

# Effect of Extract of *Emblica officinalis* on Developmental Stages of *Drosophila melanogaster*

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**Abstract:** *Drosophila melanogaster* is an excellent organism to test on ayurvedic medicines. It also remains one of the most studied organisms in biological research, particularly in genetic analysis. The objective of our study was to explore the potential of *Emblica officinalis* on the life span of *Drosophila melanogaster* using the adult feeding method. *Emblica officinalis* commonly known as the Indian gooseberry is an important medicinal plant in the traditional Indian system of Ayurvedic medicine. In the present study, we noted the phenotypic effect of *Emblica officinalis* extracts on the different developmental stages of *Drosophila melanogaster*. The fruit flies were grown on 10-gram culture media supplemented with a concentration of *E. officinalis* (0.1ml). Further, the size and growth of different life stages of *D. melanogaster* were observed and total protein estimated from it. The increase in the size and protein concentration in different life stages of controlled *D. melanogaster* were recorded.

**Keywords:** *Drosophila melanogaster*, *Emblica officinalis*, Protein, Lifespan

## 1. Introduction

India has been known to be a rich depot of medicinal plants. More than 35% of the entire plant species, at one time or other, were used for medicinal purposes. Plants have always been an important source of drugs (Yakob et al., 2012). A large number of the world's populations, especially in developing countries, depend upon medicinal plants as an alternative and complementary drugs therapy for various diseases.

Medicinal plants such as Aloe (*Aloe vera*), Tulsi (*Ocimum tenuiflorum*), Neem (*Azadirachta indica*), Amla (*Emblica officinalis*), Turmeric (*Cucurma longa*), and Ginger (*Zingiber officinale*) cure several common maladies. These are considered home remedies. The extractor plant is identified to be curative against diversified ailments and several pathophysiological or antioxidant property which prevents the damage of cellular compartments from oxidative stress. In Ayurveda "Swaasthasya Rakshanam" is one of the goals of treating disorders, maintaining or preserving good health (Charaka Samhita Sutrasthana, 2000a). However, serious efforts are required in systemic research to identify, isolate and evaluate the chemical constituents for nutritional and therapeutic potentials.

*Emblica officinalis* is commonly known as Indian gooseberry, Amla in Marathi, and Amalaki in Sanskrit is a deciduous tree of the family Phyllanthaceae. In Ayurveda, Amla is considered to be a potent rejuvenator and immune modulator effective in stalling degenerative processes and senescence and promoting longevity, enhancing digestion, treating constipation, reducing fever and cough, alleviating asthma, strengthening the heart, benefiting the eyes, stimulating hair growth and enliven the body. *E. officinalis* contain high amounts of ascorbic acid (vitamin C) and have a slightly bitter taste that may derive from a high density of ellagitannins, such as emblicanin A (37%), emblicanin B (33%), punigluconin (12%), and pedunculagin (14%). Amla also contains punicalofin and phyllanemblinin A, phyllanemblin other polyphenols, such as flavonoids,

kaempferol, ellagic acid, and gallic acid (Rawal S, et.al., 2014).

*Drosophila melanogaster* is a species of fly in the family Drosophilidae. The species is known generally as the common fruit fly or vinegar fly. *D. melanogaster* as the test system proved to be an excellent organism to test the effect of many drugs and other chemicals. This is cosmopolitan mostly found in temperate regions. In *Drosophila* and other insects body size is positively linked with mating success, longevity, fecundity, and other fitness characteristics also the best phenotypic heritable characteristics (Santos et al., 1992). All these conclusions demonstrated the advantage of size in mating success and fitness. Various researchers demonstrated the fitness studies in *Drosophila* such as fecundity, fertility (Long et al., 1980), and longevity (Cordts & Partridge, 1996, Partridge Tower, 2008). The species shares large numbers of homologous genes with mammals, 13,601 with humans. These have been studied to identify sequences related to those causing human diseases (Reiter et al. 2001). The present study was carried out with an objective to explore the potential of *Emblica officinalis* drug, on the fitness of *D. melanogaster* using the adult feeding method and found an increase in the life span of *D. melanogaster* flies.

## 2. Materials and Methods

**Collection and culture of *Drosophila melanogaster* fly:** *D. melanogaster* has a cosmopolitan distribution. These flies were collected on ripe banana fruit and fed on them then transferred to a conical flask by covering the open mouth of the flask with a muslin cloth so that flies were easily collected with a brush.

For studying *Drosophila* etherization process is used and for culturing those etherized and re-etherized bottles are used. Flies become inactive and we studied them under a microscope. The flies were cultured on potato, dextrose, and agar culture media.

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**Preparation of *Embllica officinalis* (Amla) Extract:** For making ethanolic *E. officinalis* extract about 415.76gm of powdered material of *E. officinalis* was taken in a clean, flat-bottomed glass container and soaked in 1300ml of 80% ethanol. This container was sealed and kept for 2days accompanied by occasional shaking and stirring. Then the whole mixture is filtered with filter paper and evaporated the filtrate at room temperature. The gummy concentrate was designed as an ethanolic extract.

**Experimental setup:** Take five culture vials having 0.1 ml of *E. officinalis* extract and 10 gm of normal culture media. *Drosophila* flies are anesthetized with diethyl ether and transferred into culture vials and each vial contains 10 flies (95 females and 5 males). The vials were covered with a cotton plug and placed on the working table. The flies were laid eggs in cultures, hatched, and completed the life cycle within 10 to 15 days. Collecting the larval stages for measurement of total length and protein estimation was done by De Lowry's (1951) method.

The measurements of the size of the various stages of *Drosophila melanogaster* were carried out by the oculometer. The photographs were taken in Carl Zeiss binocular microscope and stereo zoom microscope. All results are presented a mean  $\pm$  SD. Test of significance (t-test) was used to analyze the data collected.

### 3. Observation and Result

It has been observed that *E. officinalis* plant has potent

antioxidant activity. Thus to determine whether supplementation of Amla powder with food, provides any nutritional support, survivorship assay of control (without *E. officinalis* and with *E. officinalis*) fed flies was performed. For this, an equal number of male and female flies were fed on control, and *E. officinalis* mixed food.

In the present study, the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, pupal stage and adult stage flies showed a normal size along with normal growth and development. The concentration of 0.1ml showed extremely significant change by making an increase in size at each stage of development. The percent change of 36.1%, 5%, 95.6%, 87.29%, and 88% was observed in 1<sup>st</sup> instar, 2<sup>nd</sup> instar, 3<sup>rd</sup> instar, pupa, and adult respectively over the control (Table).

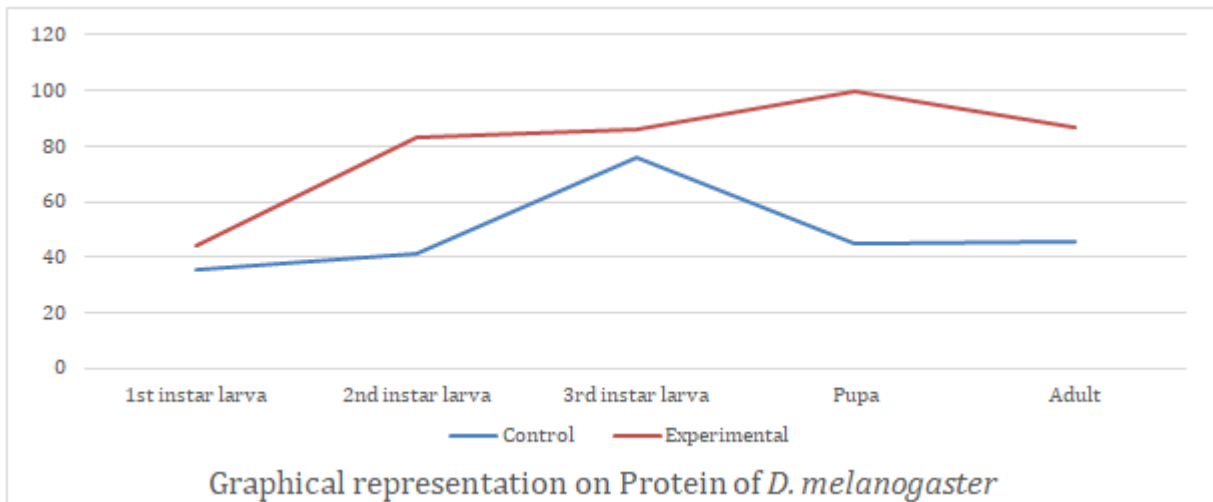
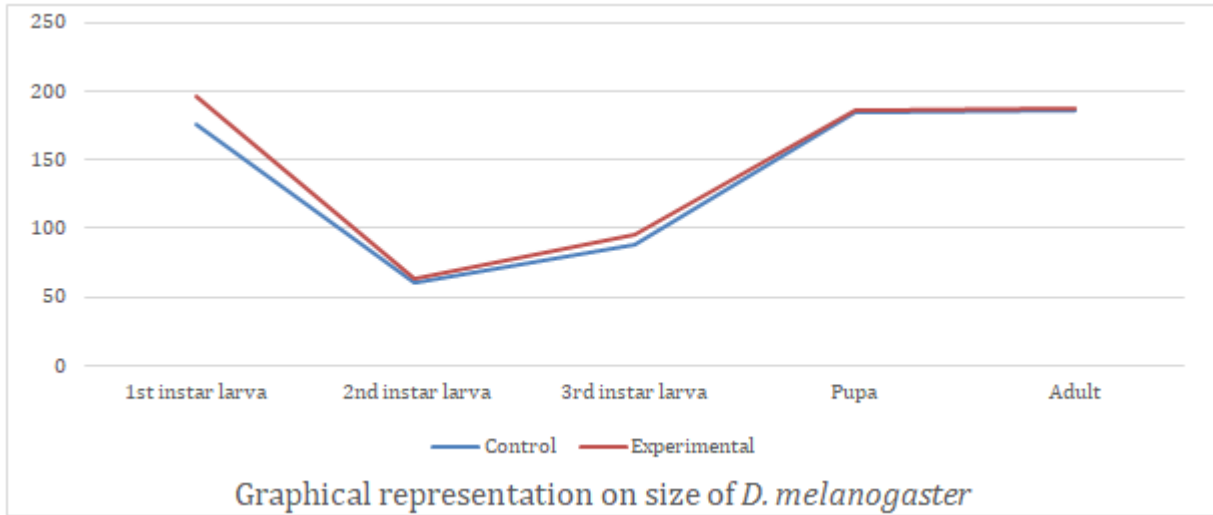
Whereas in protein concentration, significant increases were found in an experimental setup. In 1<sup>st</sup> instar larvae 55.63%, in 2<sup>nd</sup> instar larvae 17.21%, in 3<sup>rd</sup> instar larvae 14.07%, in pupal stage 0.374% and in adult stage 13.648% changes were noted over the control (Table).

The phenotypic characteristics were altered in the developmental stages of the experimental setup. In 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> larvae increased in size, faded transparency, yellowish to brownish color, and high segmentation was noticed in the experimental setup as compared to normal fruit flies. In pupal and adult stages, increased size, brownish color, no transparency, and high pigmentation were found over the control.

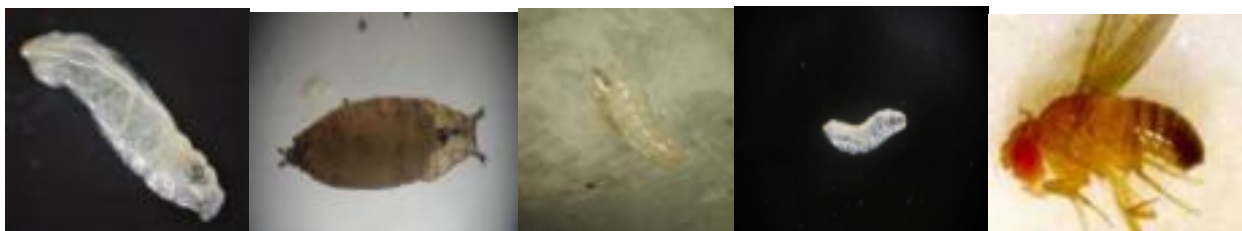
**Table:** Measurement of size ( $\mu\text{m}$ ) & Protein ( $\mu\text{g}$ ) from various larval stages of *Drosophila melanogaster*.

| Developmental stages         |              | Size                           | Protein                           |
|------------------------------|--------------|--------------------------------|-----------------------------------|
| 1 <sup>st</sup> instar larva | Control      | 175.6 $\pm$ 10.65              | 35.256 $\pm$ 1.57                 |
|                              | Experimental | NS<br>195.6 $\pm$ 9.85 (36.1%) | NS<br>44.37 $\pm$ 2.46 (55.63%)   |
| 2 <sup>nd</sup> instar larva | Control      | 60.45 $\pm$ 8.20               | 41.488 $\pm$ 0.98                 |
|                              | Experimental | NS<br>63.9 $\pm$ 5.19 (5%)     | NS<br>82.788 $\pm$ 2.78 (17.21%)  |
| 3 <sup>rd</sup> instar larva | Control      | 87.7 $\pm$ 13.49               | 75.6 $\pm$ 10.65                  |
|                              | Experimental | NS<br>95 $\pm$ 12.27 (95.6%)   | NS<br>85.928 $\pm$ 2.26 (14.072%) |
| Pupa                         | Control      | 184.5 $\pm$ 15.34              | 45.054 $\pm$ 0.26                 |
|                              | Experimental | NS<br>186 $\pm$ 12.44 (87.29%) | NS<br>99.626 $\pm$ 1.88 (0.374%)  |
| Adult                        | Control      | 186.5 $\pm$ 15.64              | 45.458 $\pm$ 0.69                 |
|                              | Experimental | NS<br>188 $\pm$ 11.15 (88%)    | NS<br>86.352 $\pm$ 2.62 (13.648%) |

Mean  $\pm$  S.D. of five flies <0.05 (\*), P<0.01(\*\*), and P<0.001(\*\*\*) and NS= not significant. The value mentioned in parenthesis is the % change over the control.



**Normal development of *D. melanogaster*:**



**Experimental development of *D. melanogaster*:**



**4. Discussion**

Amla (*Emblica officinalis*) has a hallowed position in Ayurveda- an Indian indigenous system of medicine. In the present study, we reveal that there was significant increase

in size of experimental set up as compared to the normal flies. It may be due to the beneficial effect of *Emblica officinalis* extract.

Shilpa Rawal (2014) reported the intake of food and

nutrition plays a major role in affecting aging process and longevity. However, the precise mechanisms underlying the aging process are still unclear. To this respect, diet has been considered to be a determinant of aging process. In order to better illustrate this, they used *Drosophila melanogaster* as a model and fed them orally with different concentrations of two commonly used Indian medicinal plant products, *Cucurma longa* (rhizome) and *Emblca officinalis* (fruit). Similar type of results is found in the present study.

The lifespan of *D. melanogaster* was increased in the study after the treatment of *Emblca officinalis*.

Lee K. S (2010) reported that curcumin extended the life span of two different strains of *Drosophila melanogaster*, an effect that was accompanied by protection against oxidative stress, improvement in locomotion, and chemopreventive effects. Life span extension was gender and genotype-specific. Curcumin also modulated the expression of several aging-related genes, including mth, thor, InR and JNK. The observed positive effects of curcumin on the life span of two different *D. melanogaster* strains demonstrate a potential applicability of curcumin treatment in mammals. Whereas in the present study the protein concentration increases were recorded in an experimental setup. (Akhare et al., 2021) reported the protein concentration in control *D. melanogaster* was normal but in experimental protein, the concentration was changed after the effect of neem extract.

In the present study the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, pupa, and adult stage flies showed extremely significant change by making an increase in size at each stage of development. Furthermore, the study was get concluded on the phenotypic character change in the development if *Drosophila melanogaster* in an experimental setup. Like as the phenotypic character involved the change in the transparency of the *Drosophila* body. Along with that, the segmentation on the body surface occurred in each of the stages. The segmentation showed the overall impact of body features on a fly. The effect of *Emblca officinalis* extract showed no pigmentation in an experimental setup but in the control setup, it does. The intake of 0.1ml concentration showed a color change too. All changes in the phenotypic character may be due to the activity of *Emblca officinalis* extract.

Many researchers have made studies on the effect of different chemicals on fertility in *D. melanogaster* (Vasudeva and Krishnamurthy1983). The rate of development is another parameter, which is used to examine some chemicals clinically. In that studies, the genetic constitution, amount of the food, temperature, and space were kept constant. The differences in the development must have been determined by the chemical used and not by the other factors. This type of result on the developmental time by different chemicals in *Drosophila melanogaster* has been shown by Luning (1966).

## 5. Conclusion

The conclusion of our experiment suggests that extract of *Emblca Officinalis* enhances the development of *D. melanogaster*. This noticeably increases longevity, fertility, fecundity, number along with developmental time. The

Influence of the above fruit extract is found in the parents and F1 generation and not in the F2 generations. The impact of the *E. officinalis* is not carried in further generations after the F1 generation. Lastly, here concludes there is a linear interrelationship between sexual activities and fitness parameters in experimental culture. So, along with life span, other reproductive fitness characteristics of flies like size, pigmentation, segmentation, and protein concentration were undertaken for this study so as to explore the unknown principles of *Emblca Officinalis* extract therapy which improves the quality of the life.

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