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# Effects of *Terminalia chebula* on the Larval Crawling Ability of *Drosophila melanogaster*

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**Abstract**

The study investigates the impact of *Terminalia chebula (T. chebula*) on the larval crawling ability and overall locomotion of *Drosophila melanogaster*. As concentrations of *T. chebula* extract increase, a significant reduction in both crawling ability and general locomotor activity of the larvae is observed. This decline in motor function and locomotion is hypothesized to result from factors like lipid accumulation due to enhanced feeding and potential neurotoxicity induced by certain phytoconstituents present in the extract. The lipid accumulation is suggested to impair energy metabolism and physical movement, thereby reducing overall locomotion, while the neurotoxic effects at higher concentrations might directly affect the nervous system, leading to impaired motor coordination and crawling ability. Despite these findings, the precise molecular mechanisms underlying these observed effects remain unclear and warrant further research. Elucidating these mechanisms could provide valuable insights into the biological activity of *T. chebula* and its implications for locomotor and neurological health. This study underscores the importance of a detailed analysis of the interaction between phytochemicals and physiological processes in model organisms like *Drosophila melanogaster*.

**Keywords:** *Terminalia chebula, Drosophila melanogaster,* Larval Crawling

## Introduction

Locomotion is one of the more complex physiological functions in animals. It is an integrative approach that connects an organism's neural system to its host body, its environment, and its interactions with other bodies is necessary for a comprehensive theory of locomotory behaviour (Pearson *et al.,* 2006) [16].

A well-coordinated locomotor activity can be linked to the constant relay of information (stimulus). A thick network connects the muscles to the central nervous system. Peripheral neurons and neurotransmitters facilitate molecular communication. It is rising increasingly and is now clear that a number of genetic and environmental variables have an important role in appropriate reception of inputs, creation of appropriate responses, conveyance, and release of neurotransmitters at the axon terminals.

Despite such significant advances, the factors that modulate neuronal and muscular activity are still elusive. Disorders (including Parkinson's disease, Alzheimer's disease, Huntington's disease, etc.) and other neuropathological disorders that predispose an individual to develop a neuromuscular impairment. Neuromuscular deficits result in disoriented movement, such altered behaviour can be used as phenotypic readings (McGurk *et al*. 2015) [12].

*Drosophila melanogaster* has emerged to be one of the most successful, tractable, and versatile model systems to explore and comprehend the relationship between disoriented behaviour. This study examines the rectilinear crawling behaviour of *D. melanogaster* larvae, a model organism that has been the subject of numerous molecular, cellular, genetic, and behavioural investigations employing a range of experimental probes (Suster and Bate, 2002) [24]; locomotion, or crawling. In the third instar stage, the larva is a soft-bodied, cylindrical organism that is about 4 mm in length and 800µm in diameter. By taking advantage of the peristaltic propagation of muscle relaxation and contraction waves along their bodies, it travels similarly to other long, soft-bodied organisms like Earthworms and Leeches. For a century now, academics have focused on the dynamical process that initiates, coordinates, and sustains the propagation of such waves (Garrey, 1915).

So, the crawling behaviour of larvae can be used to explore the impact of medicines, altered gene expression, and olfactory malfunctioning. Furthermore, investigating these features during the larval stage helps determine precocious nature of a genetic or environmental alteration, which may be useful in developing an early diagnosis. The technique describes a quick, semi-automated, and reproducible method for providing high-throughput information. This technique was originally created to assess crawling behaviour in *Caenorhabditis elegans* (Nussbaum-Krammer *et al*. 2014) [6].

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***Terminalia chebula***

Medicinal plants are part and parcel of human society from the dawn of civilization to combat diseases and have been considered valuable and cheap source of unique phytoconstituents. Medicinal plants have been an integral part of human society and are used extensively in the development of medicines against a variety of diseases (Agarwal *et al*., 2011) [1] (Sarasa *et al*., 2012) [20] (Gupta *et al*., 2012) [8]. Several hundred plant genera are utilised medicinally, mostly as herbal concoctions in traditional systems of medicine. In several countries that have stood the test of time, modern medications have not been able to replace majority of them. The World Health Organisation reported that 80% of the global population relies primarily on traditional treatments involving the use of plant extracts or their active components (Nai *et al*., 2004) [15]. It has been estimated that in developed countries like the United States, plant medicines account for up to 25% of total medicines, while in fast developing countries, such as China and India, contribute as much as 80%. Therefore, the economic significance of therapeutic plants is much more in countries like India than elsewhere in the world. Over the last few decades, the discipline of herbal medicine has gained popularity in both developed and developing nations (Ayyanara *et al*., 2011) [3].

This is because herbal treatments are inexpensive, natural, and have a higher safety margins and minimal or no adverse effects (Aneja *et al*., 2009) [2]. *Terminalia chebula* is a blooming evergreen tree in the family Combretaceae. It has various common names, including black myrobalan, ink tree, or Chebulic myrobalan (English), Haritaki (Sanskrit and Bengali), Harad (Hindi), harada (Marathi, Gujrati), Karkchettu (Telugu), Alale/ralu (Kannada) and Kadukkaya (Tamil). In Tibet, *T. chebula* is known as the "King of Medicine" (Pulliah, 2006) [17]. Though it is a native of Asia, it can also be found in Nepal and Sri Lanka, Myanmar, Bangladesh, Egypt, Iran, Turkey, Pakistan as well as in Yunnan, Tibet, Guangdong, and Guangxi which are all Chinese provinces. In India, it grows in deciduous woods of Himachal Pradesh, Tamil Nadu, Kerala, Karnataka, Uttar Pradesh, Andhra Pradesh and West Bengal. It is termed as 'Haritaki' because it carries away all diseases or it is considered sacred to God Siva (Hara). Haritaki has numerous interesting synonyms such as 'pathya', because it removes obstacles from the routes and canals in the body; 'abhaya', since it provides fearlessness; 'amrta' denotes an ambrosia; 'divya' means a divinity herb;'medhya' signifies nerve tonic; 'pranada' implies lifesaving: 'Jivaniya' signifies a vitalizing herb, while 'vayahstha' means one that Promotes lifespan and keeps youth; 'rasayana phala' denotes a fruit rejuvenation, etc., According to Indian mythology, this plant is derived from drips of ambrosa (Amrita) that dropped on the Earth when God Indra drank it.

**Phytochemical Properties**

*T. chebula*, however, contains numerous phytoconstituents, such as tannins, flavonoids, sterols, amino acids, fructose, resins, fixed oils, etc. However, it is quite rich in various tannins (about 32%) however its tannin concentration depends on its geographical location (Srivastava *et al*., 2010) [23]. There are around 14 hydrolysable tannins, the main components of tannin are chebulic, chebulinic, chebulagic, and gallic acids, corilagin and ellagic acids which were isolated from the fruits of *T. chebula* (Juang *et al* 2004) [9]. Phytochemicals, like anthraquinones and ethaedioic acid, Sennoside, 4, 2, 4 Chebulyl-d-glucopyranose, terpinenes, and terpinenols have all been reported (Kumar, 2006) [11] Triterpenoids and their glycosides have been isolated from the stem bark of *T. chebula* (Saleem *et al*., 2002) [18].

**Research Gap**

A detailed assessment of the available literature reveals a dearth of comprehensive investigations on the effects of *Terminalia chebula* on neuro-muscular performance using larval crawling behaviour of larvae of *Drosophila melanogaster*. The current research focuses mostly on other model organisms and lacks direct comparative data with *Drosophila melanogaster*, making it impossible to draw significant conclusions about its potential behavioural effects across species.

**Materials and Methods**

***Drosophila* Culturing or Establishment of Stock**

Appropriate food medium in suitable containers is required for maintaining *Drosophila* in laboratory. Glass 'milk bottles' (250 ml or 500 ml capacity) and glass vials (2.5 cm diameter and ~ 7.5 cm long) have been conventionally used for culturing. In recent decades, reusable and autoclavable semi-transparent plastic bottles and vials have become more popular. They are plugged with non-absorbent cotton (reusable a few times after sterilization) or disposable synthetic foam plugs. Culture contamination should be avoided.

Therefore, the flies must be transferred regularly to fresh bottles/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 <50 flies in bottles avoids overcrowding and maintains a healthy culture. As a good practice, flies reared at 24°/25°C must be transferred to fresh food within 25-30 days 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, and 12h light/dark cycle. The most common diet used to culture *Drosophila* in laboratory is Semolina-Jaggery Diet. Along with this diet, different concentration of *Terminalia chebula* is used for treatment groups. 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 week if it is possible.

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

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

**Other Items**

i). Soft fine brush, forceps, etc.
ii). Petri plates with Agar-sugar medium and Yeast paste.
iii). Petri plates (8-10 cm diameter) with filter/blotting paper discs of the same size.

iv). Phosphate Buffered Saline (PBS.

**Larval Crawling Assay**

a) With the help of a wet brush, collect the age-matched third instar larvae from vials/bottle carefully and wash them with 1X PBS, in order to remove traces of food sticking to the larvae (do not leave larvae for long in PBS, since larvae tend to get lethargic and this might affect their crawling efficiency).
b) With the help of blunt forceps or soft brush, transfer third-instar larvae into a clean petri dish containing a few drops of 1X PBS solution. Care should be taken while picking the larvae to prevent rupturing of the larval cuticle and damage to the internal organs.
c) Additionally, it should be ensured that the posterior spiracles of the larvae are exposed to air for them to respire.
d) Place 5 larvae in the centre of the agar plate with Yeast paste and wait for ~1 min for larvae to acclimatize. With the help of the brush draw the larvae back to the centre for the next 1 min.
e) Observe and record the larval movement on the agar plate placed over graph and measure the distance covered by larva in given time (1min).
f) Collect tracks from at least 30 larvae following the step above. The larvae already tracked should be discarded to avoid mixing with the fresh set of larvae.
g) Repeat the procedure with larvae of different test concentration media to be examined and compared with the control type (Benzer, 1967) [5].

**Statistical Analysis**

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

**Graphs and Statistics**

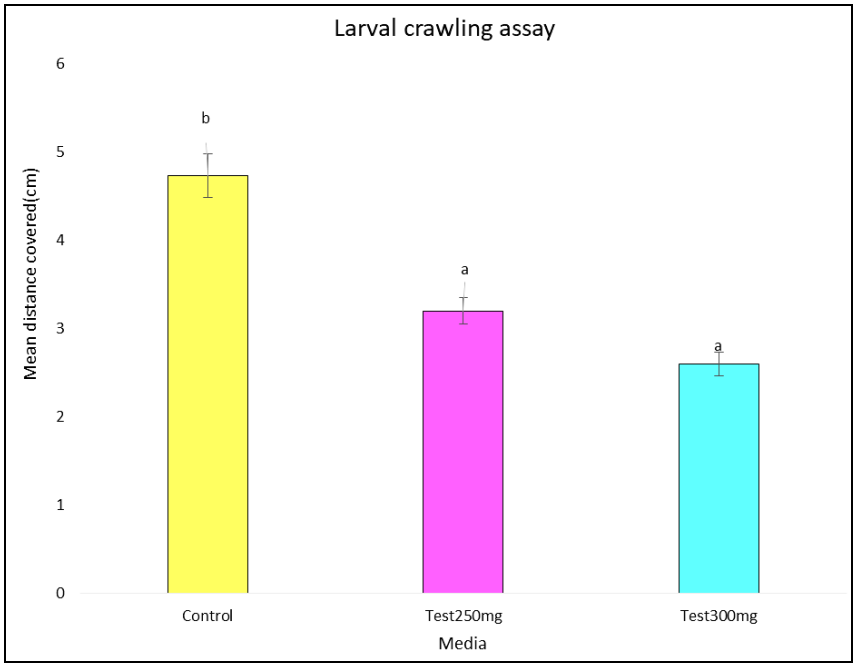


**Fig 1:** The effect of *Terminalia chebula* on the larval crawling behaviour of *Drosophila melanogaster*

**Results**

Fig. 1 represents the effect of *Terminalia chebula* on the larval crawling behaviour of *Drosophila melanogaster* raised in control and treated media. According to the data obtained, the crawling ability of larvae treated with control media is highest than both the treatment groups. The larvae of media 300mg treatment shows lesser larval crawling ability than the larvae of 250mg treatment which is significant with $p<0.05$, df= 2,87 and F =36.790.

**Discussion**

The decrease in larval crawling ability in *Drosophila melanogaster* treated with *Terminalia chebula* can be attributed to various physiological and biochemical disruptions caused by the bioactive compounds present in the extract. *Terminalia chebula* contains a range of bioactive compounds such as tannins, flavonoids, and phenolic acids, which can exhibit toxic effects on larvae at certain concentrations. Neurotoxicity is a significant factor, as these compounds can reduce neurotransmitter levels or impair receptor function, thereby impacting synaptic transmission (Mohan *et al*., 2013) [13]. Such interference with the nervous system affects muscle coordination and movement (Bag *et al*., 2013) [4]. Specifically, certain components in *Terminalia chebula* may have neurotoxic effects that impair the nervous system's ability to control and coordinate muscle movements, leading to decreased crawling ability (Sharma *et al*., 2005) [21]. Researchers found that higher concentrations of the extract significantly reduced the crawling speed and distance covered by the larvae, indicating neurotoxic effects (Kumar *et al*., 2012) [10].

The polyphenolic compounds in *Terminalia chebula* may induce oxidative stress in larvae. A study by Singh *et al*. (2015) [22] assessed oxidative stress levels and larval mobility after treatment with *Terminalia chebula* extract. Results showed increased markers of oxidative stress, such as malondialdehyde levels, and a corresponding decrease in

larval crawling speed and movement frequency. Oxidative stress can damage cells and tissues, including those involved in movement and coordination, leading to reduced crawling ability (Saleem *et al*., 2001) [19].

Research by Desai *et al*. (2017) [7] focused on the metabolic disruptions caused by *Terminalia chebula* in *Drosophila melanogaster* larvae. This study reported reduced ATP levels and altered expression of genes involved in energy metabolism, correlating with decreased crawling activity. Additionally, some experiments observed an increase in feeding behaviour in *Drosophila* larvae when exposed to certain concentrations or extracts of *Terminalia chebula*. Enhanced or overfeeding of the media can lead to an imbalance in nutrient intake, potentially causing issues like lipid accumulation or hyperglycaemia, which negatively affect muscle function and overall health, leading to decreased mobility (Musselman *et al*., 2013) [14].

## Conclusion

These studies collectively highlight the detrimental effects of *Terminalia chebula* on the crawling ability of *Drosophila melanogaster* larvae, attributing the decline to neurotoxicity, oxidative stress, and metabolic disruptions. Each study provides specific quantitative data on how the extract Impacts larval behaviour, supporting the overall conclusion. Further research is needed to elucidate the underlying molecular mechanisms and to determine if these effects are translatable to other species.

## References


1. Agarwal M, Sharma P, Kushwaha S. Antifertility efficacy of 50% Ethanolic extract of *Calendula officinalis* in male rats. *Int J Pharmacy Pharmaceutical Sci*. 2011; 3(5):192-196.
2. Aneja KR, Joshi R. Evaluation of antimicrobial properties of Fruit extracts of *Terminalia chebula* against dental caries Pathogens. *Jundishapur J Microbiol*. 2009; 2(3):105-11.
3. Ayyanara M, Ignacimuthu S. Ethnobotanical survey of Medicinal plants commonly used by Kani tribals in Tirunelveli Hills of Western Ghats in India. J Ethnopharmacol. 2011; 134:851-864.
4. Bag A, Bhattacharyya SK, Chattopadhyay R. R. The development of *Terminalia chebula* Retz. (Combretaceae) in clinical research, Asian Pacific Journal of Tropical Biomedicine. 2013; 3(3):244-252.
5. Benzer S, Behavioural mutants of *Drosophila melanogaster.* Proceedings of the National Academy of Sciences. 1967; 58(3):1112-1119
6. Carmen I, Nussbaum-Krammer, Richard I, Morimoto, *Caenorabditis ele*gans as a model system for studying non-cell-autonomous mechanisms in protein-misfolding diseases, The company of biologists, 2014; 7(1):31-34.
7. Desai P., Kumar R., Bhattacharya, S. *Terminalia chebula* extract affects energy metabolism and reduces larval locomotion in *Drosophila melanogaster*. PLOS ONE, 2017; 12(8):e0183098.
8. Gupta PC, *Withania coagulans* Dunal-An Overview, *Int J Pharmaceutical Sci Review Research*. 2012; 12(2):68-71.
9. Juang LJ, Sheu SJ and Lin TC. Determination of hydrolyzable tannins In the fruit of *Terminalia chebula* Retz., By high-performance Liquid chromatography and capillary electrophoresis, *J Sep Sci.* 2004; 27(9):718-24.
10. Kumar D, Kumar S, Singh J. Neurotoxic effects of *Terminalia chebula* extract on Drosophila melanogaster larvae. *Journal of Toxicological Sciences*, 2012; 37(4):715-725
11. Kumar KJ, Effect of geographical variation on contents of tannic Acid, gallic acid, chebulinic acid and ethyl gallate in *Terminalia chebula.* Natural Products, 2006; 2(3-4):170-75.
12. Mc Gurk L, Berson A, Bonini N.M, *Drosophila* as an in-vivo model for human neuro-degenerative disease, Genetics Oxford Academics, 2015, 377-402.
13. Mohan J, Ramesh N. *Terminalia chebula*-induced changes in synaptic transmission and motor function in *Drosophila* larvae. *Journal of Neurochemistry*, 2013; 125(4):583-591.
14. Musselman LP, Fink JL, Ramachandran PV, Patterson BW, Okunade AL, Maier E and Baranski TJ. Role of fat body lipogenesis in protection against the effects of caloric overload in *Drosophila*. *Journal of Biological Chemistry*, 2013; 288(10):8028-8042.
15. Naik GH, Priyadarsini KI, Naik DB, Gangabhagirathi R, Mohan, Studies on the aqueous extract of *Terminalia chebula* as a potent antioxidant and a probable radioprotector, Phytomedicine. 2004; 11:530-538.
16. Pearson K, Ekeberg O, Buschges A, Assaying sensory function in locomotor systems using neuromuscular simulations, Trends in neurosciences. 2006; 29(11):625-631.
17. Pulliah T, Encyclopedia of world medicinal plants, New Delhi, India, Regency Pub, 4, 1931-1934.
18. Saleem A, Husheem M, Harkonen P and Pihlaja K. Inhibition of Cancer cell growth by crude extract and the phenolics of *Terminalia chebula* Retz. Fruit. *J Ethnopharmacol*, 2002, 81, 327
19. Saleem A, Ahotupa M. *Terminalia chebula* fruit extract reduces the susceptibility of LDL to oxidation *in vitro* and ex vivo. Free Radical Research, 2001; 34(3):305-311.
20. Sarasa D, Sridhar S, Prabakaran E, The effects of an antidiabetic. *Trigonella foenum-graecum* Extract on Normal and Alloxan Mice were induced to develop diabetes, *International Journal of Pharmacy and Pharmaceutical Science*. 2012; 4(1):63-65.
21. Sharma A, Rathor H. S. *Terminalia chebula* fruit may have neuroprotective effects by reducing the levels of neurotoxic agents in the brain. *Journal of Ethnopharmacology*. 2005; 97(3):563-568.
22. Singh N, Rajini PS, Jain SK. Oxidative stress and impaired larval mobility in *Drosophila melanogaster* following exposure to *Terminalia chebula* extract. Environmental Toxicology and Pharmacology, 2015; 39(2):668-675.
23. Srivastava A, Chandra A, Singh M, Jamal F, Rastogi P, Rajendran SM, Bansode FW, Lakshmi V. Inhibition of hyaluronidase Activity of human and rat spermatozoa *in vitro* and Antispermatogenic activity in rats *in vivo* by *Terminalia chebula*, a flavonoid rich plant, Reproductive Toxicol, 2010; 29:214-224.
24. Suster M. L, Bate M, Embryonic assembly of a central pattern generator with sensory input, Nature, 2002, 174-178.
25. World Health Organization. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram. 1993; 28:13-14.