



Mini Review

Drosophila melanogaster as a Promising Model Organism in Toxicological Studies

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Abstract

The common fruit fly, *Drosophila melanogaster*, has been extensively studied for decades. In effect, it was introduced as a decisive model in biology about a century ago. The fly shares several basic biological, biochemical, neurological and physiological similarities with mammals. It is documented that about 75 % of human disease-causing genes have functional homolog in *D. melanogaster*. The fly can effectively be maintained at low cost in the laboratory, and it has been recommended as an alternative model to vertebrate usage. Consequently, it has attracted the attention of toxicologists. In the present review, we presented and discussed the opportunities available to toxicologists who wish to use *D. melanogaster* as a model to study neurodegenerative diseases, oxidative stress and antioxidant markers, cancer, inflammation and metabolic disorders. We also included vital information such as the FlyBase resource that will assist toxicologists interested in using *D. melanogaster* as an alternative organism to other animal models..

Keywords: *Drosophila melanogaster*, oxidative stress, fly base, toxicology

Introduction

The arthropod *Drosophila melanogaster*, belonging to the family *Drosophilidae*, is a dipteran insect (i.e., a member of an order of insects containing the two-winged or so-called true flies). This insect was introduced as a model in biology about 100 years ago and it was decisive for the development of genetic and related fields (Sepel and Loreto, 2010). This fly has been classically used as a model of genotoxicity, and only recently, it has been included as a potential model for studying systemic toxicology or as an alternative model for studying toxicology (Dean 1985; Barale 1991; Brusick *et al.*, 1998; Cummings and Kavlock, 2005; Whitworth *et al.*, 2006; Rand 2010; Paula *et al.*, 2013). *D. melanogaster* displays anatomical characteristic features such as wings and compound eyes. It has a lifespan of between 40-120 days depending on diet and environmental stress conditions (e.g., temperature and population density).

Diet such as cornmeal prolongs the lifespan of the fly, while diets with high quantities of free available

carbohydrates (saccharides) and cholesterol can reduce life expectancy (Hirth, 2010). In addition, overcrowding has been shown to reduce the longevity of the fly (Joshi and Mueller, 1997). The similarities of molecular processes involved in the control of lifespan and aging between *D. melanogaster* and human, coupled with good degree of genetic homology between the two species, makes *D. melanogaster* an interesting model system for toxicologists. Of particular importance, more than 65-70% of human disease genes are present in *D. melanogaster* (Reiter *et al.*, 2001; Pandey and Nichols, 2011; Poddighe *et al.*, 2013), making it an important model to understand not only how the genes induce diseases, but also the discovery of the relation of such genes to diseases (Fortini *et al.*, 2000; Fortini and Bonini, 2000). Compared with other models, *D. melanogaster* offers rapid generation time, ease of use, and easy to maintain in the laboratory in a large quantity due to its tiny body size and short lifespan.

D. melanogaster is widely used as a model organism in genetics, biochemistry, cell biology, and developmental biology. Since the last decades, it has been used as a model to elucidate human diseases, and incipiently for toxicological studies (Rand, 2010; Singh *et al.*, 2011; Sharma *et al.*, 2012; Rand *et al.*, 2012; Paula *et al.*, 2012; Sudatti *et al.* 2013).

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Due to the success achieved in the use of *D. melanogaster* in experimental studies, it has met the standard of the European Centre for the Validation of Alternative Methods (ECVAM): Reduction, Refinement and Replacement (3Rs) of laboratory animal usage (Festing *et al.*, 1999). *D. melanogaster* as a model raises few ethical concerns and its genome can be easily manipulated in the study of a particular gene of interest under a defined condition. Interestingly, the post genomic sequencing of *D. melanogaster* generated a great deal of attention of toxicologists because it revealed functional conservation of majority of the genes present in mammals. As a result of this, it has been used to obtain mechanistic insights in toxicological studies (Adams *et al.*, 2000). Thus, its use in toxicological studies will continue to generate valuable data.

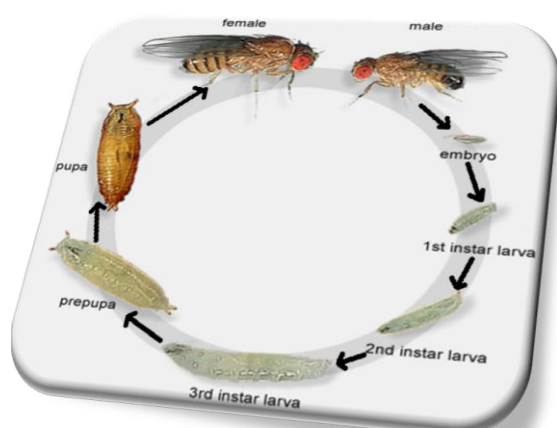


Figure 1:
Life Cycle of *Drosophila melanogaster*

The life cycle of *D. melanogaster* is rapid because a fertile mating pair can generate hundreds of genetically similar offspring at 25°C within 10 to 12 days (Figure 1). Another interesting attribute of the fly is that it is a multiple model organism. Thus, its embryo, larva, pupa and adult can be used as models in different toxicological settings. For instance, the embryo and the pupa can be used as models in developmental toxicological studies, while the larva can be used to study physiological and behavioural processes. Interestingly, the adult fly possesses sophisticated and complex systems. It has structures that can mimic the equivalent functions of mammalian reproductive tract, heart, kidney, gut and lung. Additionally, the brain of the fly possesses more than 100,000 neurons that are important in flight navigation, circadian rhythms, memory, feeding, courtship and aggression. Importantly, *D. melanogaster* responds to several central nervous system drugs in a similar way to mammals (Nichols *et al.*, 2002; Rothenfluh and Heberlein, 2002; Satta *et al.*, 2003; Wolf and Heberlein, 2003; Andretic *et al.*, 2008).

In the present mini review, we attempt to briefly show the use of *D. melanogaster* as a promising model in toxicology.

The History of *D. melanogaster*

The history of *D. melanogaster* in science is over a century. As a result of this, it is difficult to do a complete write-up

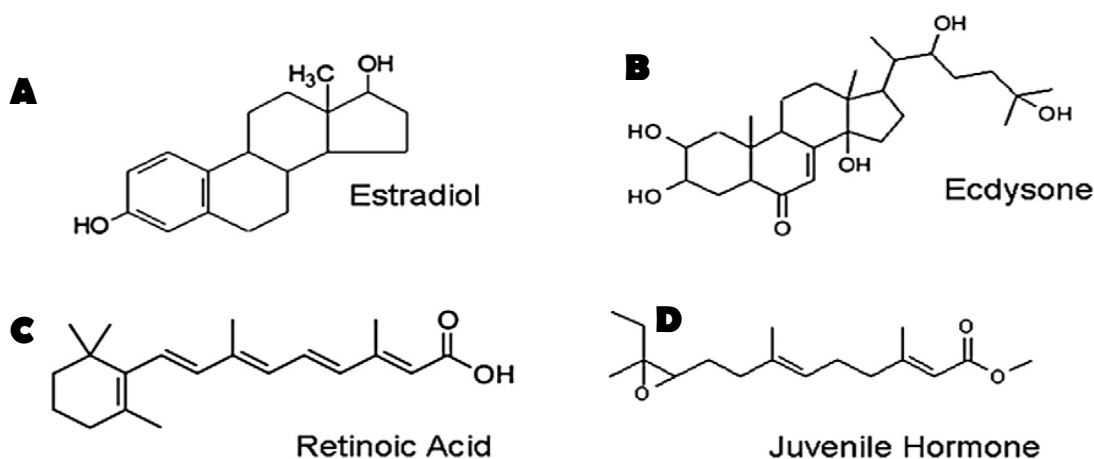
in this review. Nonetheless, it is important to highlight some important historical aspects of the fly. The first person to use the fly was widely accepted as Charles W. Woodworth, who also suggested its use to W.E. Castle. Its usage has thereafter spread dramatically. *D. melanogaster* has been used to discover several milestone findings in genetics. The notion that hereditary traits are carried on the chromosomes was first developed in *D. melanogaster*. As a matter of fact, in 1994, Nobel Prize for Physiology and Medicine was awarded to Ed Lewis, Eric Weischaus and Christiane Nusslein for their ground-breaking studies on *D. melanogaster* that led to the discoveries ranging from defining gene structure, to identification of vital genes involved in embryogenesis. Subsequently, these genes have been found to be associated with normal mammalian development. The first major complex organism to have its genome completely sequenced is *D. melanogaster* (Adams *et al.*, 2000). This incidence immensely bring to fore the observed homologies between the fly genome and the human genome when it was sequenced a few years later. This later buttressed the use of the fly as a model in toxicology and to understand human biology and disease processes. The fly has continued to gain attention of toxicologists because molecular studies are often first elucidated in simple organism models, such as the fly, and later translated to mammalian systems (Bhan and Nichols, 2011).

Drosophila Culture and media

In order to incorporate *D. melanogaster* for toxicological studies, it is important to maintain cultures of flies for backup since they have a short lifespan. For ease of culturing and transferring of flies, uniform bottles and vials are preferable. It is also important to completely clean and sterilize the bottles and vials to prevent outbreak of diseases. There are many standard formulations for *D. melanogaster* media. In our laboratory for instance, we maintain and rear flies on cornmeal medium (1%, w/v brewer's yeast; 2%, w/v sucrose; 1%, w/v powdered milk; 1%, w/v agar; 0.08%, v/w nipagin) at constant temperature and humidity (23±1 °C; 60% relative humidity, respectively) under 12 h dark/light cycle. Li *et al.* (2007) and Peng *et al.* (2009) have used basal diets containing 105 g of cornmeal, 21 g of yeast, 105 g of glucose, and 13 g of agar; then, 0.4% of Ethyl 4-hydroxybenzoate was added to the diet in order to prevent mould growth (Hugo and Peter, 2008).

Drosophila melanogaster Endocrinology

The endocrine glands of *D. melanogaster* are derived from epithelial tissues and, at molecular and cellular levels, they function in a similar way to those of vertebrate glands. For instance, *D. melanogaster* possesses circulating hormones, plasma membrane receptors (PMRs) and nuclear receptors (NRs) governed by the same chemical and biological mechanisms found in the hormonal system of vertebrates. The genome of *D. melanogaster* codes for PMRs and NRs that correspond to diverse subclasses of human receptors, including those involved in neurotransmission (Riddiford *et al.*, 1993).



Scheme 1:

Comparison of Human and *Drosophila* Hormones. Human Estradiol (A) vs. *Drosophila* Ecdysone (B), and Human Retinoic acid (C) vs. *Drosophila* Juvenile hormone (D).

D. melanogaster possesses two major hormonal systems: Ecdysone (Ecy, scheme 1B) and Juvenile Hormone (JH, scheme 1D). Ecdysone is a steroid hormone produced in the prothoracic glands, and it is homologous to the steroid hormones such as estradiol (scheme 1A) synthesised from cholesterol. Juvenile Hormone is a sesquiterpenoid produced in the corpora allata in the brain of *D. melanogaster*, and it shares some traits with retinoic acid (scheme 1C) (King- Jones and Thummel, 2005).

The moult in *D. melanogaster* is preceded and controlled by Ecy. The production of JH ceases at the end of the third instar larva to allow for the peak in Ecy to initiate pupation, cell death, and the development of new cellular structure. JH later returns in the adult stage and regulates spermatogenesis, lifespan, locomotor behaviour, feeding, secondary sexual differentiation and courtship. It also interacts with Ecdysone to promote fertility (Helmut *et al.*, 2010).

As pointed out above, the regulation of fruit fly development shares several chemical and biological features with vertebrate, however, the influence of classical developmental toxicants in mammals have been little explored in *D. melanogaster* (Lee *et al.*, 1989; Carmora *et al.*, 2008; Rand *et al.*, 2010).

Drug Administration Routes in *D. melanogaster*

Drugs can be administered to *D. melanogaster* via different routes depending on the type of study, the nature of the drug and the developmental stage of the fly. During the embryonic stage, drugs can be administered by permeabilization (Rand *et al.*, 2010); whereas, in the larva stage, it can be added to the media in the solid state for long exposures or in a media containing yeast paste for short exposures. In the adult flies, numerous routes of exposure are available (Figure 2). If the drug is volatile for example, it can be administered as a vapour using suitable vehicle like ethanol (Moore *et al.*, 1998). The drug can also be mixed with the diet of the flies using appropriate ratio, or using filter saturated with the drug in the presence of sucrose (Nichols *et al.*, 2002). Some researchers prefer to

administer certain drugs to the flies by injecting the drugs directly into the exposed nerve cord of decapitated flies (Torres and Horowitz, 1998), or directly into the abdomen in order to aid rapid diffusion into the tissues of the flies (Dzitoyeva *et al.*, 2003). The taste of the drug should be taken into consideration before administering the drug, because flies tend to avoid drugs with aversive taste (Ja *et al.*, 2007). The common practice in this case, is to mix the drug with substrates that taste well such as sucrose or yeast paste, or to starve them for a period of time before exposure. It is important to carry out pilot studies to examine different concentrations with different duration of exposure. In our laboratory, we sometimes carry out survival curve experiments using different concentrations of the drugs to assist in choosing the doses of the drugs to be used, and the duration of exposure. Occasionally, we use different vehicles too for certain drugs to choose the best vehicle with the drugs. In our recent studies, we observed that corn oil (2.5%) is not a good vehicle for introducing 4-vinylcyclohexene (VCH) in the basal diet (unpublished results) (see *D. melanogaster* Media section above), even though it had been used as vehicle in rat models. This is because the mortality recorded in the control flies (corn oil) was similar to the VCH-treated groups. The reason for the dietary toxicity of corn oil was not investigated, but flies normally can tolerate up to 1% of fat in the diet (Miquel *et al.*, 1982). Ethanol gave better results when used as vehicle with the flies at final concentration of 2.5%. Indeed, fruit flies can tolerate up to 5% of ethanol in the diet, but they prefer diets containing lower concentration of ethanol (3-4%).

Another method to be used in exposing flies to drugs that have aversive taste is to starve the flies for 17 hours, and then transfer the flies to the vial containing the drug for a few minutes, so that the flies can rapidly consume the drug. This strategy can help to quickly determine the acute effects of the drug. Generally, if the flies are maintained on food saturated with the drug for longer than 24 hours, it allows for a steady state to be achieved in the flies, however, it has the disadvantage of possible adaptive

mechanisms due to prolonged exposure due to down-or up-regulation of target genes in the drug disrupted pathway(s) (Pandey and Nichols, 2011).

If an acute effect is under investigation, it is important to test the flies immediately they are removed from the vials containing the drug, because metabolism and elimination of the drug can affect the final outcome of the assay.



Figure 2:
Some routes of drug administration in *D. melanogaster*

Areas of Opportunities to Use *D. melanogaster* in Toxicology

The Use of *D. melanogaster* to Assess Oxidative stress and antioxidant Biomarkers: In biomedical sciences, we are living in the era of free radical and oxidative stress. In fact, experimental evidences have implicated oxidative stress in the pathophysiology of several disease conditions (El-bab *et al.*, 2013; Hwa, 2013), however, the precise role of free radicals and oxidative stress in the pathology of diseases are far from being known. Consequently, the study of free radicals and oxidative stress in animal models are of particular importance at the present time.

Several articles have been published in the literature indicating the use of *D. melanogaster* to assess oxidative stress and antioxidant markers. Each assay may require different approach depending on the levels of the markers of interest to the toxicologist. For instance, the procedure of homogenisation of the treated and control flies in appropriate buffers, centrifugation at appropriate speed and temperature, and using the separated supernatants to determine biochemical and molecular markers of interest to the toxicologist, can be employed (Golombieski *et al.*, 2008; Sudati *et al.*, 2013).

***D. melanogaster* in Neurodegenerative Diseases:** Human neurodegenerative diseases largely affect the elderly and it is associated with devastating illnesses. Important neurodegenerative conditions such as Alzheimer, and Parkinson's diseases are associated with pathogenic oligomers due to misfolded proteins, ultimately resulting in gradual loss of neurons in the nervous system and brain (Hirth, 2010). Due to the limitation of human genetics, it is necessary to use model systems to investigate the affected genes. For several years now, genetically amenable *D.*

melanogaster has been used as suitable model system in the study of human neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's diseases, Sleep, Seizure and Cognitive disorders (Hirth, 2010). Interestingly, toxicologists can use *D. melanogaster* as a model to screen several compounds for their potential to induce or ameliorate neurodegenerative diseases.

***D. melanogaster* in Cancer study:** *D. melanogaster* has been increasingly used as a model system in cancer research. In the past, cancer research was limited to mammalian-based systems such as tissue culture and whole-animal studies (Nagaraj and Banerjee, 2004). The use of *D. melanogaster* in cancer research has facilitated several discoveries such as the signalling pathway that coordinates cell proliferation and death, cell competition and apoptosis-induced compensatory proliferation, as well as the mechanism of oncogenes and tumour suppressor cooperation (Vidal and Cagan, 2006).

***D. melanogaster* in Cardiovascular diseases:** The fruit fly has been used successfully to model certain aspects of cardiovascular diseases caused by environmental factors. This is because the fly's heart development depends on certain genes that are conserved up through mammals (Bryantsev and Cripps, 2009; Reim and Frasch, 2010). Several heart dysfunctions, some of which are age related, such as cardiomyopathies, structural defects and arrhythmias have been observed in flies (Ocorr *et al.*, 2007). Using several methods beyond the scope of this review, *D. melanogaster* has made it easy to determine effects of pharmacological agents on heart function (Dasari *et al.*, 2007; Neckameyer *et al.*, 2007).

***D. melanogaster* in Inflammation and Infectious Diseases:** Because *D. melanogaster* is constantly exposed to pathogens in the environment, it has developed sophisticated immune response that is instrumental to the understanding of human inflammatory conditions. In a similar way to mammals, *D. melanogaster* has been used to model certain forms of inflammatory conditions such as asthma (Hirth, 2010).

***D. melanogaster* in Metabolic Disorders and Diabetes:** Recent studies have shown that *D. melanogaster* can be used as a model to study certain aspects of human metabolism such as glucose homeostasis, and endocrinology. It possesses insulin-like-peptide secreting cells in the brain (Nassel and Winther, 2010), and additional glucagon analog secretory cells, making it to exhibit similarity with vertebrates endocrine system at the physiological level (Wang *et al.*, 2007; Haselton and Fridell, 2010). In fact, flies have been used to study metabolic process related to type II diabetes (Pendse *et al.*, 2013), however, its use is limited in this field due to the lack of liver and pancreas.

FlyBase Resource in the study of *D. melanogaster*

Several online resources are available to toxicologists interested in *D. melanogaster* as a model. FlyBase database (<http://flybase.org>) is a very helpful and comprehensive internet-based reserve for toxicologists interested in this

model. Vital information about available mutant alleles, knockdown lines, human disease homologs, and whole-genome sequences in *D. melanogaster* can be found in the FlyBase. In addition, FlyBase also has links to several stock centres of different institutions where specific request for the fruit fly can be obtained (Hirth, 2010). The FlyBase home page (<http://flybase.org>) is as shown in Figure 3.

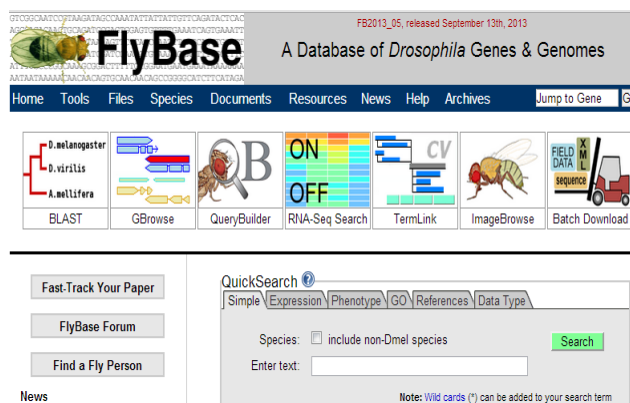


Figure 3: FlyBase Home Page (www.flybase.org)

Concluding remarks

Conclusively, it is important to take into account potential differences in pharmacokinetics and pharmacodynamics which may cause differences in drug levels and tissue distribution profiles between mammals and *D. melanogaster*, when extrapolating toxicological data from *D. melanogaster* to mammals. For instance, in toxicological studies involving the central nervous system, there may be differences in blood-brain permeability (Mayer *et al.*, 2009). In addition, despite the fact that there is a strong correlation of toxicity between the fly and mammals, metabolic differences may occur in which some toxicants may cause toxicity in flies and not in humans (Rand, 2010). However, this assumption can be applied to all experimental models of human toxicology. In spite of this, the use of flies in toxicology must be encouraged to determine common or universal pathway or targets of a given toxicant or class of toxic agents. In effect, the establishment of pathways in simple animal models that could be used as early “complex molecular targets” that could predict the potential toxicity of toxicants in mammals would be important both from a rational (economical) and ethical point of view. In this case, the research using simple organism models such as worms (*Caenorhabditis elegans*) and insects (particularly *D. melanogaster*) should be further stimulated (Leung *et al.* 2008; Rand, 2010; Li *et al.* 2013; Rodrigues *et al.* 2013) to recognize which pathways should be considered initially in the toxicological studies.

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