EFFECT OF DIAGNOSTIC RADIATION ON THE EXPRESSION PATTERN OF DrICE AND DCP-1 mRNAS IN *Drosophila melanogaster*

BY

USMAN MIRACLE ESHIOFUNEH

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

SCHOOL OF BASIC MEDICAL SCIENCES,

COLLEGE OF MEDICAL SCIENCES,

UNIVERSITY OF BENIN,

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE(BMLS), UNIVERSITY OF BENIN, BENIN CITY.

SUPERVISED BY

DR. A.I. ARUOMAREN

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CERTIFICATION

This is to certify that this project work was done by **USMAN MIRACLE ESHIOFUNEH** with the Matriculation number **BMS2101534** under the supervision of **DR. A.I. ARUOMAREN** in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science degree.

_____ _____

DR. A.I. ARUOMAREN DATE

(Project Supervisor)

_____ _____

DR.(MRS) Z. OMORUYI DATE

(Head of Department)

_____ _____

DR ADEDIRE ADEYINKA

(External Examiner) DATE

DEDICATION

I dedicate this project work to God Almighty for His wisdom and guidance during the course of this project and also to my family for their care and support.

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ABSTRACT

Diagnostic radiation (X-rays, CT scans) generates reactive oxygen species and DNA damage, affecting apoptotic gene expression in Drosophila melanogaster, a model for cellular responses. Effector caspases DrICE and Dcp-1 regulate apoptosis under stress. The aim of this study was to assess the effect of acute (X-ray) and chronic (CT room) radiation over 7 and 14 days on DrICE and Dcp-1 mRNA expression in Drosophila. The flies were divided into 4 groups; X ray exposure (7days), X ray exposure (14days), CT room exposure (7days), CT room exposure (14days). DrICE and Dcp-1 mRNAs expression were determined using Polymerase chain reaction. The data obtained was analyzed using graphpad prism (version 8.02, California, USA). The result showed that DrICE mRNA increased significantly ($p<0.001$) in all exposure groups (X-ray/CT room, 7/14 days) when compared to the control (66.16± 0.31), but decreased ($p<0.01$) in CT room at 14 days (75.84± 2.17) when compared to X-ray (14 days) (83.29± 1.14) and CT room (7 days)(86.18±1.84). Dcp-1 mRNA showed no significant change ($p>0.05$) with X-ray (7/14 days) when compared to the control, but decreased ($p<0.01$) in CT room at 14 days (57.16±2.37) when compared to control (71.29±1.13) and X-ray 7 and 14 days (74.51±2.66 and 69.06±1.72) with a milder drop ($p<0.05$) at 7 days(61.48±1.15). In conclusion, DrICE upregulation shifts to suppression under chronic CT exposure, indicating an adaptive response. Dcp-1 stability under X-ray contrasts with CT suppression, showing dose-rate effects. This suggests radiation modulates apoptosis, with potential for pest control, needing further protein studies.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Low energy doses (0.01–10 mSv) are delivered by diagnostic ionizing radiation, mainly X-rays used in imaging procedures like radiography and computed tomography (CT), to examine internal structures with little harm to tissue (Brenner and Hall, 2007). Diagnostic IR produces sparse ionization, which results in DNA double-strand breaks (DSBs), oxidative stress through reactive oxygen species (ROS), and cellular reactions like DNA repair, cell cycle arrest, or, in rare cases, apoptosis, in contrast to therapeutic radiation, which targets cancer cells with high doses (Azzam *et al*., 2012). Although usually safe, repeated exposures can raise stochastic hazards, such as cancer, hence ALARA (As Low As Reasonably Achievable) principles and dosage optimization are necessary (ICRP, 2007). Commonly employed in diagnostic imaging, X-rays are sparsely ionizing, dispersing small energy doses to reveal interior structures with little harm to tissue (Baskar *et al*., 2012). Long-term hazards like cancer may result from this energy deposition's induction of DNA double-strand breaks and oxidative stress, which sets off cellular reactions like DNA repair or cell cycle arrest (Azzam *et al.*, 2012).

Caspases such as DrICE and DCP-1 are essential for the strictly controlled process of apoptosis, or programmed cell death, which destroys damaged or superfluous cells in Drosophila. Effector caspases DrICE and DCP-1 cleave cellular substrates to break down cells; they are triggered downstream of initiator caspases such as Dronc

are(Hay and Guo, 2006). Damage to DNA caused by ionizing radiation can cause apoptosis through pathways that may involve p53-dependent or p53-independent mechanisms. It has been demonstrated that ionizing radiation causes apoptosis in the imaginal discs of Drosophila larvae, a process that depends on caspase activation (Wichmann *et al*., 2006). However, the specific regulation of DrICE and DCP-1 expression in response to different types and doses of ionizing radiation, such as X-rays and other diagnostic radiation, is less well understood and merits further exploration.

Ionizing radiation exposure in Drosophila has been shown to cause major cellular and molecular alterations, including an increase in apoptosis. For example, Sudmeier *et al.* (2015) examined the neurotoxic effects of ionizing radiation on Drosophila larvae and discovered that adult brains containing activated DCP-1 increased in a dose-dependent manner after larval irradiation with doses of 40 Gy or more. Higher levels of apoptosis were linked to this rise in DCP-1 activity, indicating that ionizing radiation causes caspase-mediated cell death in brain tissues (Sudmeier *et al*., 2015). Additionally, the study found that younger larvae responded less well to ionizing radiation and had a lower chance of surviving to adulthood, highlighting the impacts of radiation that vary by developmental stage. But this study was more concerned with DCP-1 protein activity than gene expression levels, which raised concerns regarding DCP-1 and its homolog DrICE's transcriptional control.

Fewer studies have examined DrICE's function in ionizing radiation-induced apoptosis, although it is known to cooperate with DCP-1 to cause cell death. The pro-apoptotic genes reaper, hid, and grim mediate the ionizing radiation-induced apoptosis in Drosophila imaginal discs, which is dependent on caspase activity, including DrICE and DCP-1, as

demonstrated by Wichmann *et al.* (2006). Remarkably, their research showed that apoptosis still happens in mutants without Drosophila Chk2 or p53 homologs, albeit slowly. p53-independent pathways that trigger caspases like DrICE and DCP-1 in reaction to ionizing radiation (Wichmann *et al*., 2006). Drosophila is a useful model for p53-independent apoptosis research, which is important for comprehending radioresistance in p53-deficient human malignancies, according to this finding. Although these investigations shed light on caspase activation, little is known about how DrICE and DCP-1 are transcriptionally regulated in response to ionizing radiation exposure. Ionizing radiation may change Drosophila's gene expression profiles, according to some research. A threshold effect was observed in the genome-wide analysis of gene expression in Drosophila exposed to X-rays (10 to 20,000 roentgens) by Antosh *et al.* (2014). This means that while low doses had little effect on gene expression, higher doses significantly changed genes related to metabolism, stress response, and reproduction. While neither DrICE nor DCP-1 were directly investigated in this work, it does indicate that ionizing radiation doses affect gene expression in a dose-dependent manner, which may also affect genes linked to apoptosis. Although caspase genes were not the main focus of another work by Moskalev *et al.* (2015), which investigated the transcriptome impact of diverse stressors, including ionizing radiation (144–864 Gy), they did find differential expression of stress response genes such Hsp70 and GstD2.

The expression of the caspase gene may also be influenced by the type of radiation. The energy deposition and biological consequences of therapeutic gamma radiation, which is frequently derived from cesium-137 sources, and sparsely ionizing X-rays are different. Low-dose gamma radiation causes different genetic and epigenetic modifications in

Drosophila than high-dose radiation, according to a study by Tanaka *et al.* (2021). This suggests that the molecular response to ionizing radiation is dose- and type-specific (Tanaka *et al.,* 2021). It has been demonstrated that neutrons, a different type of ionizing radiation, are more efficient than gamma rays at causing chromosomal abnormalities and sterility, which may have an indirect impact on caspase activation (Scientific Archives, 2022). Nevertheless, there is a crucial gap in the literature since no research has directly contrasted how X-rays and therapeutic radiation affect DrICE and DCP-1 expression. The function of oxidative stress and cellular repair processes in regulating the effects of ionizing radiation is another crucial factor to take into account. Reactive oxygen species (ROS), which are produced by ionizing radiation, have the ability to increase DNA damage and alter gene expression (Azzam *et al.*, 2012). Ionizing radiation-induced ROS generation in Drosophila was demonstrated to be higher in axenic flies than in conventional flies, indicating that commensal microorganisms may reduce oxidative stress and perhaps affect the expression of genes linked to apoptosis (Kim *et al*., 2021). Although it is unknown, it is an interesting field for research to determine whether ROS directly impacts DrICE and DCP-1 transcription.

The precise effects of X-rays and therapeutic radiation on DrICE and DCP-1 gene expression are not entirely understood, despite the fact that Drosophila melanogaster has been crucial in the research of ionizing radiation-induced death, especially through caspase activation. Although previous research has demonstrated that ionizing radiation causes caspase-dependent apoptosis and that high dosages of DCP-1 increase its activity, there are few in-depth examinations of the transcriptional alterations in DrICE and DCP-1. More focused studies are required to elucidate the links between these genes and factors such radiation type, dose, developmental stage, and oxidative stress. Gaining knowledge of these

processes in Drosophila may help improve radiotherapy and create plans to shield human healthy tissues from ionizing radiation harm.

1.2 Justification of Study

This work is crucial because it investigates the effects of ionizing radiation (IR), such as X-rays and therapeutic radiation, on the expression of the genes DrICE and DCP-1, which are important regulators of apoptosis in *Drosophila melanogaster*. By illuminating the ways in which radiation causes cell death, an understanding of these impacts helps enhance cancer radiotherapy by determining the best ways to target tumors while preserving healthy tissues. Because of its ease of study, short life cycle, and genetic resemblance to humans, Drosophila makes an excellent model. There is currently a dearth of comprehensive knowledge regarding the regulation of these caspase genes by ionizing radiation, particularly with regard to variations in radiation types and dosages. Addressing this gap could help improve radiation therapy approaches and provide guidance on how to prevent radiation damage in environmental and medical settings.

1.3 Aim of Study

The aim of this study is to investigate the effect of diagnostic radiation on the expression levels of DrICE and DCP-1 genes in *Drosophila melanogaster.*

1.4 Specific Objectives

- To analyze all samples for DrICE and DCP-1 gene expression

- To investigate the effect of diagnostic radiation on the expression level of DrICE gene in *Drosophila melanogaster* compared to the control.

- To evaluate the effect of diagnostic radiation on the expression level of DCP-1 gene in *Drosophila melanogaster* compared to the control.

1.5 Research Questions

1) How does diagnostic radiation affect the expression patterns of DrICE mRNA in *Drosophila melanogaster*?

2) How does diagnostic radiation affect expression levels of DCP-1 mRNA in *Drosophila melanogaster*?

3) What is the impact of diagnostic radiation on the expression pattern of DrICE and DCP-1 mRNAs in *Drosophila melanogaster* compared to unirradiated controls?

4) Are there differences in the transcriptional response of DrICE and DCP-1 mRNAs to diagnostic radiation in *Drosophila melanogaster*?

1.6 Research Hypothesis

Null Hypothesis (H0)

- Diagnostic radiation has no effect on the expression pattern of DrICE mRNA in Drosophila *melanogaster.*

- Diagnostic radiation has no effect on the expression pattern of DCP-1 mRNA in *Drosophila melanogaster.*

- There are no differences in the transcriptional response of DrICE and DCP-1 mRNAs to diagnostic radiation in *Drosophila melanogaster.*

- There are no impact of diagnostic radiation on the expression pattern of DrICE and DCP-1 mRNAs in *Drosophila melanogaster* compared to the unirradiated controls.

Alternate Hypothesis (H1)

- Diagnostic radiation does have an effect on the expression pattern of DrICE mRNA in *Drosophila melanogaster.*
- Diagnostic radiation does have an effect on the expression pattern of DCP-1 mRNA in *Drosophila melanogaster*.
- There are differences in the transcriptional response of DrICE and DCP-1 mRNAs to diagnostic radiation in *Drosophila melanogaster.*
- There is an impact of diagnostic radiation on the expression pattern of DrICE and DCP-1 mRNAs in *Drosophila melanogaster* compared to the unirradiated controls.

CHAPTER TWO

LITERATURE REVIEW

2.1 Ionizing Radiation

Ionizing radiation is a potent energy source that can remove electrons from atoms to produce ions, which are charged particles. Because of this mechanism, it can have enormous effects on living tissues, making it both extremely helpful and potentially dangerous. The term "ionizing radiation" describes electromagnetic waves or particles that have sufficient energy to cause electrons to be dislodged from their orbits and ionize atoms. Examples of these include particle radiation (such as alpha and beta particles), gamma rays, and X-rays. Since ionizing radiation can enter tissues and alter cells, unlike non-ionizing radiation (such visible light or radio waves), it is both a useful medical tool and a health problem (World Health Organization, 2023). Natural and man-made sources both produce ionizing radiation.

Ionizing radiation used in medical imaging to diagnose illnesses is referred to as "diagnostic radiation." It creates fine-grained pictures of inside organs, tissues, and bones using high-energy beams like gamma or X-rays. These images are obtained using ionizing radiation in procedures such as computed tomography (CT) scans and X-ray radiography, which help identify abnormalities, infections, or fractures. Multidetector CT and other advanced techniques reduce radiation exposure to patients while improving picture precision (Smith and Dillon, 2024). Human-made diagnostic radiation is produced by specialized machinery in medical institutions, such as CT scanners and X-ray machines.

High-energy electrons striking a metal target, usually in an X-ray tube, produce X-rays, a form of electromagnetic radiation. Since they may pass through soft tissues but are absorbed by denser materials like bone, producing clear images, they are frequently employed in diagnostic imaging, such as for identifying fractures or dental problems. For instance, physicians may identify lung problems with amazing accuracy using a chest X-ray (Smith-Bindman and Kwan, 2023). X-rays are created by humans, produced in regulated environments such as hospitals, and their use is strictly managed to reduce exposure. Ionizing radiation can come from natural sources such as radon gas or cosmic rays. Diagnostic radiation, on the other hand, stands out due to its carefully regulated use in medicine, providing vital diagnostic information while controlling any possible radiation exposure dangers. (World Health Organization, 2023).

Fig 2.1: Diagram of a modern CT scanner (Al- Ameen and Sulong 2016)

2.2 Biological Effects of Ionizing Radiation

The biological effects of ionizing radiation depend on the dose, exposure duration, and tissue type. These effects can be immediate (acute) or delayed (chronic), and they are a key focus of research due to their implications for health.

1. DNA Damage and Cellular Effects: Ionizing radiation can cause mutations by directly breaking DNA strands or indirectly producing free radicals that do the same. According to a 2018 study, even low radiation dosages, such those from diagnostic X-rays, can result in epigenetic alterations that eventually raise the risk of cancer (Burgio *et al*., 2018). This is especially problematic for radiation-sensitive cells that divide quickly, such as those found in bone marrow or the intestinal lining.

2. Acute symptoms: Skin burns, nausea, and acute radiation sickness are among the rapid symptoms of high doses, such as those used in radiotherapy or from unintentional exposure. Therapeutic radiation, for example, can cause side effects including weariness or localized inflammation in addition to targeting cancer cells (Saleh *et al*., 2023).

3. Long-Term Risks: Recurring low-dose exposure over an extended period of time, such as years of X-rays, may increase the risk of developing malignancies like thyroid or leukemia. Pediatric imaging adheres to stringent dose-reduction standards since children's developing tissues make them particularly sensitive (American Journal of Neuroradiology, 2009). Medical practitioners are guided by the ALARA principle (As Low As Reasonably Achievable) to reduce radiation exposure while preserving the effectiveness of diagnostic or therapeutic procedures (Smith-Bindman and Kwan, 2023).

4. Diagnostic Benefits vs. Risks: The objective of diagnostic imaging is to minimize radiation exposure to patients while obtaining precise images of inside structures to help diagnose

problems. New developments, such digital X-ray technology and low-dose CT scans, improve image quality with less ionizing radiation, increasing the precision of diagnosis for ailments like lung disorders or fractures (Smith and Dillon, 2024). Researchers are still weighing the trade-offs to maximize safety, but even diagnostic radiation has dangers, such as a slight chance of radiation-induced cancer years later.

2.3 Mechanism of DNA Damage Induced by Ionizing Radiation

Ionizing radiation, such as X-rays and gamma rays, damages DNA through two primary mechanisms: direct and indirect effects, which disrupt the DNA's structure and function, potentially leading to mutations, cell death, or diseases like cancer.

2.3.1 Direct Effects

Ionizing radiation directly strikes DNA molecules, transferring high energy that breaks chemical bonds. This results in:

1.Single-Strand Breaks (SSBs): When one strand of the DNA double helix is broken, it can usually be repaired, but if it occurs frequently, it can build up and affect cellular function (Burgio *et al*., 2018).

2. Double Strand Breaks: According to Lomax *et al.* (2013), double-strand breaks (DSBs) are a severe type of damage that can result in chromosomal abnormalities or cell death since both DNA strands are disrupted. They are also challenging to precisely repair. Direct damage is more likely when high-energy radiation, like alpha particles, produces dense ionization tracks (Hall and Giaccia, 2018). In radiotherapy, for instance, DSBs are a major worry because they have the ability to stop the growth of cancer cells while simultaneously running the risk of damaging healthy cells.

2.3.2 Indirect Effects

The majority of ionizing radiation-induced DNA damage happens indirectly, as reactive oxygen species (ROS) are produced. Radiation causes water radiolysis, which produces free radicals like hydroxyl radicals (OH•), when it interacts with water molecules, which make up over 70% of a cell's composition. These compounds' reactive properties result in:

1. Base Damage: Chemical changes to DNA bases that hinder transcription and DNA replication, such as guanine oxidation (Azzam *et al*., 2012t).

2. Strand Breaks and Cross-Links: To further destabilize the molecule, ROS can cause SSBs, DSBs, or aberrant cross-links between DNA strands or DNA and proteins (Burgio et al., 2018). 60–70% of DNA damage is caused by indirect effects, especially when low-energy radiation is utilized, such as X-rays, for medical imaging (Hall and Giaccia, 2018). ROS-induced oxidative stress can also interfere with cellular signaling, which can lead to long-term consequences such epigenetic modifications (World Health Organization, 2023).

Radiation type, dose, and cell sensitivity are some of the variables that affect the degree of damage. While low doses from diagnostic X-rays may produce more subtle damage, increasing the chance of mutations over time, high doses, which are typical in radiotherapy, generate widespread DSBs (Lomax et al., 2013).

2.3.3 Cellular Response to Radiation-Induced DNA Damage

Single-strand breaks (SSBs), double-strand breaks (DSBs), and base alterations are among the DNA damage caused by ionizing radiation, such as X-rays or gamma rays. To lessen these consequences, cells react by detecting, repairing, and determining their fate.

Damage Detection: ATR recognizes base damage or SSBs, triggering cell cycle checkpoints to stop division and start repair, while proteins such as ATM detect DSBs (Blackford and Jackson, 2017).

DNA Repair

1. Base Excision Repair (BER): This process replaces and eliminates damaged bases, which are frequently brought on by free radicals generated by radiation (Azzam et al., 2012).

2. Using the undamaged DNA strand as a template, Single-Strand Break Repair fixes SSBs (Burgio et al., 2018).

3. Double-Strand Break Repair: While homologous recombination (HR) employs a sister chromatid for precise repair, non-homologous end joining (NHEJ) quickly reconnects damaged ends, increasing the possibility of mistakes (Lomax et al., 2013).

Fate of Cells:

1. Cell Cycle Arrest: Checkpoints interrupt division to permit repair, mediated by p53 (Hall and Giaccia, 2018).

2. Apoptosis: To stop mutations, irreparable damage causes programmed cell death (Saleh *et al.*, 2023).

3. Senescence or Mutagenesis: Inadequate repairs might result in mutations or irreversible cell arrest, which can lead to cancer (World Health Organization, 2023).

Although the goal of these reactions is to restore cellular function, they may not be successful, which could result in tissue damage or cancer.

2.4 Apoptosis

Often known as programmed cell death, apoptosis is a tightly controlled process that is essential for immune system function, tissue homeostasis, and embryonic development. It is typified by recognizable morphological alterations, such as nuclear condensation, membrane blebbing, cell shrinkage, and fragmentation into apoptotic bodies that are phagocytosed without inducing inflammation. Apoptosis necessitates energy and includes complex biochemical pathways, most notably a family of cysteine proteases known as caspases, in contrast to necrosis, which is a passive, energy-independent process. In both physiological functions and pathological conditions, apoptosis is essential. According to Favalo *et al*. (2012), dysregulation can result in either insufficient or excessive cell death, which can fuel cancer or neurological disorders. The molecular mechanisms—specifically, the intrinsic and extrinsic pathways—and their potential therapeutic applications have been further explored in recent years.

2.4.1 Mechanisms of Apoptosis

The two main methods by which apoptosis is carried out are the intrinsic (mitochondrial) and extrinsic (death receptor-mediated) processes. These pathways combine to activate caspases, which causes cellular disintegration.

1. The Extrinsic Route: Death ligands, such Fas ligand or tumor necrosis factor-alpha (TNF-α), attach to cell surface death receptors to start the extrinsic route. In order to create the death-inducing signaling complex (DISC), which in turn triggers initiator caspase-8, this interaction enlists adaptor proteins like as FADD (Fas-associated death domain). Apoptosis is then brought on by caspase-8's cleavage of downstream effector caspases, including

caspase-3. Furthermore, Bid, a pro-apoptotic Bcl-2 family protein, can be cleaved by caspase-8, connecting the intrinsic and extrinsic pathways (Wong, 2011).

2. Intrinsic route: The Bcl-2 protein family controls the intrinsic route, which reacts to intracellular stressors such oxidative stress or DNA damage. Cytochrome c is released into the cytosol by pro-apoptotic members (e.g., Bak, Bax) increasing the permeability of the outer membrane of the mitochondria. Together with Apaf-1 and procaspase-9, cytochrome c forms the apoptosome, activating caspase-9, which in turn activates effector caspases (Elmore, 2007). By stopping the release of cytochrome c, anti-apoptotic proteins like as Bcl-2 and Bcl-xL block this process.

3. Alternative Routes: The perforin/granzyme pathway, in which granzyme A causes caspase-independent cell death and granzyme B causes caspase-dependent apoptosis, is one of the less frequent mechanisms. In certain situations, such chronic illnesses, the endoplasmic reticulum (ER) stress pathway involving caspase-12 is also observed (Chen *et al*., 2024).

2.4.2 Relevance to Disease

Because it balances cell death and survival, apoptosis is linked to a variety of diseases:

1. Cancer: Insufficient apoptosis, frequently brought on by Bcl-2 overexpression or p53 downregulation, enables cancerous cells to avoid death, fostering tumor growth and chemotherapy resistance (Wong, 2011). These deficiencies are the focus of therapeutic approaches such BCL-2 inhibitors (e.g., venetoclax) (Tang *et al.*, 2021).

2. Neurodegenerative Diseases: In disorders such as Parkinson's and Alzheimer's, excessive apoptosis leads to the loss of neurons (Favaloro *et al.*, 2012).

3. Cardiovascular Disorders: Apoptosis plays a role in myocardial infarction, especially during ischemia-reperfusion injury, where tissue damage is caused by a number of cell death pathways, including necroptosis and apoptosis (Galluzzi *et al*., 2023).

4. Infectious Diseases: Pathogens can modulate apoptosis to evade immune responses, while excessive apoptosis can exacerbate tissue damage in infections (Favaloro *et al*., 2012).

2.4.3 Role of Caspases in Apoptosis

Apoptosis, a type of planned cell death essential for development, tissue homeostasis, and disease prevention, is mostly carried out by the conserved enzyme family known as caspases (cysteine-aspartic proteases). These proteases orchestrate the morphological and biochemical changes that are typical of apoptosis, including DNA fragmentation, chromatin condensation, cell shrinkage, and the creation of apoptotic bodies, by cleaving certain substrates at aspartic acid residues (Elmore, 2007). Through two main pathways — the intrinsic (mitochondrial) and extrinsic (death receptor-mediated) pathways — caspases participate in both the initiation and execution stages of apoptosis.

2.4.4 Classification and Structure of Caspases

Based on their functions in apoptosis, caspases are divided into two groups:

1.The activation of initiator caspases (e.g., CASP2, CASP8, CASP9, and CASP10) is facilitated by their lengthy pro-domains, which engage with adaptor proteins in multiprotein complexes such as the death-inducing signaling complex (DISC) or apoptosome. By triggering downstream caspases, they start the apoptotic cascade (Shalini *et al*., 2015).

2. Initiator caspases activate effector caspases, such as CASP3, CASP6, and CASP7, which have brief pro-domains. They cause cellular disintegration by cleaving structural and

regulatory proteins to carry out apoptosis (McIlwain *et al.,* 2013). Caspases are produced as procaspases, which are inactive zymogens that must be cleaved or dimerized in order to become active. A cysteine residue in their active sites ensures specificity by targeting peptide linkages after aspartic acid (Elmore, 2007).

2.5 Organism of Study

2.5.1 *Drosophila melanogaster*

Drosophila melanogaster, sometimes referred to as the fruit fly or vinegar fly, is a small dipteran insect that is frequently used as a model organism in studies of apoptosis, developmental biology, and genetics because of its short life cycle, simple genome, and conserved metabolic pathways. Because of its easily manipulable nature and well-characterized genetics, it is perfect for researching processes like caspase-driven programmed cell death, which is mediated by Dronc and Drice (Steller, 2008).

2.5.2 Groupings

As an insect with a single set of wings, *Drosophila melanogaster* is categorized under the order Diptera and is a member of the kingdom Animalia. Here is its taxonomic hierarchy:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera (true flies)

Family: Drosophilidae

Genus: Drosophila

Species: *Drosophila melanogaster*

Although there are more than 1,500 species in the genus Drosophila, D. melanogaster is the most researched because of its well-annotated genome, which has about 14,000 genes, and genetic tractability. Its biological specialization as a saprophytic feeder, which is frequently seen on rotting fruit, is reflected in its classification within the Drosophilidae.

2.5.3 Morphology

The three primary parts of *Drosophila melanogaster's* body plan — the head, thorax, and abdomen — are typical of insects. It has unique traits that make genetic and developmental research easier, and its shape is tailored for its small size and quick reproductive cycle.

1. Head: They have large compound eyes that are usually brilliant red because of pteridine pigments, though they can also have white eyes if they are a mutant (for example, a white gene mutant). Each of the roughly 800 ommatidia that make up the compound eyes functions as a separate visual entity. They have feathery aristas and three-segmented aristate antennae that are employed for mechanoreception and smell. The mouthpart (a proboscis) has labellar structures for tasting and is designed to feed on liquid substrates, such as fermenting fruit juices.

2. Thorax: They have a single set of translucent wings with a distinctive cross and longitudinal vein pattern. Wings are employed for courting displays and flight. Vestigial and other mutants have smaller wings, which helps with genetic research. Each of its three pairs of segmented legs has chaetae, or bristles, which are employed for grooming and sensory purposes. In developmental biology, bristle patterns are frequently researched and are

genetically regulated. Additionally, they have halteres, which are modified hindwings that serve as gyroscopic organs to help them stay balanced while flying.

3. Abdomen: The chitinous cuticle covers the six to eight segments that make up an adult's abdomen. The reproductive and digestive organs are located in the abdomen. There is clear sexual dimorphism, with females having lighter, pointed abdomens with an ovipositor and males having darker, rounder abdomens.

2.5.4 Physical Characteristics

Due to its unique physical characteristics, *Drosophila melanogaster* is a tiny insect that can be used in experimental settings. Adults are about 2 and 3 mm long, with females being somewhat bigger than males. Wingspan is roughly 4 to 5 mm. The abdomen usually has black transverse lines, while the body is yellowish-brown (tan). Genetic mutations and environmental factors cause modest variations in the cuticle's pigmentation. In courting, males have sex combs, which are rows of dark bristles, on their first pair of legs. They also have a darkly colored genital arch at the tip of their abdomen. In order to deposit eggs, females have an ovipositor on their pointed abdomen and no sex combs. Three pairs of autosomes and one pair of sex chromosomes (XX in females and XY in men) make up the genome. The polytene chromosomes in larval salivary glands are large and banded, aiding cytogenetic studies.

2.5.5 Life Cycle of *Drosophila melanogaster*

Complete metamorphosis occurs in *Drosophila melanogaster*, which goes through four different life stages: egg, larva, pupa, and adult. Because of its quick life cycle—which lasts 10–14 days at 25 °C on average—it is perfect for genetic and developmental research.

Temperature, humidity, and nutrition are environmental elements that affect how long each stage lasts. Higher temperatures (like 29°C) shorten the cycle to roughly 8–10 days, whereas lower temperatures (like 18°C) prolong it to 19–20 days.

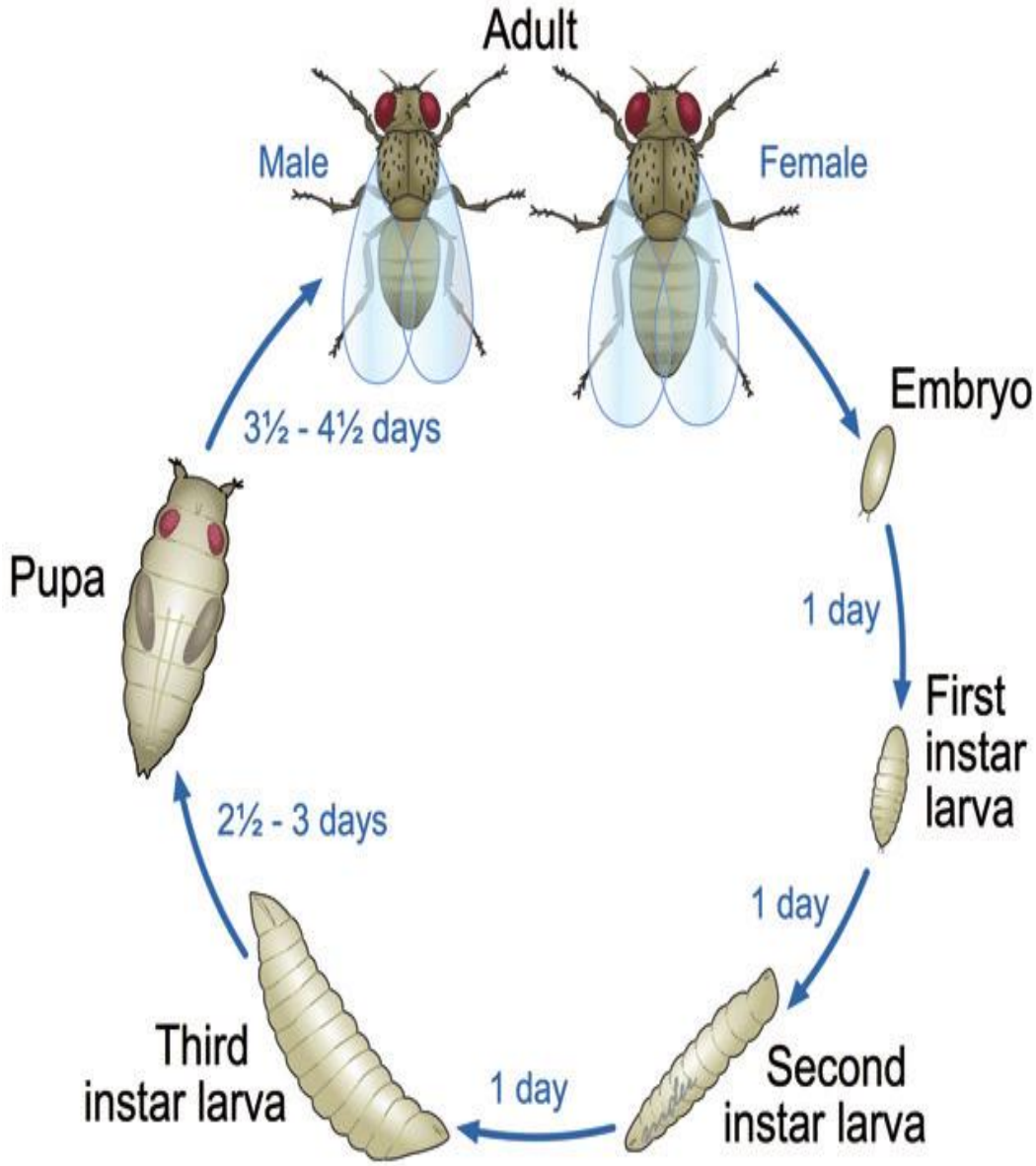

Fig 2.2: Life cycle of *Drosophila melanogaster* (Ong *et al.*, 2014)

1. Egg Stage

The eggs are kept at 25 °C for around 22 to 24 hours. The oval-shaped, white, and tiny (about 0.5 mm long) eggs include two anterior respiratory appendages (filaments) that facilitate gas exchange. Females normally deposit 20 to 50 eggs each day, with a lifetime total of up to 500 eggs, on moist, fermenting substrates such as decomposing fruit. As the embryo grows inside the egg, gastrulation and cell division happen quickly. The embryo is complete and prepared for hatching after 24 hours.

2. Larval Stage

- It has three instars (first, second, and third) and lasts for roughly four to five days at 25° C;
- The initial 24-hour instar: Larvae are tiny (approximately 1 mm), translucent, and eat a lot of the substrate's carbohydrates and yeast after hatching. They molt into the second instar and grow quickly.
- 24 hours into the second instar: Larvae continue to feed, doubling in size, and grow more pronounced spiracles (respiratory holes) and mouth hooks. The third instar is when they molt.
- The largest larval stage, reaching up to 4 – 5 mm, is the third instar (48 – 72 hours). As they get ready to pupate, larvae engage in vigorous eating and roaming behavior. For genetic research, the salivary glands produce polytene chromosomes. Larvae have a segmented body with a chitinous cuticle but no compound eyes or wings.

3. Pupal Stage

At 25 °C, the duration is roughly 4 – 5 days. Pupation begins when the third-instar larva develops a puparium, a solid case from the larval cuticle. The organism changes into an

adult structure within the puparium, undergoing metamorphosis. Imaginal discs mature into adult tissues (such as wings, eyes, and legs) during the prepupal (0–12 hour) and pupal phases, whilst larval tissues (such as the midgut and salivary glands) undergo programmed cell death. Hormonal cues like ecdysone induce internal remodeling in the immobile pupa, which has a brown, opaque puparium.

4. Adult Stage

Adults can survive for 30 to 60 days at 25°C, depending on their genetic makeup and the surrounding environment. Adults have a yellowish-brown body, black bands across the abdomen, and red compound eyes. They are 2-3 mm long. They are sexually dimorphic; females have a pointed abdomen with an ovipositor, while males have sex combs on the forelegs and a darker, rounder abdomen.

Within 8 to 12 hours of eclosion (emerging from the puparium), adults reach sexual maturity and start mating and laying eggs. They engage in behaviors including courting and phototaxis and feed on fermenting substrates. Genetic mutations that alter morphology (such as wing form or eye color) and behavior are studied in the adult stage.

Factors Influencing the Life Cycle

I. Temperature: 25° C is ideal; lower temperatures (like 18° C) prolong development, while higher ones (like 29° C) speed it up.

II. Nutrition: Larval growth depends on having access to carbohydrates and yeast. Adult size is decreased and development is delayed by poor nutrition.

III. Genetics: Developmental problems can result from mutations in genes linked to apoptosis, such as Dronc, Drice, and reaper, which can disrupt particular stages, especially the pupal stage (Steller, 2008).

2.5.6 Methods of Breeding *Drosophila melanogaster*

Large amounts of *Drosophila Melanogaster* are naturally found on soft fruits like bananas, mangos, and plums, particularly when the fermentation process has started and the fruit is overripe. Instead of eating the fruit, they usually eat the yeast that is growing on it. Cornmeal, yeast, sugar, agar, and preservatives such as propionic acid should be combined in distilled water to create a food medium for breeding *Drosophila melanogaster*. The mixture should be heated until it thickens, cooled slightly, and then poured into sterile vials to solidify, making sure the mixture is moist but not too wet; it can be stored at 4°C for up to a month. 5–10 adult flies (male and female) should be placed in vials with the medium for rearing. The females will lay eggs in 24–48 hours at 25°C, 60–70% humidity, and a 12:12 light–dark cycle. The eggs will hatch into larvae in 24–30 hours, which will feed on the medium for 4–6 days before pupating on the vial walls for 4–5 days before the adults emerge. While keeping an eye out for contamination, move adults to new vials every three to five days to avoid crowding and remove parent flies after seven to ten days to avoid mixing generations (Ashburner *et al*., 2005).

2.6 Importance of *Drosophila melanogaster* as a Model Organism

The fruit fly, or *Drosophila melanogaster*, is a fundamental model organism in biological study because of its special qualities that make it easier to conduct investigations in the fields of genetics, developmental biology, neuroscience, and disease modeling. It is significant for a number of reasons:

1. Genetic Simplicity and Manipulation: Although Drosophila's genome is smaller than that of humans (around 14,000 genes versus 20,000–25,000 genes), it shares roughly 60% of human genes, including 75% of those linked to human disorders. Due to the complete sequencing of its genome, precise genetic manipulations are possible through the use of tools such as the GAL4-UAS system for targeted gene expression.

2. Short Life Cycle and High Fecundity: The fruit fly can produce large sample numbers for investigations quickly because its life cycle lasts 10–12 days at 25°C. Studies can benefit from statistical robustness because a single female can deposit hundreds of eggs (Ashburner *et al.*, 2005).

3. Cost-Effectiveness and Maintenance Ease: Drosophila is affordable to keep in lab settings, requiring little room and basic food media, which makes it available to researchers all over the world (Jennings, 2011).

4. Conserved Biological Processes: Drosophila and higher creatures, including humans, share many basic biological processes, including behavior, development, and cell signaling. This makes it possible to gain understanding of intricate processes like immunology, neurodegeneration, and cancer (Bier, 2005).

5. Ethical Benefits: Drosophila research is less constrained by ethical standards than mammalian models, enabling high-throughput screening and genetic alterations without facing legal obstacles (Pandey and Nichols, 2011).

2.7 Apoptosis in *Drosophila melanogaster*

In multicellular animals, apoptosis, also known as programmed cell death, is a conserved biological mechanism that is essential for growth, tissue homeostasis, and illness prevention.

Because of its genetic tractability and conserved pathways, apoptosis in the fruit fly *Drosophila melanogaster* provides a useful model for researching the genetic and molecular mechanisms underlying programmed cell death.

2.7.1 Apoptotic Pathway in *Drosophila melanogaster*

In the fruit fly, *Drosophila melanogaster*, apoptosis, or programmed cell death, is a strictly controlled mechanism that is essential for growth, tissue homeostasis, and reaction to cellular stress. Drosophila is a great model for researching cell death mechanisms because of its highly conserved apoptotic pathway compared to humans.

Caspases are cysteine proteases that cleave cellular substrates to cause apoptosis. The apoptosome activates Dronc, the initiator caspase (homologous to mammalian caspase-9), which sets off effector caspases downstream. The effector caspases Drice and Dcp-1, which are similar to human caspase-3/7, break down cellular structures and cause cell death (Hay and Guo, 2006).

Dronc is activated by a protein complex called the apoptosome. Similar to the mammalian Apaf-1/caspase-9 complex, it is made up of Dark (Apaf-1 homolog) and Dronc, which come together in response to pro-apoptotic cues (Yuan and Akey, 2013).

The main caspase inhibitor, Diap1 (Drosophila Inhibitor of Apoptosis Protein 1), binds to Dronc, Drice, and Dcp-1 to stop premature apoptosis. Ubiquitin-mediated degradation controls the activity of Diap1 (Steller, 2008).

Apoptosis is primarily induced by the Reaper, Hid, and Grim (RHG) proteins. By attaching themselves to Diap1, they inhibit it, encourage its breakdown, and release caspases to

activate it. Developmental or stress cues, like DNA damage or p53 activity, control the transcription of these proteins (Brodsky *et al*., 2000).

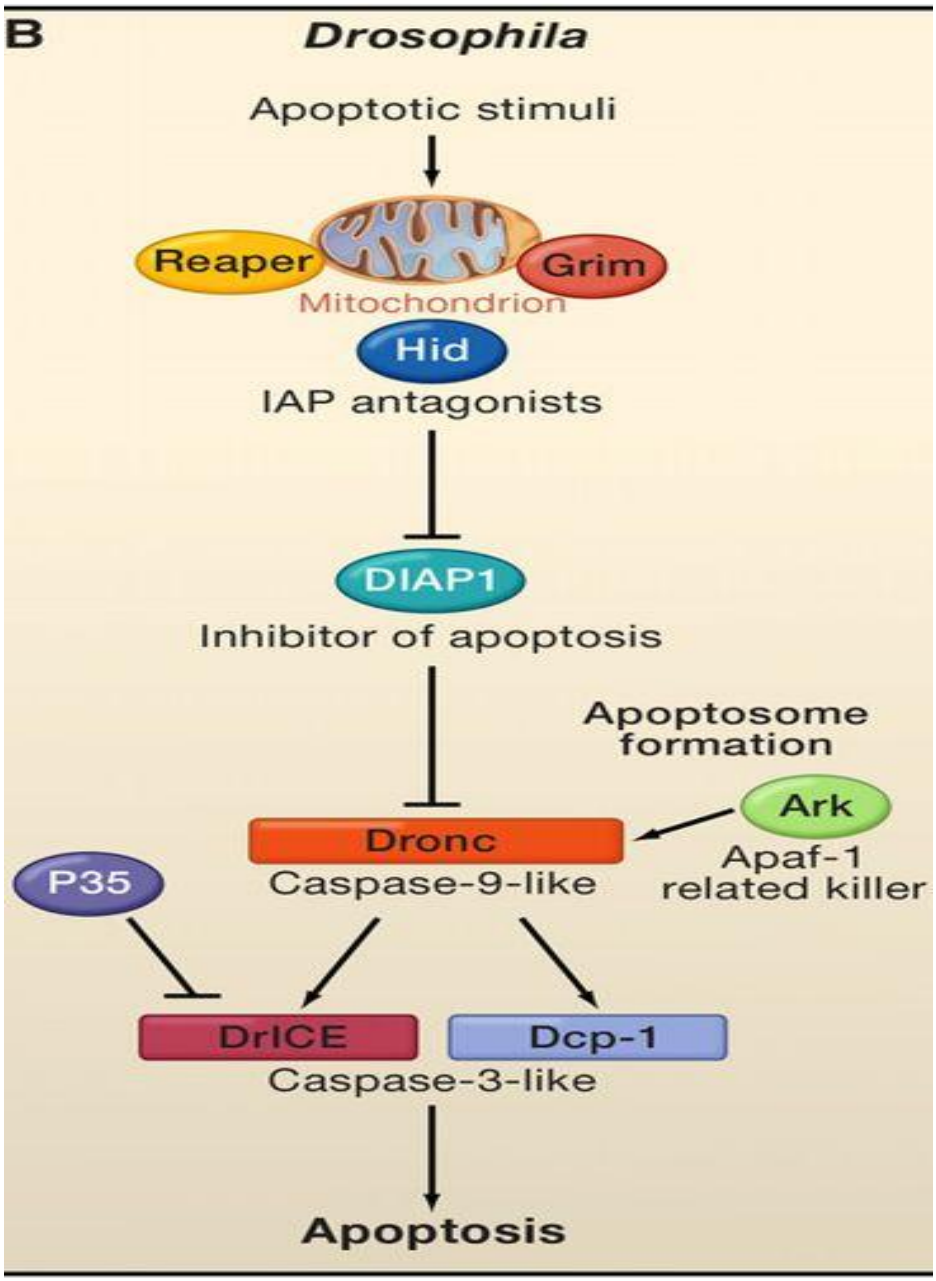

Fig 2.3: Apoptotic pathway in *D. melanogaster* (Fuchs and Steller 2011)

2.7.2 Regulation of the Apoptotic Pathway

- Transcriptional Control: Developmental cues or external signals (such as UV radiation or DNA damage) activate the RHG genes. For instance, in response to genotoxic stress, p53 directly triggers reaper expression. RHG expression is controlled by other routes, including the Hippo signaling pathway and the JNK (c-Jun N-terminal kinase) pathway, which govern apoptosis during stress or development.

- Post-Translational Regulation: RHG proteins or other signals cause ubiquitin-mediated proteasomal degradation, which regulates the levels of Diap1. Feedback loops regulate caspase activity, guaranteeing accurate regulation of cell death (Hay and Guo, 2006).

- Extrinsic and Intrinsic Signals: While Drosophila does not have a well-defined extrinsic pathway, such as the mammalian death receptor system, it does have intrinsic signals that drive the creation of apoptosomes and the activation of caspase, such as developmental cues or mitochondrial stress (Steller, 2008).

2.8 DrICE Gene

The DrICE gene (Death related ICE-like caspase) in *Drosophila melanogaster* (fruit fly) encodes a major effector caspase, a cysteine protease critical for programmed cell death (apoptosis) and non-apoptotic cellular processes.

2.8.1 Gene Structure

i. i. Location and Composition: The DrICE gene is found in the Drosophila melanogaster genome on chromosome 3R (positions 29,799,658 – 29,801,584, forward strand). It has a single exon, one annotated polypeptide, no introns, and a single transcript (splice variant) (FlyBase, 2025).

ii. Molecular Characteristics: The protein that DrICE encodes is a member of the peptidase C14A family and is distinguished by its heterotetrameric structure, which is made up of two anti-parallel heterodimers, each of which is made up of a 12 kDa (p12) and a 21 kDa (p21) component. The inactive pro-form has the ability to homodimerize, and DrICE forms stable complexes with other proteins such as Diap2 (via its BIR3 domain) and Dronc (an initiator caspase) (FlyBase, 2025).

iii. Domains: The protein's function in apoptosis and protein interactions depends on its functional domains, which include ubiquitin protein ligase binding, cysteine-type peptidase activity, cysteine-type endopeptidase activity, and BIR domain binding (FlyBase, 2025).

2.8.2 Functions

1.Apoptotic Role: In Drosophila, DrICE is a crucial effector caspase required for damage-induced and developmental apoptosis. It cleaves substrates like baculovirus p35 and lamin DmO to cause cell death. It is triggered downstream of the initiator caspase Dronc and the apoptosome component Dark. Research indicates that DrICE undergoes proteolytic processing into active p21 and p12 subunits when apoptosis is induced by cycloheximide, irradiation, or reaper (rpr) expression. The majority of apoptotic activity is eliminated when DrICE is immunodepleted from S2 cell lysates, demonstrating its crucial function. DrICE is specifically needed in some cell clusters, although it also functions redundantly with another effector caspase, Dcp-1, in some situations, such as optic lobe formation. It also facilitates corpse clearance by activating mechanisms that remove dying cells (Muro *et al*., 2015).

2. DrICE has non-apoptotic roles, particularly in tracheal morphogenesis, where it controls the endocytic trafficking of proteins downstream of the Hippo Network, including Crumbs,

Uninflatable, Kune-Kune, and Serpentine. This function, which is unrelated to cell death, involves a pool of DrICE that influences tracheal tube elongation by co-localizing with Clathrin (McSharry and Beitel, 2019). By creating a complex with Diap2 that results in its destruction, DrICE inhibits Diap2-mediated NF-κB activation in the colon, hence reducing chronic inflammation. Inflammation and Diap2 buildup occur when DrICE is lost, especially in reaction to local microorganisms (Kietz *et al*., 2022). Additionally, DrICE is essential for spermatid individualization, a non-apoptotic process that produces functional sperm by removing cytoplasm through caspase activity.

3. Regulation: Inhibitors such as Diap1 and Diap2 strictly regulate DrICE. While Diap2 exclusively regulates DrICE in both apoptotic and non-apoptotic settings, requiring a functioning RING finger domain for ubiquitination, Diap1 suppresses DrICE to prevent undesired apoptosis (Ribeiro *et al.*, 2007). DrICE acts downstream in caspase cascades, and the connection with Dronc and Dark guarantees precise activation. Pro-apoptotic genes including rpr, hid, and grim also affect its activity.

2.9 Gene DCP-1

An effector caspase, a cysteine protease essential for both non-apoptotic processes like autophagy and planned cell death (apoptosis), is encoded by the DCP-1 gene (Death caspase-1) in the fruit fly Drosophila melanogaster.

2.9.1 Gene Structure

i. i. Location and Composition: In the genome of Drosophila melanogaster, the DCP-1 gene is found on chromosome 2L (positions 4,671,104 – 4,672,567, forward strand).

It is composed of a single, intron-free transcript with a single exon that codes for a single, 322 amino acid polypeptide (FlyBase, 2025).

ii. ii. Molecular Characteristics: DCP-1 is a member of the C14A family of peptidases, which are distinguished by their cysteine-type endopeptidase activity and caspase domain. During apoptosis, the protein's inactive zymogen is broken down into the active p22 and p13 subunits. By forming a heterotetrameric structure, these subunits facilitate substrate cleavage.

iii. Interactions: DCP-1 is controlled by apoptosis protein (IAP) inhibitors such as Bruce and Diap1 and interacts with the initiator caspase Dronc. Additionally, it influences mitochondrial dynamics by binding to proteins like SesB. (DeVorkin *et al*., 2014).

2.9.2 Functions

1. Apoptotic Role: In Drosophila apoptosis, DCP-1 plays a crucial effector caspase role by cleaving important substrates such as poly(ADP-ribose) polymerase (PARP) downstream of the initiator caspase Dronc, which results in the execution of cell death. DCP-1 is crucial for embryonic apoptosis, as evidenced by the development of melanotic tumors and larval mortality when its activity is lost. It is especially important in germline cell death, where apoptosis is inhibited by P-element insertions that disrupt DCP-1. While DCP-1 and DrICE work in tandem in the majority of cell clusters in the developing optic lobe, certain neurons are absolutely dependent on DrICE, and DCP-1 only plays a supporting role until DrICE activity is decreased (Muro *et al*., 2015).

2. Non-Apoptotic Functions: Control of Autophagy: During Drosophila oogenesis, starvation-induced autophagy is positively regulated by DCP-1. DCP-1 mutants exhibit decreased autophagic flux in stage 8 egg chambers and germaria under nutrient-deprivation

conditions, suggesting that autophagy induction is required (Hou *et al*., 2008). In order to encourage autophagy, DCP-1 lowers ATP levels by negatively regulating the mitochondrial adenine nucleotide translocase SesB. According to DeVorkin *et al*. (2014), this non-apoptotic function relates caspase activity to cellular homeostasis and mitochondrial shape. During spermatid individualization, a non-apoptotic phase crucial to sperm maturation, DCP-1 and DrICE participate in caspase-dependent activities (Arama *et al.*, 2007).

3. Regulation: IAPs, especially Bruce, tightly regulate DCP-1, preventing it from acting during autophagy and apoptosis. In oogenesis, starvation-induced autophagy is modulated by the ratio of DCP-1 to Bruce (Hou *et al*., 2008). It ensures precise control of cell death pathways by activating downstream of the apoptosome component Dronc and pro-apoptotic genes reaper, hid, and grim (Xu *et al*., 2006).

2.10 Commonalities and Dissimilarities Between the Genes of DrICE and DCP-1

In Drosophila melanogaster, the DrICE and DCP-1 genes encode effector caspases that are essential for both non-apoptotic and planned cell death (apoptosis). Despite having structural and functional similarities and being members of the caspase family, they have different functions and regulatory systems.

2.10.1 Similarities

1. Gene and Protein Structure:

- The peptidase C14A family, which encodes cysteine proteases with caspase domains that cleave substrates after aspartate residues, includes DrICE and DCP-1 (FlyBase, 2025).

- One Exon: Both genes only have one exon and no introns. DCP-1 encodes a 322-amino-acid protein on chromosome 2L (positions 4,671,104 – 4,672,567), whereas DrICE encodes a 339-amino-acid protein on chromosome 3R (positions 29,799,658 – 29,801,584) (FlyBase, 2025).

- Both are produced as inactive zymogens and cleaved into active heterotetrameric forms (two big and two tiny subunits) as part of the activation mechanism. Substrate cleavage during apoptosis is made possible by the formation of p21/p12 subunits by DrICE and p22/p13 subunits by DCP-1.

2. Apoptotic Functions:

- Both effector caspases cleave substrates such as poly(ADP-ribose) polymerase (PARP) to cause cell death in the apoptotic cascade, acting downstream of the initiator caspase Dronc and the apoptosome component Dark (Xu *et al.,* 2006).

- The functions of DrICE and DCP-1 in developmental apoptosis largely overlap, especially in the optic lobe, where either caspase can make up for the loss of the other in the majority of cell clusters (Muro *et al.*, 2015).

- Both aid in the production of mature sperm by eliminating extra cytoplasm through non-apoptotic caspase activity during sperm differentiation (Arama *et al*., 2007).

3. Regulation: Diap1 and Diap2, which stop improper caspase activation, are inhibitors of apoptosis proteins (IAPs) that inhibit both. Reaper, hid, and grim are pro-apoptotic genes that alleviate this inhibition and cause apoptosis (Ribeiro *et al*., 2007).

2.10.2 Differences

1. Gene Structure and Expression:

Genomic Location: Representing different genomic contexts, DrICE is located on chromosome 3R and DCP-1 is located on chromosome 2L (FlyBase, 2025).

Protein Size: The somewhat longer polypeptide of DrICE (339 amino acids) compared to DCP-1 (322 amino acids) may have an impact on binding affinity or substrate selectivity (FlyBase, 2025).

Expression Patterns: DCP-1 plays a relatively limited role, especially in germline cells and autophagy control, whereas DrICE is more widely expressed and essential in the majority of apoptotic situations.

2. Functional Roles:

Apoptotic Specificity: In most tissues, DrICE is the main effector caspase; in S2 cell lysates, its removal eliminates the majority of apoptotic activity. Although DCP-1 has a secondary function, it is particularly crucial for germline cell death, as its interference results in melanotic tumors and larval mortality.

3. Non-Apoptotic Functions:

a) By regulating the endocytic trafficking of proteins such as Crumbs and influencing NF- κ B signaling in the gut to prevent inflammation, DrICE regulates tracheal morphogenesis (McSharry and Beitel, 2019; Kietz *et al*., 2022).

b) DCP-1: Regulates mitochondrial dynamics through SesB to promote starvation-induced autophagy in oogenesis; DrICE has not been shown to play this role. In germaria and egg chambers, DCP-1 mutants exhibit impaired autophagic flow (Hou *et al*., 2008; DeVorkin *et al*., 2014). While DCP-1's function is optional unless DrICE is

impaired, some neuronal clusters in optic lobe development strictly require DrICE (Muro *et al*., 2015).

4. Regulation and Interactions:

Diap1 and Diap2 are the main regulators of DrICE; in non-apoptotic settings, Diap2 forms a stable complex to adjust its activity (Ribeiro et al., 2007). Different regulatory networks are highlighted by the IAP Bruce's unique regulation of DCP-1 during autophagy, especially in oogenesis (Hou *et al*., 2008). While DCP-1 interacts with SesB to affect mitochondrial ATP levels, a function unrelated to DrICE, DrICE interacts with Diap2's BIR3 domain and Dronc to create stable complexes (DeVorkin *et al*., 2014; FlyBase, 2025).

2.11 DrICE in Apoptosis

The majority of developmental and stress-induced apoptosis in Drosophila is dependent on DrICE, the primary effector caspase. It disassembles cellular structures during apoptosis by cleaving substrates such baculovirus p35 and lamin DmO, acting downstream of the initiator caspase Dronc and the apoptosome component Dark. Most caspase activity is eliminated with immunodepletion of DrICE from S2 cell lysates, highlighting its critical function. Pro-apoptotic cues such as reaper (rpr), hid, or radiation cause the proteolytic cleavage of DrICE into the p21 and p12 subunits. Inhibitors of apoptosis proteins (IAPs), specifically Diap1 (which inhibits unintended cell death) and Diap2 (which modulates DrICE via its BIR3 domain), closely regulate its activity (Ribeiro *et al*., 2007). As the optic lobe develops, In particular neuronal clusters, DrICE plays a non-redundant role in cell death. By triggering processes to eliminate dead cells and maintain tissue homeostasis, it also makes corpse disposal easier (Muro *et al*., 2015). DrICE is the predominant caspase for carrying out

apoptosis in the majority of Drosophila tissues due to its strong activity and widespread expression; its absence significantly disrupts cell death pathways (Xu *et al.*, 2006).

2.12 DCP-1 in Apoptosis

An effector caspase with a more limited function, DCP-1 aids DrICE in apoptosis. It contributes to the execution of cell death by cleaving substrates such as poly(ADP-ribose) polymerase (PARP) into p22 and p13 subunits. Germline cell death, especially during oogenesis, depends on DCP-1. DCP-1 plays a crucial role in particular developmental situations, as evidenced by P-element insertions that disrupt it, which results in faulty apoptosis in germline cells, causing larval mortality and melanotic tumors (Laundrie *et al.,* 2003). In the majority of cell clusters in the optic lobe, DCP-1 works in tandem with DrICE to make up for DrICE's absence. Its secondary contribution in many tissues is highlighted by the fact that its involvement only becomes crucial when DrICE activity is disrupted (Muro *et al.*, 2015). Similar to DrICE, DCP-1 is activated downstream of pro-apoptotic genes (reaper, hid, grim) and Dronc, and is controlled by IAPs (e.g., Diap1, Bruce). In the germline, Bruce's control of it is especially noteworthy since it strikes a balance between autophagy and apoptosis (Hou *et al*., 2008).

2.13 DrICE and Dcp-1 Expression Under Normal Conditions

DrICE's function as the main effector caspase is demonstrated by its widespread expression in Drosophila throughout a range of tissues and developmental stages under normal circumstances. According to FlyBase (2025), it can be found in the nervous system, imaginal discs, and somatic tissues in both embryos and larvae as well as adult tissues. DrICE mRNA and protein are extensively dispersed throughout embryos, especially in organs like the epidermis and central nervous system that are going through developmental remodeling.

According to Muro *et al.* (2015), it is expressed in the optic lobe during the larval and pupal phases, where it promotes programmed cell death for healthy neuronal development. In order to minimize unnecessary apoptosis, DrICE normally maintains its low baseline activity in an inactive zymogen form known as pro-DrICE. Inhibitors of apoptosis proteins (IAPs), such Diap1, carefully control its expression, guaranteeing that caspase activation only happens when necessary (Ribeiro *et al*., 2007). Under normal circumstances, DrICE expression in intestinal and tracheal cells promotes non-apoptotic processes, including endocytic trafficking during tracheal morphogenesis and immunological homeostasis maintenance through NF-κB signaling control (McSharry and Beitel, 2019; Kietz *et al.*, 2022).

DCP-1 shows more limited expression under normal settings than DrICE, with higher levels in certain tissues, especially the germline. During oogenesis, it is highly expressed in the ovary, supporting developmental activities, particularly in germaria and stage 8 egg chambers (FlyBase, 2025). In most somatic tissues, DCP-1 mRNA and protein are found, albeit at lower quantities than DrICE, in embryos and larvae. According to Muro *et al.* (2015), DCP-1 and DrICE are expressed together in the optic lobe, supporting redundant apoptotic processes during brain development. Under normal circumstances, DCP-1 is an inactive zymogen (pro-DCP-1), similar to DrICE. IAPs, particularly Bruce in the germline, inhibit its action. According to Hou *et al.* (2008), this guarantees regulated caspase activity for tissue maintenance without inducing cell death. Under normal circumstances, the ovary's production of DCP-1 promotes autophagy during oogenesis, especially in preserving cellular homeostasis. Even when there is no stress, it still interacts with mitochondrial proteins like SesB to control mitochondrial dynamics (DeVorkin *et al*., 2014).

2.14 Radiation-Induced Changes in DrICE mRNA Expression Level

1. Upregulation and Activation: In Drosophila embryos, larvae, and cell lines (such as S2 cells), ionizing radiation, such as gamma or UV, dramatically increases DrICE expression. Pro-apoptotic genes (reaper, hid, and grim) are activated by radiation and block Diap1, a crucial inhibitor of apoptosis protein (IAP). Increased quantities of DrICE mRNA and protein result from this, and pro-DrICE is then proteolytically cleaved into active p21 and p12 subunits, facilitating substrate cleavage and the execution of apoptosis. When DrICE is immunodepleted, radiation-induced apoptosis in S2 cell lysates is almost completely eliminated, demonstrating its crucial function in radiation-induced cell death. Studies reveal that DrICE activity rises within hours of irradiation, cleaving substrates such baculovirus p35 and lamin DmO.

2. Tissue-Specific Effects: Gamma irradiation increases the expression of DrICE in the developing optic lobe, especially in neuronal clusters, which mediates damage-induced apoptosis and corpse clearance. According to Muro *et al.* (2015), DrICE is specifically needed in some neuronal populations, where its activation guarantees the accurate removal of injured cells. UV irradiation causes DrICE expression in embryonic tissues such as the nervous system and epidermis, which aids in the elimination of radiation-damaged cells in the early stages of development (Ribeiro *et al*., 2007).

3. Mechanistic insights: The initiator caspase Dronc is activated by radiation, and it cleaves and activates DrICE in conjunction with the apoptosome component Dark. Feedback loops involving pro-apoptotic genes enhance this cascade, guaranteeing strong DrICE activation in

response to DNA damage (Xu *et al.,* 2006). According to quantitative research, DrICE activity increases in a dose-dependent manner after radiation, with stronger upregulation and quicker apoptosis occurring at higher doses (such as 40 Gy gamma radiation) (Muro *et al.*, 2015).

2.15 Radiation-Induced Changes in DCP-1 mRNA Expression Level

1. Upregulation and Secondary Role: Radiation mostly affects tissues where DCP-1 has a redundant function in apoptosis, causing DCP-1 expression to be induced to a lesser degree than DrICE. Gamma irradiation raises DCP-1 mRNA and protein levels in the optic lobe, but its secondary role in cell death is only noticeable when DrICE activity is decreased (Muro *et al.*, 2015). DCP-1 expression can be increased in the germline by UV or gamma irradiation, especially in germaria and egg chambers, where it promotes damaged cell death. According to Laundrie *et al*. (2003), this reaction is essential for preserving reproductive integrity under stress.

2. Activation Mechanism: After radiation-induced overexpression of reaper, hid, and grim, Dronc and Dark activate DCP-1, just like DrICE does. After the pro-DCP-1 zymogen is broken down into active p22 and p13 subunits, substrates such as poly(ADP-ribose) polymerase (PARP) can be cleaved. In most somatic tissues, DCP-1's activation is weaker than DrICE's, but it is more noticeable in the germline, where P-element disruptions of DCP-1 hinder radiation-induced apoptosis.

3. Tissue-Specific Effects: DCP-1's radiation-induced expression in the optic lobe promotes DrICE's redundant apoptotic actions, but it is optional in some neuronal clusters where DrICE is absolutely necessary (Muro *et al.,* 2015). Radiation-induced DCP-1 expression is less pronounced in larval tissues than DrICE, but it still plays a role in apoptosis in imaginal discs, especially when paired with other stressors (Xu *et al*., 2006).

2.16 Relevance of Radiation Study on genes in Drosophila for Human Health

Researching how radiation affects the effector caspase genes DrICE and DCP-1 in Drosophila melanogaster offers important insights into human health, especially in relation to apoptosis, DNA damage responses, and associated disorders. Because of its simpler caspase system, conserved molecular pathways, and genetic tractability—all of which mimic human caspase functions — Drosophila is a potent model organism. Among its implications for human health are;

1. Conserved Apoptotic Pathways:

a) Human effector caspases, including caspase-3 and caspase-7, which are essential for apoptosis in response to radiation-induced DNA damage, are similar to DrICE and DCP-1 (Xu *et al.*, 2006). Similar to how p53 and BCL-2 family proteins activate caspase-3/7 in humans, radiation upregulates DrICE and DCP-1 in Drosophila by activating pro-apoptotic genes (reaper, hid, grim) and releasing IAP inhibition (Muro *et al*., 2015). Knowing these Drosophila systems helps to clarify conserved pathways in human cells.

b) DNA Damage Response: When radiation damages DNA, apoptosis is triggered to get rid of the damaged cells. Similar to caspase-3's dominance in human cells, studies conducted on Drosophila demonstrate that DrICE is the main effector caspase in this reaction, with DCP-1 having a redundant function (Muro *et al*., 2015). When humans are exposed to radiation, like during cancer radiotherapy, this parallelism aids in identifying molecular targets for regulating apoptosis.

2. Cancer Research and Radiotherapy:

a) Apoptosis in Cancer Cells: Radiation is a key component of cancer therapy because it causes tumor cells to undergo apoptosis. Studies in Drosophila provide light on the dynamics of caspase activation by demonstrating how DrICE and DCP-1 drive radiation-induced cell death. Drosophila models aid in identifying variables (such as IAPs) that affect caspase activity, which informs methods to improve the efficacy of radiation. Dysregulation of caspase-3/7 in human tumors can result in radioresistance (Ribeiro *et al*., 2007).

b) Tumor Suppression: Similar to human cancers where caspase deficits increase tumor survival, DCP-1 mutations in Drosophila result in melanotic tumors because they inhibit apoptosis. Treatment results can be improved by identifying methods to restore apoptosis in human cancers through research on radiation-induced caspase activation in Drosophila.

3. Radiation-Induced Tissue Damage:

a) a) Normal Tissue Protection: Radiation therapy frequently causes adverse consequences by damaging healthy tissues. According to Drosophila research, DrICE reduces inflammation by mediating corpse clearance after radiation (Muro et al.,

2015). Radiation toxicity in humans can be made worse by excessive caspase activation in healthy tissues. By modifying caspase activity, Drosophila insights may direct the creation of treatments that save healthy tissues (Kietz *et al.,* 2022).

b) Neuroprotection: DCP-1 has a supporting function in the Drosophila optic lobe, whereas radiation-induced DrICE activation guarantees accurate neuronal pruning (Muro *et al.,* 2015). For patients receiving brain radiation therapy, comparable processes in human neural tissues are essential for reducing radiation-induced neurotoxicity.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out at the University of Benin (UNIBEN), located in Benin City, Edo State, Nigeria. Founded in 1970, UNIBEN is a well-known public research university, widely respected throughout Nigeria.

3.2 Study Location

The research was carried out in a specialized laboratory designed for rearing and studying *Drosophila melanogaster*, equipped with suitable infrastructure and tools. The facility, known as the Biomedical Toxicology Chemicals Safety (BIOTOXCS) Research Laboratory, Central Biomedical Research is located at the University of Benin (UNIBEN), Benin City, Edo State, Nigeria. The laboratory's controlled environment ensures optimal conditions for maintaining the flies and performing the experiments.

3.3 Study Population

In this research, Drosophila melanogaster served as the model organism, sourced from the Drosophila Laboratory at the University of Ibadan, Department of Biochemistry, Oyo State, Nigeria. Prior to initiating feeding and transfer protocols, the flies were allowed to undergo an acclimatization period.

3.4 Feed Formulation and Handling of *Drosophila melanogaster*

The flies were substaind on a standard cornmeal-based diet, composed of cornmeal (52 g), brewer’s yeast (5 g), glucose (0.5 g), agar (7.9 g), nipargin (1 g), ethanol (2 ml), and distilled water (850 ml). The water used to prepare the meal was distilled water. The flies were

randomly picked from vials with 1-2days old flies. Care was taken when handling the flies to avoid excape and death of flies.

3.5 Procedure for Meal Preparation

To prepare the culture medium for Drosophila melanogaster, the following steps are meticulously followed to achieve optimal nutrient content and consistency:

1. Dissolve 52g of cornmeal in 150 ml of room-temperature water, stirring to ensure no clumps form. Separately, dissolve brewer's yeast in a small volume of hot water to facilitate mixing.

2. Boil 700ml of water, add agar to boiling water and let it stand for about 10 minutes to fully hydrate and activate.

3. Gradually incorporate the pre-dissolved cornmeal into the boiling agar solution, stirring constantly to maintain a smooth texture.

4. Continue stirring the mixture for 5–10 minutes to achieve a uniform consistency.

5. Add the dissolved yeast and 3.5 g of glucose to the mixture, stirring thoroughly to combine all components.

6. Allow the mixture to simmer for an additional 15–20 minutes to ensure proper integration of ingredients.

7. Dissolve Nipagin in 1–2 ml of absolute ethanol to create a preservative solution.

8. Remove the main mixture from heat, let it cool slightly, and slowly add the Nipagin solution while stirring continuously for even distribution.

9. Pour the prepared medium into vials, ensuring it retains a semi-solid, flowable consistency during transfer.

10. Allow the medium to set, forming a firm yet pliable substrate suitable for Drosophila melanogaster culture.

3.6 Experimental Design

The experiment employs *Drosophila melanogaster* to investigate the impact of diagnostic radiation and X-rays. Five groups of flies were studied in separate vials.

Group 1: Control group

Group 2: Exposed to X- Ray for 7days

Group 3: Exposed to X-ray for 14days

Group 4: Exposed to CT for 7days

Group 5 : Exposed to CT for 14days

Group 1(the control) was housed under standard laboratory conditions without radiation exposure, group 2 was placed in an X-ray room for seven days, group 3 was placed in the X-ray room for fourteen days, group 4 was placed in the computer tomography (CT) room for seven days, group 5 was placed in the CT room for fourteen days.The flies were not directly exposed to the X-ray beam but experienced indirect exposure to ambient radiation in the diagnostic imaging room. The study ensured an evaluation of the effect of radiation on the apoptotic genes; DrICE and DCP-1 in *Drosophila melanogaster*. To ensure consistency, all groups were maintained under identical environmental conditions. Following the exposure periods, the flies were evaluated for changes in growth, survival, reproduction, and biochemical markers.

3.7 Laboratory Assay

3.7.1 Experiment 1: DrICE and DCP_1 mRNA Assay

3.7.2 RNA Extraction and Semi-Quantitative Polymerase chain reaction (PCR)

The experimental procedure began with extracting RNA from the flies using Trizol Reagent, a common chemical for RNA isolation, which was carried out in an Eppendorf tube (ThermoFisher Scientific). This extracted RNA was then converted into complementary DNA (cDNA) through a process called reverse transcription, utilizing the ProtoScript II first-strand synthesis kit(New England BioLabs). Following this, the genes were amplified using polymerase chain reaction (PCR) with One Taq® 2X Master Mix (New England BioLabs).

Procedure

Wild-type Drosophila melanogaster (Harwich strain) was cultured on a standard cornmeal-agar medium at 25°C with a 12-hour light/dark cycle. Adult flies, aged 3–5 days post-eclosion, were selected for experiment. Following radiation exposure, flies from each group (Control, X-ray, and diagnostic Radiation) were briefly anesthetized on ice. For each time point, 20 flies were randomly chosen and pooled per replicate, with three biological replicates per group. The flies were quickly placed into RNase-free microcentrifuge tubes, flash-frozen in liquid nitrogen and stored at −80°C until RNA extraction. To prevent RNA degradation, handling time was minimized, and sterile, RNase-free tools were used. All samples were processed under identical environmental conditions to maintain consistency across time points and treatment groups. RNA extraction from *Drosophila melanogaster* typically involves isolating high-quality RNA for downstream applications like gene expression analysis.

To extract the RNA, the frozen flies were disrupted in a guanidinium-based lysis solution (e.g., TRIzol) using a sterile pestle or bead homogenizer. samples were kept chilled to protect RNA. Chloroform was added, it was mixed thoroughly and centrifuged at 4°C to isolate the RNA-containing aqueous phase. This phase was transfered to a fresh tube, RNA was precipitated with isopropanol, and centrifuged to form a pellet. The pellet was washed twice with 75% ethanol, air-dried briefly and dissolved in RNase-free water or TE buffer, optionally warming to facilitate resuspension. This method yields high-quality RNA for further analysis.

cDNA Synthesis

The cDNA synthesis was performed by reverse-transcribing 500–1000 ng of high-quality RNA into cDNA using a commercial kit, such as iScript, Bio-Rad, with oligo-dT and random hexamer primers, a method commonly applied in Drosophila studies. The reactions were carried out in a 20 µl volume, incubated at 42°C for 60 minutes, and the enzyme was inactivated at 85°C for 5 minutes. The resulting cDNA was diluted 1:10 to optimize the template concentration for subsequent qRT-PCR.

Primers

The primer sequence for the genes and the housekeeping gene are as follows;

DrICE Forward primer : 5'-CGAGATCGTGATCGAGGAG-3'

Reverse primer: 5'-GCTTGTCGTAGATGCGGTTC-3'

DCP-1 Forward primer: 5'-ATCGAGCACGAGATTGAGAC-3'

Reverse primer: 5'-CGTGTCCAGCTTCTTGATGT-3'

GAPDH Forward primer: 5'-CTCCCTGGAGAAGAGCTATGA-3'

Reverse primer: 5'-AGGAAGGAAGGCTGGAAGA-3'

Gene Amplification

The qRT-PCR reactions were prepared in 10 µl volumes, consisting of 5 µl SYBR Green p mix (e.g., Bio-Rad iTaq), 0.3 µM of each primer, and 1 µl of diluted cDNA. The annealing temperature was optimized between 58–62°C, and primer concentrations were adjusted from 0.2–0.4 µM to achieve amplification efficiencies of 90–110%, which were calculated using standard curves generated from serial cDNA dilutions. The reactions were run in triplicate on a qRT-PCR system (e.g., Bio-Rad CFX) with cycling conditions set at 95°C for 3 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 20 seconds, and 72°C for 20 seconds, and concluded with a melt curve analysis from 65–95°C at 0.5°C increments.

Gel Electrophoresis

The gel electrophoresis process was carried out by initially preparing a 1–2% agarose gel mixed with an appropriate amount of ethidium bromide or a safe DNA stain in TAE or TBE buffer to enable DNA visualization. The gel was poured into a casting tray with a comb inserted to form wells, then allowed to solidify for approximately 20–30 minutes at room temperature. Following solidification, the comb was removed, and the gel was placed in an electrophoresis chamber filled with the same buffer. DNA samples, combined with a loading dye, were carefully loaded into the wells, alongside a DNA ladder for size reference. Electrophoresis was performed by applying a voltage of 80–120 V for 30–60 minutes, depending on the gel size and desired separation, until the dye front migrated an adequate distance. The gel was then visualized under UV light using a transilluminator to observe the DNA bands, and images were captured for analysis.

Statistical Analysis

The statistical data from this study were presented and evaluated using GraphPad Prism 8.0 software. Analysis of Variance (ANOVA) was employed to compare differences among the

treatment groups (California, USA). When significant differences were detected in continuous variables, a Tukey HSD post hoc test was conducted. Bar charts were utilized to depict the mRNA gene expression patterns identified in the ANOVA results. Barcharts where used to represent the gene expression patterns, a p-value below 0.05 was deemed statistically significant. Error bar presented shows mean ± standard error of mean.

CHAPTER FOUR

RESULTS

Figure 4.1. shows the PCR and agarose gel analysis expression pattern of DrICE mRNA. In this study, DrICE mRNA was analyzed in *Drosophila melanogaster* exposed to radiation at different duration. There was a significant increase ($p<0.001$) in all groups; X ray exposure (7 days and 14days), CT room exposure (7days and 14days) compared to the control. Also,

there was a significant decrease ($p<0.01$) in CT room exposure (14days) compared to X ray exposure (14days) and CT room exposure (7 days).

Figure 4.2. shows the PCR and agarose gel analysis expression pattern of DCP-1 mRNA. In this study, DCP-1 mRNA was analyzed in Drosophila melanogaster exposed to radiation at different duration. Also, there was no significant difference ($p>0.05$) in DCP-1 mRNA expression in Drosophila melanogaster exposed to X ray (7days and 14days) when compared to the control. There was no significant difference ($p>0.05$) in X ray exposure (14days) compared to CT room exposure (7days). There was a significant decrease ($p<0.05$) in CT room exposure (7days) compared to the control and X ray exposure (7days). There was also a significant decrease ($p<0.01$) in CT room exposure (14days) compared to the control, X ray exposure (7days) and X ray exposure (14days)

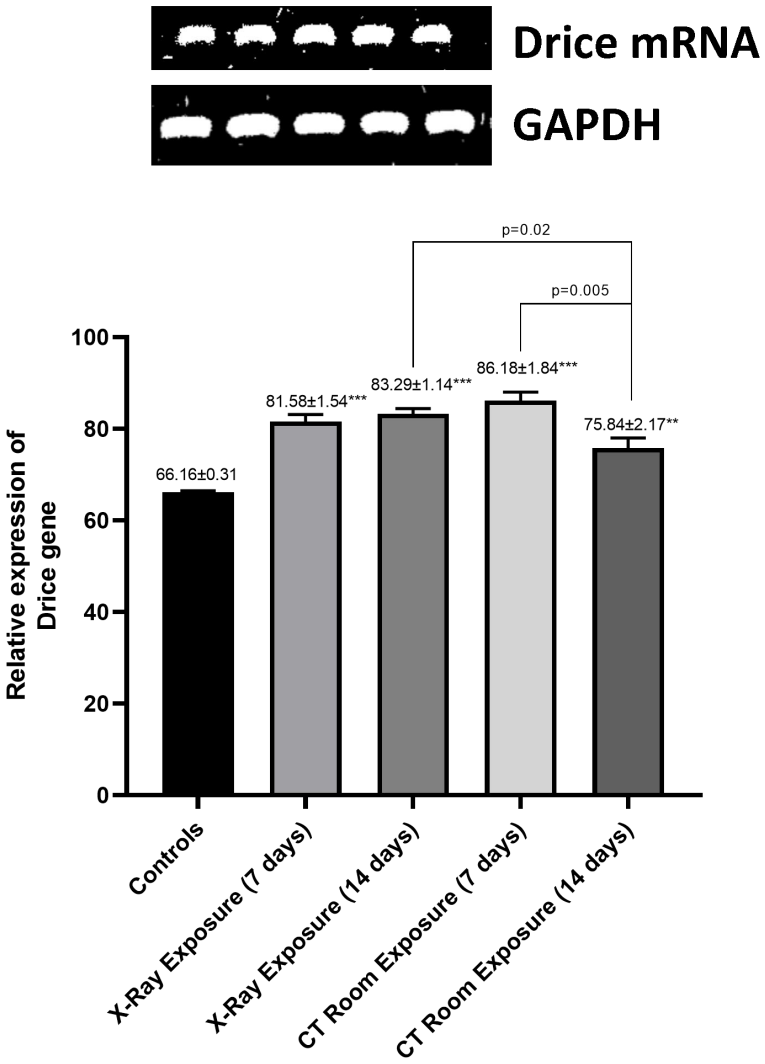

Figure 4.1: PCR and agarose gel analysis of DrICE mRNA from *Drosophila melanogaster* exposed to diagnostic radiations at different duration. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, **p<0.01, ***p<0.001)

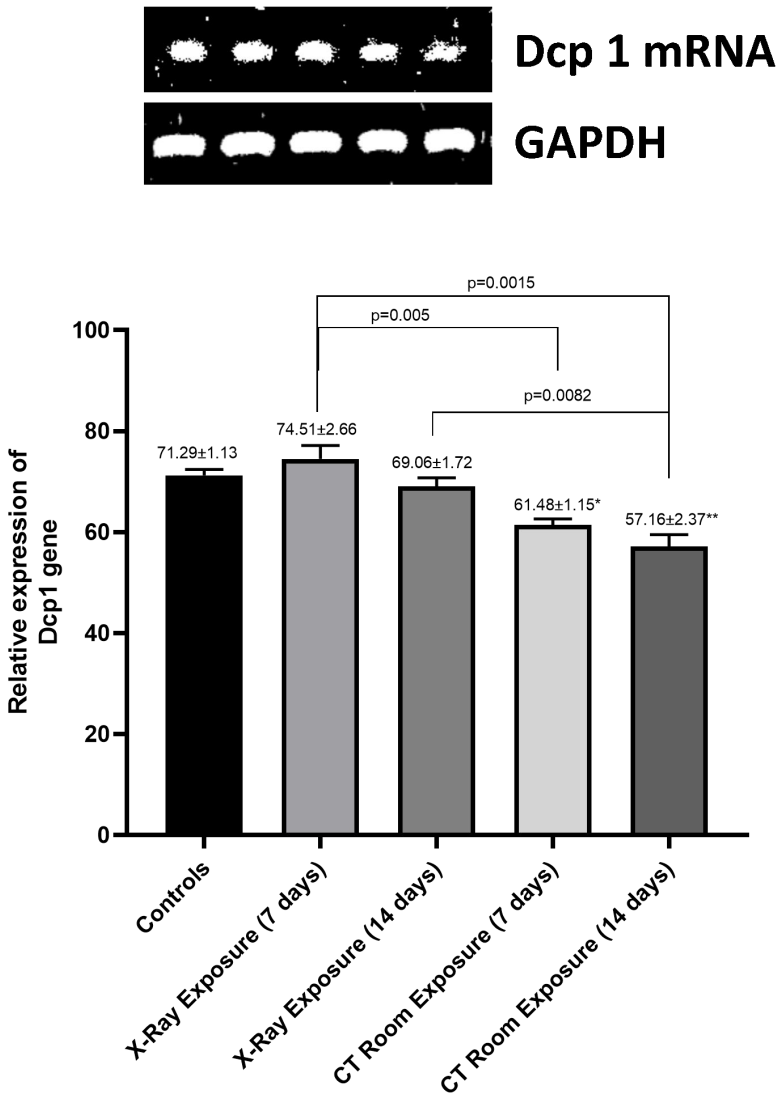

Figure 4.2: PCR and agarose gel analysis of DCP-1 mRNA from *Drosophila melanogaster* exposed to diagnostic radiations at different duration. Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, **p<0.01, ***p<0.001)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Many medical and research settings use diagnostic radiation, such as computed tomography (CT) scans and X-rays, which expose organisms to ionizing radiation that damages DNA and produces reactive oxygen species (ROS) (Li *et al.*, 2016). Such radiation can alter the expression of genes, especially those related to apoptosis, a conserved mechanism for destroying damaged cells, in Drosophila melanogaster, a model organism used to research biological responses. Because of their functions in preserving tissue homeostasis under stress, DrICE and Dcp-1 — effector caspases essential to the execution of programmed cell death downstream of initiator caspases — are particularly noteworthy among them. Apoptosis, corpse clearance, and autophagy are all impacted by changes in the DrICE and DCP-1 genes, which encode effector caspases in Drosophila melanogaster. Due to redundancy with DCP-1, mutations that decrease DrICE expression (e.g., drICE17) partially reduce apoptosis, leaving residual cell death (Li *et al*., 2006). DrICE and DCP-1 double mutants show roles specific to cell types: While either caspase is sufficient for type II cells, DrICE is essential for type I cell death (Mendoza *et al*., 2015). Egg chamber removal is disrupted by DCP-1 null mutants, which also affect germline apoptosis during starvation-induced oogenesis. Genetic interactors can alter the apoptotic effects of DCP-1 overexpression in the eyes (Kamber Kaya *et al*., 2010). Due to impaired phagocytic signaling, DrICE or DCP-1 mutations in optic lobe development cause glia to take longer to eliminate corpses (Mendoza *et al.*, 2015). According to DeVorkin *et al*. (2014), DCP-1 mutations also decrease autophagic flow by failing to cleave SesB, which raises ATP and prevents

autophagy. SesB reduction can restore autophagy. DrICE causes more pronounced apoptotic effects than DCP-1, and these changes interfere with embryonic processes like as neurogenesis and oogenesis. This work examines how acute (X-ray) and chronic (CT room) exposures over 7 and 14 days affect these apoptotic regulators by examining the impact of diagnostic radiation on the mRNA expression patterns of DrICE and Dcp-1.

The apoptotic pathway in Drosophila melanogaster appears to be more activated in response to diagnostic radiation, as evidenced by the significant increase in DrICE mRNA expression ($p<0.001$) seen in all groups — X-ray exposure (7 and 14 days) and CT room exposure (7 and 14 days) — when compared to the control. This upregulation most likely represents a cellular stress response in which the effector caspase DrICE is triggered to carry out programmed cell death, possibly to destroy damaged cells and preserve tissue homeostasis after reactive oxygen species (ROS) generation or radiation-induced DNA damage (Li *et al.,* 2016). Even low-dose, chronic radiation (such as that found in a CT room) causes this response, as evidenced by the uniformity across exposure types and durations. This response may be triggered by conserved signaling pathways like JNK or p53 homologs that control caspase production under stress. Literature showing that ionizing radiation quickly increases apoptotic genes in Drosophila supports this conclusion. For example, Fogarty *et al*. (2015) found that DrICE mRNA is increased within 6 hours of low-dose irradiation (0.5 Gy) in Drosophila optic lobes, promoting apoptosis to eliminate injured cells. Expression peaks early before stabilizing. This is consistent with the notable rise observed here, pointing to an early spike in DrICE transcription as a defense mechanism. In contrast to Fogarty *et al*. (2015), who found that expression returned to baseline within 24 hours, the current study's persistence of this increase over 14 days suggests that diagnostic radiation

may sustain DrICE upregulation for a longer period of time, perhaps as a result of cumulative stress or delayed feedback regulation.According to Ribeiro *et al.* (2007), the significant decrease in CT room exposure at 14 days ($p<0.01$) when compared to X-ray and 7-day CT exposure further suggests a time-dependent modulation, possibly reflecting an adaptive downregulation after initial activation. This pattern is consistent with hormetic responses, where sublethal doses enhance resilience. This dual response—an initial rise followed by a possible fall — emphasizes how DrICE is dynamically regulated in response to diagnostic radiation, indicating the need for more research into the molecular switches causing these changes. This effector caspase is downregulated in response to prolonged low-dose radiation in Drosophila melanogaster, as evidenced by the significant decrease in DrICE mRNA expression ($p<0.01$) in the 14-day CT room exposure compared to the control, 7-day CT room exposure, and 14-day X-ray exposure. This decrease most likely reflects an adaptive or compensatory mechanism in which, following an initial activation phase, prolonged exposure to ambient radiation (such as that found in a CT room) may reduce apoptotic signaling. In order to lessen the cumulative consequences of radiation-induced stress, this may indicate a switch from apoptosis to alternative survival mechanisms like autophagy or tissue repair, which may be regulated by feedback inhibition through ROS or JNK signaling pathways (Ribeiro *et al*., 2007). A dose-rate impact is highlighted by the contrast with X-ray exposure at 14 days, which sustains greater expression. Acute X-ray doses maintain apoptotic preparedness, but chronic CT exposure gradually dampens the response. Literature examining the long-term impact of radiation on gene expression supports this conclusion. According to Jiao *et al*. (2022), Ephestia elutella exposed to low-dose X-ray radiation for an extended period of time showed transgenerational resilience and decreased apoptotic activity in later stages. This suggests that prolonged exposure may downregulate

effector caspases like DrICE to improve reproductive fitness and survival. This is consistent with the current finding, which indicates a notable decrease following a 14-day CT room exposure. This could suggest adaptive suppression to avoid excessive cell death. A biphasic response, in which early apoptosis is followed by a protective downregulation, is further supported by the first increase at 7 days and the subsequent drop at 14 days.

This effector caspase's transcriptional levels in Drosophila melanogaster are not significantly changed by acute diagnostic X-ray radiation during the observed time period, as evidenced by the lack of a significant difference ($p>0.05$) in Dcp-1 mRNA expression between the control and X-ray exposure at 7 and 14 days. Due to compensatory mechanisms or redundancy with other caspases, such as DrICE, which may preserve apoptotic function without necessitating significant mRNA upregulation, this stability may suggest that Dcp-1 expression is tightly regulated under acute radiation stress (Amcheslavsky *et al*., 2019). In contrast to higher therapeutic doses, the lack of change may also be the result of a threshold effect, in which diagnostic doses (likely low, 0.1–1 Gy) are insufficient to elicit a robust transcriptional response in Dcp-1. This could be because of rapid protein turnover or activation of alternative survival pathways (Li et al., 2016). Conversely, some research emphasizes dynamic shifts in the expression of Dcp-1. Due to proapoptotic gene activation (e.g., reaper, hid), Song *et al*. (1997) found that Dcp-1 mRNA in Drosophila can rise within hours of irradiation, especially at higher doses, suggesting a dose-dependent response. Since therapeutic doses probably overpowered compensating mechanisms in Song *et al*. (1997)'s experimental circumstances, the current study's lack of substantial change may be due to the diagnostic X-rays' lower intensity. The possibility of post-transcriptional regulation in this instance is further supported by Chang *et al.* (2016), who observed

variable protein accumulation associated with apoptosis in Bactrocera dorsalis under X-ray exposure. This suggests that mRNA stability may not always correlate with protein activity. In contrast to the marked decline seen with CT room exposure, this stability in Dcp-1 mRNA under X-ray exposure suggests that Dcp-1 transcription may be suppressed by long-term low-dose radiation exposure, although acute exposure preserves baseline levels. Understanding radiation tolerance is affected by this, especially in applications such as the sterile insect approach for pest control, where viability after radiation may be supported by steady caspase expression. To ascertain whether Dcp-1 activity corresponds with its stable mRNA expression, future research could examine protein levels.

5.2 CONCLUSION

In Drosophila melanogaster, this study found that diagnostic radiation dramatically changes the expression of DrICE and DCP-1 mRNAs, with DrICE being upregulated and DCP-1 being downregulated over a range of exposure times. These results add to our understanding of caspase responses to cellular stress and demonstrate the intricate regulation of apoptosis under low-dose radiation. The downregulation of DCP-1 might point to a defense mechanism to balance cell death, whereas the elevation of DrICE implies increased apoptotic activity. These discoveries have practical significance for maximizing the safety of diagnostic radiation in medical practice as well as theoretical implications for apoptosis research.

5.3 RECOMMENDATION

Medical professionals should take into account the cumulative consequences of diagnostic radiation exposure, especially for patients who have imaging procedures often. Clinical evaluation of radiation-induced cellular stress may be aided by tracking apoptotic

biomarkers like caspase expression. Patient safety during diagnostic procedures may also be improved by the development of radioprotective drugs that control caspase activity. For healthcare professionals, wearing primary protection equipment (PPE) is also advised.

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APPENDIX I

MATERIALS AND REAGENTS USED

MATERIALS USED

Centrifuge

Forceps

Eppendorf container

Automated Pipette

Laboratory coast and rubber gloves

Digital weighing balance

Funnel and Whatman's fliter paper

Falcon tubes

Homegenizing stick

REAGENT USED

TriZol

Chloroform

Phosphate buffer

Primers used were synthesized by Inquaba Biotec, South Africa

Zymo DNA extraction kit

Loading dye

EZ-Vision

TBE buffer

Nuclease free water

Agarose

All purchased from Inquaba Biotec

APPENDIX II