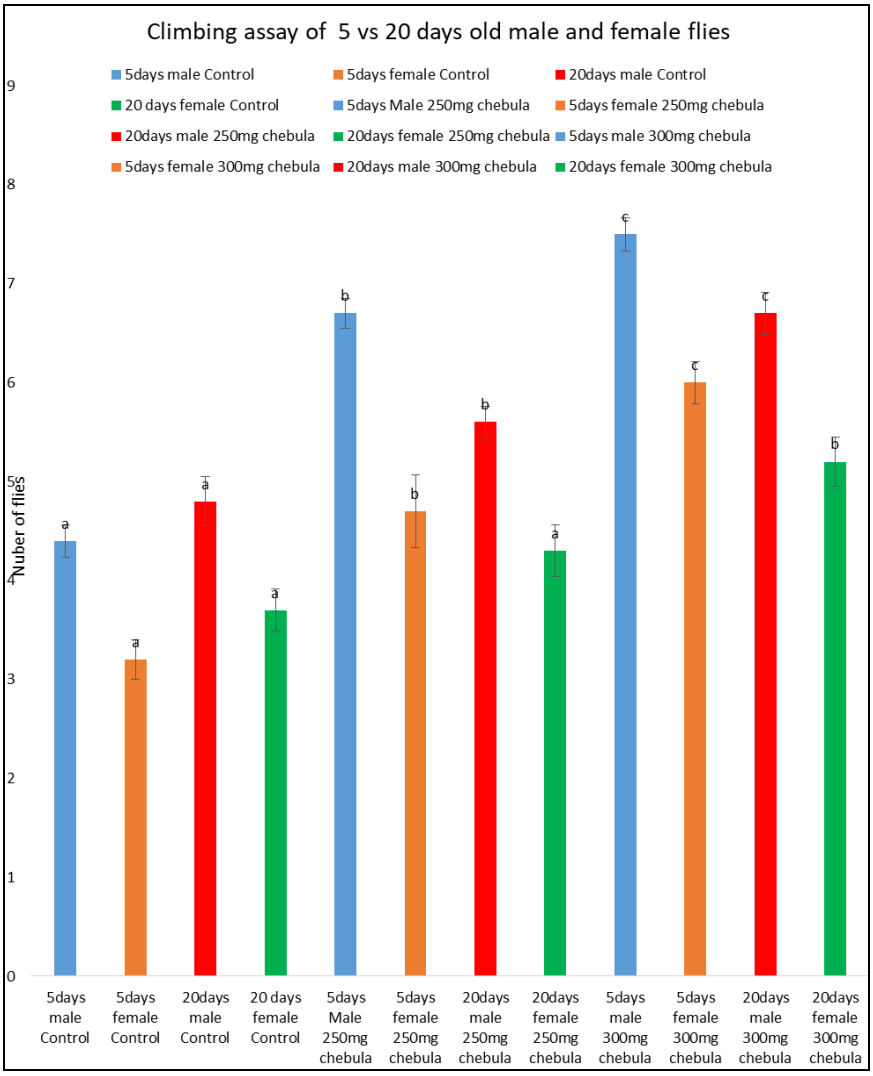

Fig 9: Effect of *Terminalia chebula* on climbing behaviour in 5 vs 20days old males and females of *D. melanogaster*

Results

The results of the climbing assay, which assessed the effect of *Terminalia chebula* on the climbing behavior of *Drosophila melanogaster* are summarized below:

5-day-old males treated with *Terminalia chebula* exhibited a significant improvement in climbing ability compared to the control group. Both T1 (250 mg) and T2 (300 mg) groups showed increased climbing activity, with T2 showing a more pronounced effect. 5-day-old females treated with *Terminalia chebula* also demonstrated improved climbing performance with the T2 group exhibiting the highest climbing ability, followed by the T1 group and the control. When comparing 5-day-old males and females, both sexes showed significant improvements in climbing ability with *Terminalia chebula* treatment with males showing pronounced ability than females. 20-day-old males treated with *Terminalia chebula* showed a notable improvement in climbing ability. The T2 group again exhibited the most significant increase in climbing activity compared to the control and T1 groups. 20-day-old females treated with *Terminalia chebula* demonstrated improved climbing behavior, with the T2 group showing the highest improvement, followed by the T1 group and the control. Both 20-day-old males and females showed enhanced climbing abilities with *Terminalia chebula* treatment. Comparing 5-day-old and 20-day-old males, younger males (5 days old) showed higher climbing activity overall. However, *Terminalia chebula* treatment improved climbing ability in both age groups, with the T2 group showing the greatest effect. When comparing 5-day-old and 20-day-old females, the younger females (5 days old) exhibited higher climbing ability. *Terminalia chebula* treatment enhanced climbing performance in both age groups, with the T2 group showing the most significant improvement. Overall, younger flies (5 days old) had

1. **Effect of *Terminalia chebula* on Climbing Behavior in 5-Day-Old Males (Fig. 1):**
   - The climbing ability of 5-day-old male *Drosophila melanogaster* was significantly higher in flies raised on media treated with *Terminalia chebula* compared to the control group.
   - The 300 mg treatment group exhibited the highest climbing ability, which was statistically significant ($p < 0.05$, df = 2, 27, F = 99.900).
2. **Effect of *Terminalia chebula* on Climbing Behavior in 5-Day-Old Females (Fig. 2):**
   - 5-day-old female *Drosophila melanogaster* showed significantly higher climbing ability in the treated media compared to the control.
   - The 300 mg treatment group had a significantly higher climbing ability than the 250 mg group ($p < 0.05$, df = 2, 27, F = 26.909).
3. **Sex Differences in Climbing Behavior of 5-Day-Old Flies (Fig. 3):**
   - Males exhibited a significantly higher climbing ability than females in both control and treated groups ($p < 0.05$, df = 1, 54, F value between sex = 85.784).

- The treatment groups showed significant differences (F value = 109.500, df = 2, 54, $p < 0.05$).
- The interaction between sex and treatment was not significant (F = 1.500, df = 2, 54, $p < 0.05$).

4. **Effect of *Terminalia chebula* on Climbing Behavior in 20-Day-Old Males (Fig. 4):**
   - The climbing ability of 20-day-old males was significantly higher in treated media compared to the control group.
   - The 300 mg treatment group showed the highest climbing ability ($p < 0.05$, df = 2, 27, F = 20.306).
5. **Effect of *Terminalia chebula* on Climbing Behavior in 20-Day-Old Females (Fig. 5):**
   - 20-day-old females displayed significantly higher climbing ability in treated media compared to the control group.
   - The 300 mg treatment group outperformed the 250 mg group ($p < 0.05$, df = 2, 27, F = 9.741).
6. **Sex Differences in Climbing Behavior of 20-Day-Old Flies (Fig. 6):**
   - Males exhibited significantly higher climbing ability than females in both control and treated groups ($p < 0.05$, df = 1, 54, F value between sex = 49.065).
   - Significant differences were observed between treatment groups (F value = 28.258, df = 2, 54, $p < 0.05$).
   - The interaction between sex and treatment was not significant (F = 0.387, df = 2, 54, $p < 0.05$).
7. **Age Differences in Climbing Behavior of Males (Fig. 7):**
   - Younger males (5 days old) showed higher climbing ability compared to older males (20 days old).
   - *Terminalia chebula* treatment improved climbing ability in both age groups, with the 300 mg group showing the greatest effect.
8. **Age Differences in Climbing Behavior of Females (Fig. 8):**
   - Younger females (5 days old) exhibited higher climbing ability compared to older females (20 days old) in treated media.
   - *Terminalia chebula* treatment enhanced climbing performance in both age groups, with the 300 mg group showing the most significant improvement.
9. **Age and Sex Differences in Climbing Behavior (Fig. 9):**
   - Overall, younger flies (5 days old) had better climbing performance than older flies (20 days old).
   - *Terminalia chebula* treatment significantly improved climbing ability across all groups.

Discussion

The results of this study indicate that *Terminalia chebula* has a significant positive effect on the climbing behavior of *Drosophila melanogaster*. Both male and female flies, regardless of age, showed improved motor function with *Terminalia chebula* treatment. This improvement was more pronounced in the T2 (300 mg) treatment group compared to the T1 (250 mg) group.

Age-related differences in climbing ability were evident, with younger flies (5 days old) generally outperforming older flies (20 days old). However, *Terminalia chebula* treatment was effective in enhancing climbing ability across all age groups. This suggests that *Terminalia chebula* may mitigate some aspects of age-related decline in motor function.

Sex differences were also observed, with males generally exhibiting slightly higher climbing performance than females, particularly in the T2 group. This could be due to inherent physiological differences between male and female flies, or it may indicate that males are more responsive to the effects of *Terminalia chebula*.

The investigation into the effects of Ayurvedic herbs, particularly *Terminalia chebula*, on the climbing ability of *Drosophila melanogaster* (fruit flies) is gaining attention among researchers. Climbing ability in *Drosophila* serves as an important indicator of neuromuscular function and general health. Notably, the administration of *Terminalia chebula* has been linked to a significant improvement in the climbing ability of these flies. This enhancement is potentially due to several factors related to the medicinal properties of the herb.

Terminalia chebula is known for its neuroprotective effects and overall health benefits, largely attributed to its rich content of tannins, polyphenols, and flavonoids. These compounds possess strong antioxidant properties, which protect neurons from oxidative stress-a major contributor to neurodegenerative diseases-thereby enhancing motor performance and climbing ability (Naik *et al*., 2004) [20].

Gallic acid, a potent antioxidant found in *Terminalia chebula*, helps safeguard cells from oxidative damage and apoptosis by neutralizing free radicals (You *et al*., 2010) [28]. Furthermore, the herb's anti-inflammatory effects, due to ellagic acid and chebulinic acid, inhibit inflammation and reduce oxidative stress, key factors in the pathogenesis of neurodegenerative diseases. This protection leads to improved neuronal health and plasticity (Saleem *et al*., 2002; Han *et al*., 2006) [25, 8].

Chebulagic acid further contributes to the herb's beneficial properties by providing antioxidant, anti-inflammatory, and anti-apoptotic effects, protecting neurons against oxidative damage and enhancing cognitive functions (Lee *et al*., 2007) [14]. Additionally, *Terminalia chebula*'s bioactive compounds are believed to improve mitochondrial function and energy metabolism, potentially leading to better physical activity and stamina in *Drosophila*, which translates to increased climbing ability (Pandey & Kumar, 2013) [21].

The observed data indicate that male flies exhibit higher climbing ability than female flies, likely due to their lower body weight, which enhances mobility and agility (Markow *et al*., 2005) [18]. Females, having larger body sizes and allocating more energy towards reproduction, may have limited physical capabilities for activities like climbing (Partridge *et al*., 1981) [22].

Conclusion

The administration of *Terminalia chebula* to *Drosophila melanogaster* has shown a significant enhancement in their climbing ability with the highest concentration (300 mg) showing the most pronounced effect. Both age and sex influence climbing ability, with younger flies and males generally performing better. This improvement is attributed to the herb's multiple beneficial properties, including its antioxidant, neuroprotective, anti-inflammatory, and metabolic-enhancing effects. These properties collectively contribute to improved neuromuscular function, reduced cellular damage and potentially enhanced energy metabolism. Overall, the positive impact of *Terminalia chebula* on *Drosophila* climbing ability underscores its potential as a valuable supplement for improving physiological functions. Further research is needed to elucidate the underlying mechanisms and to determine if these effects are translatable to other species.

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