



The Effect of *Terminalia chebula* (*Haritaki*) on starvation resistance in *Drosophila melanogaster*

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Abstract

Drosophila has been used productively as a model organism for a century to study a diverse range of biological process including genetics, inheritance, embryonic development, behaviour and aging etc. Several ancient formulations are known for its efficacy to solve several health problems in ayurveda but the scientific validations of these products are few. Hence in the present project *Terminalia chebula* has been tested for its efficacy to stress adaptogenic ability in *D. melanogaster*. *T. chebula* have 33% hydrolysable tannins namely chebulin of that 8 compounds are used for specific purpose. It is one of the constituents for nutrition in 'Triphala'. It stimulates appetite and used in cancer treatment and detoxification. Reduced starvation in *D. melanogaster* has shown the hypo lipid effect.

Keywords: *Terminalia chebula*, *Drosophila melanogaster* and starvation resistance

Introduction

Ayurveda is one of the valuable ancient medicinal systems. It was originated in India and it has 5000 years of history. Two Sanskrit word that are 'Ayur' and 'Veda' which means 'life' and 'knowledge/science' together makes up the word Ayurveda. As a result, the translation of the term meaning is "Science/wisdom of life." Ayurvedic medications are mostly derived from plants. Herbal remedies have historically served as the main source of primary healthcare globally. Plants have served as a bountiful and reliable source of effective treatments. Around 80% of the population relies on conventional therapies due to the medicinal properties of plants, which include components like saponins, tannins, alkaloids, phenols, glycol, flavonoids, sesquiterpenes, lactones, terpenoids, and phorbol ester. There is always a lot of importance and scope for these wonderful plants. Countries like India and China are the largest users of these precious medicinal plants (Naik *et al*, 2004) [20].

One such traditional plant is *Terminalia chebula*, which is referred to as the "king of medicine" in Tibet. Ayurveda, a system of medicine, claims that its Rasayana (rejuvenation) and Vayasthapana (age-sustaining) properties promote long, healthy lives and combat various diseases. *Haritaki* is acknowledged to possess a wide spectrum of phyto-chemical ingredients. *T. chebula* has a many phytoconstituents which allowed it to become the most valuable plant in ayurveda. It contains tannins, amino acids, sterols, flavonoids, fructose and resins. The study identified tannin compounds such as chebulinic acid, chebulagic acid, gallic acid, and ellagic acid, which vary depending on geology. Other compounds included flavonol glycosides, triterpenoids, and phenolics from *T. chebula* fruit (Kumar, 2006, Chattopadhyay and Bhattacharyya, 2007, Karel *et al.*, 2004) [3]. Apart from tannins, contains several nutrients and biomolecules are also present in *T. chebula* extract (Mahesh *et al.*, 2007 and Muhammad *et al*, 2012).

Terminalia chebula has several traditional uses. *Haritaki* fruit is extensively used in Thai traditional medicine for laxative, carminative, astringer and tonic effect. Routinely used in traditional medicine by tribes of Tamilnadu to cure severe disease such as fever, cough, diarrhoea, urinary tract infection, wound infections, used commonly in many

ayurvedic preparations diuretics and cardiogenic. It is used to prevent aging and impact long immunity. It is reported to cure blindness (Sonali Kad *et al*, 2023) [25]. *Haritaki* enhances digestive system function, aiding in appetite loss, abdominal pain, constipation, ascites, haemorrhoids, hepatomegaly, and parasites. *Haritaki* is also said to be useful in promoting respiratory health as it has been used to treat asthma and cough (Dodke, 2017) [10]. Along with this it has several pharmacological properties such as Anti-oxidant, Anti-lipid peroxidation, Anti-inflammatory, Anti-diabetic and anti-hyperglycaemic, Hepatoprotective, Anti-cancer, Cardioprotective, hypolipidemic and hypocholesterolaemia, Gastroprotective and anti-ulcerogenic, Neuroprotective, Wound healing activity, Antiaging, Antipsychiatry, Antibacterial, Antiviral, Antifungal, and antiparasitic activities (Bulbul *et al*, 2022) [21].

Several animals had been used as model organism to study the properties of *T. chebula*. Rats have been used to study the effect of *T. chebula* on inhibiting radiation induced lipid peroxidation (Lee *et al*, 2005), anti-arthritis activity (Pratibha *et al*, 2004), Wound healing property (Li *et al*, 2011), antidiabetic activity (Koteshwara Roa and Srinivas Nammi 2006), hepatoprotective (Tasaduq *et al*, 2003). Antimutagenic activity of *T. chebula* has been studied using *Solamanella typhirium* (Goover and Bala, 1992). Frog heart model was used to study cardioprotective activity of pericarp of *T. chebula* fruit (Reddy, 1990), Amoebic liver abscess in hamster have been used to study antiprotozoal activity (Dwivedi *et al*, 2007) and immune modulatory activity, guinea pig ileum substrate had been used to study anti-allergic activity (Pratibha *et al*, 2004). Among all the animals used as model organism, using *Drosophila* has more advantage, because experiments using mammalian models (e.g., mice or rat) requires a large number of animals to be housed, maintained and subjected to invasive testing and therefor raise both economical and ethical concerns. Furthermore, studies on the aging mammals are limited by their long lifespan. That is why invertebrate model (e.g., *melanogaster*) are used for the research in aging field (Ogeinko, 2022). *Drosophila* is easy and inexpensive to maintain in the laboratory, it can give rise to a large number

of genetically identical progeny, and it has short generation cycle which is less than 2 weeks (Narayanan and Rothenfluh, 2016). The female fly can produce 1500eggs in its lifetime thereby providing a continuous supply of new *Drosophila* for the study. *Drosophila* genome is 60% homologous to that of humans, less reductant, and about 75% of the gene responsible for human disease have homologous in flies (Uqur *et al*, 2016). They have only 4 pairs of chromosomes and 23 pairs in humans. They could be mapped easily to investigate genetic transmission. The entire genome has been sequenced and annotated as the human genome. Comparatively the fly's genome is much smaller at 5% of its size (Simmons, 2023).

Any environmental factor which affects the biological fitness of an organism is called stress. (Koehn and Bayne, 1989). Environmental stress causes natural selection to occur, because of which organism undergo specific adaptation to resist stress (Hoffman & Parsons, 1991; Randall *et al.*, 1997). Shortage of food or nutrition is one of such important cause of stress. Every organism experience starvation at least once in its life time (Mei-Hui Wang, 2007). In nature, the unpredictability of food in both time and space means that nearly all organisms will experience repeated periods of nutritional stress during their lifetime, and prolonged exposure to stress eventually cause death (Luo *et al*, 2011). Hence Food limitation became the most common environmental challenge faced by animals and the capacity of animals to survive prolonged periods of starvation and the ability of animals to withstand prolonged periods of food deprivation is called 'starvation resistance' (Lee, 2014) [18]. Many species must cope with starvation, and must evolve certain adaptations which enhances starvation resistance, otherwise it can lead to mortality. Food consumption acts as a major nutritional diet factor that plays a pivotal role in extending the stress resistance of an organism, because plants containing high antioxidant and other bioactive compounds promote tolerance capacity (Renuka prasad, 2019). Starvation resistance in animal is linked to their diet and nutritional status. Rion and Kawecki (2007) have proposed that SR can be enhanced by (i) increasing energy storage, (ii) slowing the rate at which energy is expended and (iii) lowering the minimum level of body energetic reserves required for tolerating starvation. All these physiological mechanisms are intimately linked to nutrition methodology (Lee, 2014) [18].

Drosophila melanogaster as a model organism, helps to understand how nutritional limitation affects physiology. *D. melanogaster's* short generation time and ease of maintenance have allowed experimental evolution studies on starvation resistance. that *Drosophila* species vary greatly in their ability to survive starvation stress. Van Herrewege and David (1997) found that *Drosophila* species differed up to five-fold in their survival in humid air. (Reynolds, 2013) [23]

Drosophila must ingest, digest and assimilate nutrients that are packaged in the food before allocating them to maintenance, growth, reproduction or storage (Boggs 2009). How much of which nutrients an animal can attain through these processes is a function of the rate and frequency of food intake and of the efficiencies with which ingested nutrients are digested and absorbed (Lee, 2014) [18]. Such nutrition is majorly obtained through plant materials. In this experiment '*Terminalia chebula*', one of the major

ayurvedic plant was provided to the flies to check their resistance to the stress.

The ultimate aim of the present study is to reveal the nutritional basis of starvation resistance in *Drosophila*. In this study we address about how does the *Terminalia chebula* affects the starvation resistance activity in the *Drosophila* and that is achieved by assessing the longevity of the flies. Starvation resistance and longevity has an evolutionary relationship which has been confirmed in some selection experiments and this relationship has been seen in the flies. According to the studies, flies selected to perform experiment on enhanced longevity were also resistant to starvation stress (Rose & Archer, 1996).

Materials and methods

Drosophila melanogaster stock culture

Experimental Oregon K strain of *Drosophila melanogaster* which is utilized in the experiment was collected from *Drosophila* stock centre, Department of studies in Zoology, University of Mysore, Mysuru. The Stock was cultured in the glass bottles consisting of yeast agar media. Yeast agar media was prepared by adding 100g of jaggery, 100g of Soji and 10g of agar into 1000ml of distilled water and boiled, then 7.5 ml propionic acid was added. After it got dried pinch of yeast was added. Flies were transferred to the media containing cultured bottle and maintained at optimum laboratory conditions such as 70% humidity, 12:12 dark light cycle and 22± 2°C.

Diet preparation: media prepared is as follow

- 1. Control:** yeast agar media
- 2. Treatment 1:** 100ml of yeast agar media containing 250mg of powder of *Terminalia chebula* extract
- 3. Treatment 2:** 100ml of yeast agar media containing 300mg of powder of *Terminalia chebula* extract.

Establishment of experimental stock

10 pair of male and female flies were isolated form the stock and transferred to each bottle containing three different media and are tagged as control, treatment1 and treatment 2 respectively. Such that 10 replicates of each group were established. They are maintained in the laboratory conditions and were allowed to mate and lay egg. For every 2 days the flies were transferred to new culture bottles of respective media to avoid overcrowding of flies. After a week of transfer, overall, 40 bottles of flies from each group were obtained. These flies were used for the further experimentation.

Experimental procedure

Before carrying out the starvation assay, the flies were allowed to feed on control, 250mg and 300mg *T. chebula* containing media for 5 days. 10 male flies and 10 female flies from control, treatment 1 and treatment 2 groups were transferred to new empty vials plugged with cotton without any access to food or water. 5 replicates of males and 5 replicates of females from each group were established and the vials were labelled with the name of their group, date, time and replicate number. These flies were maintained at 25°C under 12 hours light and 12 hours dark condition. Their resistance to starvation was assessed by recording the number of flies dead for every 2 hours. The assessment was

done till the death of the last fly. The data obtained from the assessment was statistically analysed to interpret the starvation resistance activity of the *Drosophila melanogaster*.

Statistical analysis

The data obtained were analysed using IBM SPSS version 29.0. Mean, standard error, one-way ANOVA, two-way ANOVA and Tukey’s Post - Hoc test were carried out. Then a graph of concentration of *T. chebula* vs survival time of flies in hours was plotted.

Results

Fig.1 Represents starvation resistance in female flies of *Drosophila melanogaster* raised in control and treated media. According to the data obtained, high starvation resistance was exhibited by the flies cultured in treated media. Among the 2 treated media, flies cultured in the media containing 250mg of *T. chebula* per 100ml of water shows high resistance to starvation.

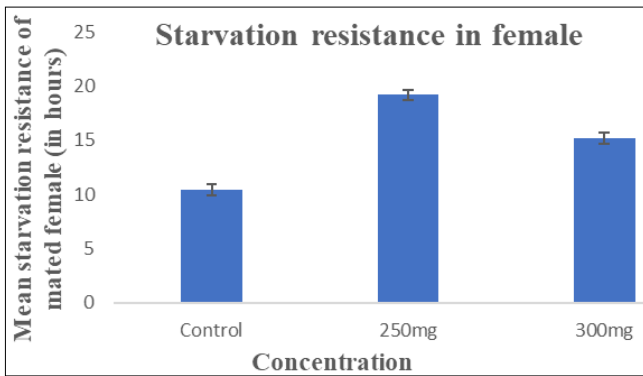


Fig 1: Effect of *Terminalia chebula* on starvation resistance in female *Drosophila melanogaster*

Control flies survived 10 hours whereas 250mg *T. chebula* treated flies survived for about 20 hours which is double the time compared to the control flies. Flies treated with 300mg *T. chebula* survival rate reduced by 5 hours compared to flies treated with 250mg *T. chebula*.

Fig.2 Represents starvation resistance in male flies of *Drosophila melanogaster* raised in control and treated media. According to the data obtained, high starvation resistance was exhibited by the flies cultured in treated media. Among the 2 treated media, flies cultured in the media containing 250mg of *T. chebula* per 100ml of water shows high resistance to starvation.

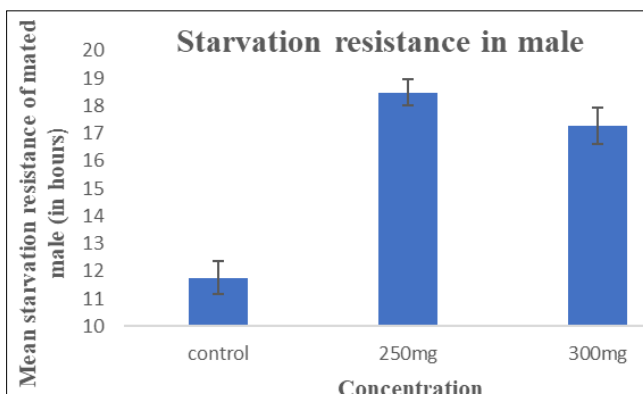


Fig 2: Effect of *Terminalia chebula* on starvation resistance in male *Drosophila melanogaster*

Similarly, to the females, starvation resistance of male *T. chebula* treated flies are very effective and survived for long time compared to control. There is 5 hours difference between 300mg *T. chebula* treated flies and control flies whereas 250mg *T. chebula* treated flies survived highest. They have survived 20 hours compared to 300mg and control flies.

Fig.3 Shows the compared means of starvation resistance in both mated male and female flies of *Drosophila melanogaster* raised in controlled and treated media. According to the data obtained, starvation resistance was high in both male and female flies cultured in the media containing 250mg/100ml *T. chebula*.

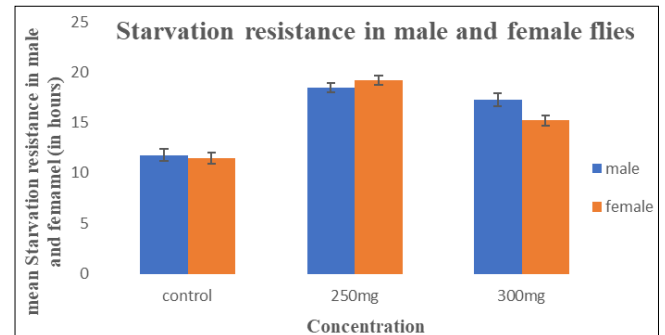


Fig 3: Effect of *Terminalia chebula* on starvation resistance in male and female *Drosophila melanogaster*

Compared to normal yeast agar media, *T. chebula* treated flies found to be more starvation resistant. Starvation resistance between male and female is non-significant and between the concentration groups, it is significant with $df=2$, $F=2.520$ and $p<0.05$.

Conclusion

Terminalia chebula is of great use in ayurveda as it has many medicinal properties. one such property is Antioxidant property. Due to the cellular mechanism or because of environmental stressors, free radical production increases in the body. When these free radicals are produced in an excess amount it creates imbalance in the body. This harmful effect of free radicals is prevented by Antioxidant substances. They either prevent the production of free radical or they remove the excess free radical to maintain balance in the body (Johnmark and Kinyi, 2021). Starvation conditions often lead to increased oxidative stress in cells due to the imbalance between free radicals (reactive oxygen species, ROS) and antioxidants. Antioxidants help neutralize ROS, thereby reducing oxidative stress and its harmful effects on cellular components such as DNA, proteins, and lipids. This protection can contribute to overall cellular health and survival during starvation periods. Studies in various organisms, including model organisms like worms, flies, and rodents, have shown that antioxidants can extend lifespan and improve survival under conditions of nutrient deprivation or starvation. Hence carrying out starvation assay is one of the best methods to study the antioxidant property.

The aim of the present study was to study the antioxidant property of *T. chebula* using *Drosophila melanogaster* as a model organism. In the present experiment *Drosophila* flies were allowed to feed on the media containing different concentration such as 250mg/100ml and 300mg/100mg

water of *T. chebula*. After 5 days of feeding the flies were kept in empty vial without any access to water or food. then their starvation resistance activity was measured by assessing how many hours they were alive without food. The data was taken till the death of the last fly.

According to the data obtained, the male flies fed on control media did not show any significant resistance to starvation. But the male flies fed on treated media exhibited significant resistance. Among the two treated media, the flies which were fed on the media containing 250mg of *T. chebula* showed high resistance to the starvation. It was significant with $p < 0.05$, $df=2$ and $F=1.054$. And the female flies exhibited the same results as male flies with significance of $p < 0.05$, $df=2$ and $F=7.404$.

This shows that the *T. chebula* was the prime constituent which allowed the flies to resist for much longer time than the control flies. *T. chebula* contains several components which allowed it to have great antioxidant property. According to a comparative study by Bhatt *et al.* 2017, *T. chebula* fruit contained a maximum value for total phenols, total flavonoids, gallic acid, catechin, chlorogenic acid, and coumaric acid among the ten wild edible fruits specimens tested (Bulbul, 2022) [21]. High performance liquid chromatography (HPLC) analysis confirmed that the fruit of *Terminalia* extract contains phenolic compounds and these compounds are good scavengers of free radicals (Muhammad, 2012).

Apart from this, when we compared starvation resistance between male and female flies, female flies had higher resistance than male. It is because under conditions of starvation stress, females stored higher amounts of body lipids and glycogen and used both of these energy sources, whereas starved males only used digested body lipids as a source of energy. Improved energy storage or a slower rate of depletion of these reserves are related to increased starvation resistance (Hoffmann and Harshman, 1999) [14].

This is good evidence of studies in *Drosophila* to show increased resistance is due increased lipid content of adults. (Jan *et al.*, 2020; Chippindare *et al.*, 1996). Further he states that lipid and starvation level in different site of liver selected for starvation or changes in life history trait. The lipid association with starvation also been documented from a set of starvation selection lines derived from a different base population (Harshman *et al.*, 1999; outman *et al.*, 2004) [14]. Present study, starvation resistance is associated with lipid contents as by personal observation the larvae and adult were compared to control, due to time constraint studies have not been done.

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