ASSESSMENT OF mRNA IN *Drosophila melanogaster* ENVIRONMENTAL EXPOSED TO DIAGNOSTIC RADIATION THERAPY

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UNIVERSITY OF BENIN
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THIS PROJECT IS SUBMITTED TO:
THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
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SUPERVISOR:

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OCTOBER, 2025

CERTIFICATION

This is to certify that this project work was satisfactory carried out by **OSHODIN PEACE OSARIEMEN (MISS)** with matriculation number: **BMS2001200** in Department of Medical Laboratory Science, University of Benin, Benin City, under my supervision in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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DEDICATION

I dedicate this project work to God Almighty, for making this work a great success, to my lovely parent **MR AND MRS OSHODIN** and family member for their constant support throughout the process.

ACKNOWLEDGMENT

With a heart full of gratitude, I first give all glory and thanks to GOD ALMIGHTY for His unfailing love, guidance, and provision, which has carried me through every stage of this project.

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ABSTRACT

Ionizing radiation from diagnostic imaging procedures has raised concerns regarding its potential biological effects, even at low doses. This study investigated the impact of diagnostic radiation exposure on mRNA expression of p53 and DIAP1 in *Drosophila melanogaster*. Healthy laboratory-reared flies (Harwich strain) were divided into five groups (n=100 per group): a control group (Group A), two X-ray–exposed groups (Group B, 0.1 Gy daily for 7 days; Group C, 0.1 Gy daily for 14 days), and two CT-exposed groups (Group D, 0.1 Gy daily for 7 days; Group E, 0.1 Gy daily for 14 days). Radiation exposure was performed under standardized laboratory conditions at the Biomedical Toxicology Chemicals Safety (BIOTOXCS) Research Laboratory, University of Benin. After exposure, flies were preserved in Trizol reagent for RNA extraction, followed by cDNA synthesis and semi-quantitative PCR analysis. Results showed a significant upregulation of p53 and DIAP1 mRNA expression in radiation-exposed groups compared to the control ($p<0.05$). Expression levels were dose- and duration-dependent, with Groups C and E (14-day exposures) exhibiting the highest increases. Gel electrophoresis and bar chart analyses further confirmed these findings, demonstrating progressive changes consistent with prolonged radiation stress. These results indicate that even low-dose diagnostic radiation can induce molecular alterations in stress- and apoptosis-related genes in *Drosophila melanogaster*. The study underscores the utility of *Drosophila* as a model organism for assessing radiation-induced genetic responses and highlights the need for further research into the implications of cumulative diagnostic radiation exposure.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Ionizing radiation such as X-rays and other other diagnosticradiation can cause significant genetic damage, leading to disruptions in cellular homeostasis and, ultimately, programmed cell death (Liu *et al*., 2022). In Drosophila, exposure to such radiation activates the Dp53 protein, a functional equivalent of the human tumor suppressor p53, which coordinates multiple cellular defense mechanisms (Proshkina *et al*., 2020). These include the activation of genes involved in apoptosis, such as reaper, hid, and grim, which dismantle protective mechanisms like DIAP1 (Xu *et al*., 2009). DIAP1, or Drosophila Inhibitor of Apoptosis Protein 1, is a critical protein that keeps cell death in check by inhibiting caspase enzymes that mediate apoptosis (Betz *et al*., 2008). However, after radiation exposure, Dp53 induces transcription of pro-apoptotic genes that directly target and suppress DIAP1, tilting the balance toward cell death (Bilak *et al*., 2014). While Dp53 generally promotes apoptosis, it also plays non-lethal roles depending on the tissue type and developmental stage, indicating its functional versatility (Ashkenazi, 2017). Interestingly, studies have shown that flies lacking functional Dp53 are more resistant to radiation-induced apoptosis, supporting its central role in mediating cell death following genotoxic stress (Sogame, 2005). On the opposite end, DIAP1 levels are tightly regulated by multiple factors beyond Dp53, including the transcription factor STAT92E, which enhances DIAP1 expression to counteract radiation damage (Betz *et al*., 2008). Research on radiation-induced loss of heterozygosity (LOH) in flies suggests that survival often depends on the interplay between anti-apoptotic signals like DIAP1 and pro-apoptotic cues from surrounding

cells (Brown and Su, 2024). If DIAP1 is suppressed, uncontrolled apoptosis occurs, especially in rapidly dividing tissues like imaginal discs (van Bergeijk *et al*., 2012). Transcriptomic analyses following X-ray exposure in Drosophila have revealed widespread gene expression shifts, highlighting upregulation of stress and DNA repair pathways, many of which are governed by Dp53 or interact with it (Liu *et al*., 2022). Genes such as E2F1, ATM, and JNK have also been found to intersect with DIAP1 pathways, influencing survival outcomes (Su *et al*., 2014). In particular, high-dose radiation induces the expression of DIAP1 repressors, rapidly triggering apoptosis unless the anti-apoptotic balance is restored (Hasan, 2017).

A study by Bilak and colleagues in 2014, highlighted an intriguing mechanism in which dying cells release protective signals that help neighboring cells upregulate DIAP1, thus ensuring tissue survival after irradiation (Bilak *et al*., 2014). This finding points to a communal defense strategy among cells, where radiation stress doesn't only act on the damaged cell but affects tissue-wide response. Understanding how Dp53 and DIAP1 function under radiation stress in Drosophila provides a conceptual framework for tackling radioresistance in human cancers, especially those lacking functional p53 (van Bergeijk *et al*., 2012). Experimental knockdowns of Dp53 and DIAP1 in fruit flies have shown synergistic effects, suggesting potential other diagnosticangles for radiosensitization strategies (Ashkenazi, 2017). Moreover, fractionated radiation therapy experiments in Drosophila show that cellular response varies significantly depending on genetic background and chromosomal context, reinforcing the complexity of radiation biology and gene-environment interactions (Cruz, 2024). Collectively, these insights confirm the value of Drosophila melanogaster as a model for dissecting the genetic and molecular dynamics behind radiation-induced apoptosis, particularly involving p53 and DIAP1 pathways.

1.2 Statement of Problem

Ionizing radiation, including diagnostic X-rays and other other diagnosticradiation, is widely employed in modern medicine for both imaging and cancer treatment (Liu *et al*., 2022), but it carries the risk of inducing serious biological effects such as DNA damage, oxidative stress, and apoptosis (Proshkina *et al*., 2020). Among the genetic pathways activated in response to radiation exposure, the tumor suppressor gene P53 plays a pivotal role in initiating DNA damage checkpoints and regulating apoptosis through transcriptional control of pro-apoptotic genes (Xu *et al*., 2009). In Drosophila melanogaster, the homologous gene Dp53 mediates these responses via activation of key effectors such as reaper, hid, and grim, ultimately leading to caspase activation and cell death (Bilak *et al*., 2014). Counterbalancing this pro-apoptotic cascade is DIAP1 (Drosophila Inhibitor of Apoptosis Protein 1), a crucial anti-apoptotic protein that inhibits caspases and ensures cell survival under normal and stress-induced conditions (Betz *et al*., 2008). Following exposure to ionizing radiation, Dp53 transcriptionally represses DIAP1 through upregulation of its inhibitors, thereby tipping the cellular balance toward apoptosis (Wichmann *et al*., 2006). However, this tightly regulated interplay between P53 and DIAP1 is not yet fully understood, particularly under different types and doses of radiation, such as the distinction between diagnostic X-rays and other diagnosticirradiation (Cruz, 2024). Although Drosophila melanogaster provides a genetically accessible and physiologically relevant model for studying radiation-induced cell death (Brown and Su, 2024), most existing studies have examined either P53 or DIAP1 in isolation, rather than exploring their dynamic interaction across different radiation conditions (Hasan, 2017). Furthermore, the effects of other diagnosticradiation doses akin to those used in clinical oncology on the P53–DIAP1 axis remain underexplored in Drosophila, especially in terms of gene expression timing, caspase regulation,

and tissue-specific responses (Liu *et al*., 2022). Recent findings indicate that in addition to directly triggering apoptosis, irradiated cells can influence neighboring cells via non-autonomous signaling that elevates DIAP1 levels as a protective mechanism (Bilak *et al*., 2014), but the regulatory feedback loops between this survival response and Dp53 activation are still unclear (van Bergeijk *et al*., 2012). Compounding this issue, studies show that Dp53 can function both as a promoter and suppressor of apoptosis depending on the developmental stage and cellular environment, suggesting the existence of context-dependent dual roles (Ashkenazi, 2017). Therefore, there exists a significant gap in understanding how P53 and DIAP1 coordinate to regulate apoptotic versus survival outcomes in response to different radiation exposures in Drosophila melanogaster (Brown and Su, 2024). This gap is particularly important as unraveling the balance between P53-mediated cell death and DIAP1-mediated cell survival could inform other diagnosticstrategies to modulate radiation sensitivity in both research and clinical contexts (van Bergeijk *et al*., 2012).

1.3 Justification of study

The selection of *Drosophila melanogaster* as a model organism is justified due to its conserved genetic pathways, particularly the apoptotic regulators p53 and DIAP1, which closely resemble mammalian systems (Akdemir, 2006). This model has been critical in elucidating radiation-induced apoptotic pathways due to its genetic tractability and well-characterized genome (Sogame, 2005). Exposure to ionizing radiation, such as X-rays, activates p53-independent and dependent pathways that orchestrate apoptosis and DNA repair, underscoring the gene's pivotal role in genomic stability (van Bergeijk *et al*., 2012). Studies demonstrate that ionizing radiation induces widespread gene expression changes, including in p53 mutants, implicating alternative apoptosis mechanisms (Liu *et al*., 2022). Importantly, the gene DIAP1, a known inhibitor of

apoptosis, is actively repressed by pro-apoptotic factors such as Rpr, Hid, and Grim following radiation exposure, establishing its role as a gatekeeper of cell survival (Wichmann *et al*., 2006). Recent findings suggest that DIAP1 can modulate radiation-induced loss of heterozygosity (LOH), with E2F1 enhancing and JNK suppressing this phenotype, highlighting the complex interplay of radiation-responsive genes (Brown and Su, 2024). Moreover, adaptive responses to low-dose ionizing radiation reveal a nuanced activation of DNA repair pathways, conditional on the genetic context and developmental stage (Koval *et al*., 2020). These insights not only validate the relevance of *Drosophila* in radiation biology but also offer translational value for other diagnosticstrategies targeting radiation resistance in human cancers (Brown and Su, 2023).

1.4 Aim of study

The study aimed to determine the expression pattern of p53 and DIAP1 in drosophila melanogaster exposed to environmental X-ray radiation and other forms of diagnostic radiation.

1.5 Specific Objectives

1. To analyze all samples for DNA analysis .
2. To evaluate the effect of X-ray and other diagnostic radiation on the expression of the apoptotic gene *P53* in *Drosophila melanogaster*.
3. To evaluate the effect of X-ray and other diagnostic radiation on the expression of the anti-apoptotic gene *DIAP1* in *Drosophila melanogaster*.

1.6 Research Hypothesis

1.6.1 Null Hypotheses (H_0)

1. X-ray and other diagnosticradiation have no significant effect on the survival rate of Drosophila melanogaster.
2. Radiation exposure does not significantly affect the developmental stages of D. melanogaster.
3. There is no correlation between radiation dose and activation or suppression of p53 and DIAP1 pathways in D. melanogaster.

1.6.2 Alternative Hypotheses (H_1)

1. X-ray and other diagnostic radiation significantly affect the survival rate of Drosophila melanogaster.
2. Radiation exposure significantly affects the developmental stages of D. melanogaster.
3. A significant dose-dependent relationship exists between radiation exposure and the regulation of p53 and DIAP1 pathways in *Drosophila melanogaster*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

2.1 Overview of Drosophila melanogaster

The fruit fly *Drosophila melanogaster* occupies diverse environmental niches globally (Atoki *et al*., 2025). Though it originally evolved in tropical regions of the Old World, human activity has led to its spread across nearly all temperate zones worldwide (Piper *et al*., 2018). Its distribution is primarily restricted by environmental temperature and the availability of moisture (Lee *et al*., 2015). Interestingly, the name *Drosophila* translates to "dew lover," reflecting the species' dependence on humid conditions (Piper *et al*., 2018). Temperature not only affects where these flies can live but also significantly impacts their developmental processes. Adults cannot survive in frigid areas such as high latitudes or elevations, where food is also scarce (Komatsu *et al*., 2010; Lee *et al*., 2015). In regions with cold winters, *Drosophila* often seeks refuge indoors, particularly in food-rich areas like fruit storage rooms or man-made shelters (Atoki *et al*., 2025; Seong *et al*., 2023). Like all Dipterans, *D. melanogaster* goes through a complete metamorphic cycle (Atoki *et al*., 2025). It possesses a body encased in chitin, segmented into three distinct regions, and supported by three pairs of jointed legs typical insect anatomy (Carnes *et al*., 2015). Reproduction occurs at a remarkable pace, with a single mating pair generating several hundred offspring in only a few weeks. Offspring attain sexual maturity in approximately seven days (Billeter *et al*., 2006; Seong *et al*., 2023). Eggs are laid directly onto fruit surfaces, where they hatch into larvae (maggots) that immediately begin consuming the fruit beneath them (Moulin *et al*., 2022; Amanullah *et al*., 2023). Males are characterized by small comb-like structures called sex combs on their forelegs, hypothesized to aid in mating. Yet, experimental removal of these combs has shown little to no effect on reproductive success (Billeter *et al*., 2006). Their

behavioral traits are relatively rudimentary; they are strongly drawn to food odors and will mate readily with any available mate of the opposite sex (Atoki *et al*., 2025; Seong *et al*., 2023).*Drosophila* senses its environment through sensitive body hairs that detect air flow, compound eyes attuned to subtle changes in light, and flight responses triggered by motion or shadows (Moulin *et al*., 2022). The flies also display phototaxis a natural tendency to move toward light sources. When cultured in a test tube, they consistently gravitate toward the side with greater light exposure (Moulin *et al*., 2022; Hill, 2024).As their name suggests, fruit flies primarily consume plant-based matter (Amanullah *et al*., 2023). Adult flies feed on decomposing fruits and vegetables, while females lay eggs on slightly ripened fruits. By the time the larvae emerge, the fruit has begun to ferment, serving as a nutritional substrate for the growing maggots (Amanullah *et al*., 2023). Due to their rapid reproductive cycles and feeding behavior, *D. melanogaster* is considered a significant agricultural pest in many parts of the world (Atoki *et al*., 2025). Under optimal conditions, the lifespan of *Drosophila* ranges from around 10 to 50 days (Carnes *et al*., 2015), with females capable of laying multiple batches of eggs during this brief period (Seong *et al*., 2023). This rapid cycle often results in fast population growth in domestic environments. As with development and distribution, temperature plays a central role in determining life expectancy (Komatsu *et al*., 2010). Genetically, *Drosophila* contains four chromosome pairs three autosomes and one sex chromosome pair (X/Y). The fourth chromosome is notably smaller and often overlooked except for the essential **eyeless** gene it carries (Carbone *et al*., 2016). The full genome, comprising roughly 139.5 million base pairs and around 15,682 genes, has been sequenced (Carnes *et al*., 2015). Surprisingly, more than 60% of this genome is non-coding but functionally vital for regulating gene expression (Carnes *et al*., 2015). Unlike human sex determination which hinges on the Y chromosome, *Drosophila* sex is

determined by the ratio of X chromosomes to autosomes (X:A) (Billeter *et al*., 2006). Despite being completely heterochromatic, the Y chromosome still houses at least 16 genes, most of which contribute to male-specific biological functions (Billeter *et al*., 2006).

2.1.1 Genetic Structure and Apoptotic Pathways in *Drosophila melanogaster*

The genomic architecture of *Drosophila melanogaster* is both compact and evolutionarily conserved, comprising four chromosomal pairs three autosomes and one pair of sex chromosomes (X and Y) (Cashio *et al*., 2005). Its genome, which has been fully sequenced, includes about 139.5 million base pairs and contains nearly 15,000 protein-coding genes, rendering *Drosophila* a powerful model system for investigating genetic regulation, development, and cell death mechanisms (Carnes *et al*., 2015). Programmed cell death, or apoptosis, plays a vital role in maintaining healthy tissue formation, immune function, and the removal of damaged or defective cells in this organism. Many of the genes and signaling networks responsible for apoptosis in *Drosophila* are evolutionarily conserved and similar to those found in mammals, including the core caspase enzymes and members of the Bcl-2 protein family (Steller, 2008). Critical pro-apoptotic genes such as *reaper (rpr)*, *head involution defective (hid)*, and *grim* promote cell death by blocking DIAP1 (Drosophila Inhibitor of Apoptosis Protein 1), thereby triggering the activation of caspases (Xu *et al*., 2009). The fly genome also includes a functional equivalent of the human tumor suppressor gene *p53*, which regulates apoptosis in response to DNA injury. Upon exposure to ionizing radiation, *p53* activates transcription of genes like *reaper* and *hid*, initiating cell death in cells with genetic damage (Brodsky *et al*., 2004). This response is coordinated by the checkpoint kinase MNK/Chk2, which detects DNA anomalies and stimulates *p53* activity to preserve genome stability. Moreover, when telomeres become dysfunctional in *Drosophila* cells, both *p53*-

dependent and independent apoptotic processes are triggered, ensuring that genomically unstable cells are eliminated from tissues (Titen and Golic, 2008). This intricate network of redundant apoptosis mechanisms underscores the organism's resilience in regulating cell survival and death. Additionally, *Drosophila* produces a protein known as Deterin, a member of the inhibitor of apoptosis protein (IAP) family, which mirrors the function of mammalian Survivin. Deterin allows cells to resist apoptosis under typical physiological circumstances (Jones *et al*., 2000). The fine balance between cell death-promoting and -inhibiting factors is essential for normal development and responses to environmental stressors.

2.1.2 Use of *Drosophila melanogaster* as a Model Organism in Radiation Biology

Drosophila melanogaster has been an indispensable model organism in the field of radiation biology, thanks to its well-characterized genome, brief generational cycle, and evolutionarily conserved DNA repair systems (Sekelsky, 2017). Its genetic tractability allows researchers to unravel intricate cellular processes activated by ionizing radiation (IR), including the repair of DNA lesions, regulation of cell cycle progression, and the initiation of apoptosis. Upon exposure to IR, *Drosophila* engages stress signaling pathways similar to those found in mammals, particularly the ATM/Chk2/p53 cascade (Shim *et al*., 2014). In this system, the p53 transcription factor plays a central role by upregulating genes that promote apoptosis in response to DNA damage, leading to targeted cell death in affected tissues (Brodsky *et al*., 2004). Interestingly, *Drosophila* also displays apoptotic responses independent of p53, indicating the presence of backup mechanisms that help preserve genome stability even in the absence of this critical regulator (Wichmann *et al*., 2006). Experimental models involving larvae with mutations in DNA repair genes such as *dATM* have shown heightened sensitivity to radiation, reinforcing the fruit fly's utility in probing gene-specific radiation responses and radiosensitivity (Song *et al*.,

2004). Moreover, investigations into low-dose IR exposure have revealed adaptive responses in *Drosophila*, where pre-exposure to minimal radiation levels enhances resilience by modulating stress-related genetic pathways. These hormetic effects appear to vary depending on the organism's genetic background (Moskalev *et al*., 2011), making *Drosophila* a compelling model for exploring long-term and transgenerational consequences of radiation. Recent research by Toyoshima-Sasatani *et al*. (2023) has further advanced our understanding of how apoptosis and mutation dynamics are jointly orchestrated to mitigate radiation toxicity. Their findings demonstrate that programmed cell death can directly curb mutagenesis under radiation stress, thereby offering protective benefits. This tight coupling between genomic integrity and cellular sacrifice allows *Drosophila* to serve as a robust system for studying tissue-level outcomes of radiation, including halted egg development, morphogenetic defects, and compensatory cell proliferation (Shim *et al*., 2014).

2.1.3 Life Cycle and Developmental Stages Relevant to Radiation Response

The developmental cycle of *Drosophila melanogaster* is composed of four primary stages: embryo, larva, pupa, and adult, each characterized by unique physiological traits and varying levels of susceptibility to ionizing radiation (Paithankar *et al*., 2017). Embryonic stages tend to exhibit increased resistance to radiation, largely due to maternally inherited proteins and DNA repair components that provide early cellular protection (Koval *et al*., 2020). In contrast, the larval and pupal phases are notably more radiosensitive, a vulnerability linked to the elevated rates of cell division and tissue remodeling that occur during these stages (Paithankar *et al*., 2018).

Particularly in larvae, the imaginal discs precursors to adult structures undergo extensive mitotic activity, rendering them highly prone to radiation-induced genetic damage and programmed cell death during key developmental windows (Zhikrevetskaya *et al*., 2015). Gamma radiation at this stage prompts the expression of stress markers such as *hsp70* and *sod*, suggesting that oxidative stress plays a central role in mediating the biological effects of radiation (Zhikrevetskaya *et al*., 2015). In response, the larval genome activates key DNA repair and checkpoint genes, including *mei-41* and *tefu*, to repair double-strand DNA breaks and prevent lethal mitotic errors (Koval *et al*., 2020). Metamorphosis in the pupal stage involves widespread cellular differentiation and reorganization, making this period particularly sensitive to radiation even at sub-lethal doses, which can disrupt organ development and lead to morphological abnormalities in the emerging adult fly (Morciano *et al*., 2018). Sustained or chronic radiation exposure during this stage can cause broad systemic consequences, such as delayed development, lower survival rates, and lifespan alterations. These outcomes are often dependent on the fly's genetic makeup and strain-specific resilience (Paithankar *et al*., 2018). Adult *Drosophila*, by comparison, show greater resistance to radiation, in part because of their lower mitotic activity and enhanced capacity to engage stress response mechanisms (Landis *et al*., 2012). Nevertheless, radiation exposure in adults can still compromise fertility, increase oxidative damage, and disrupt expression patterns of genes associated with immunity and metabolism, especially in older individuals (Parashar *et al*., 2008).

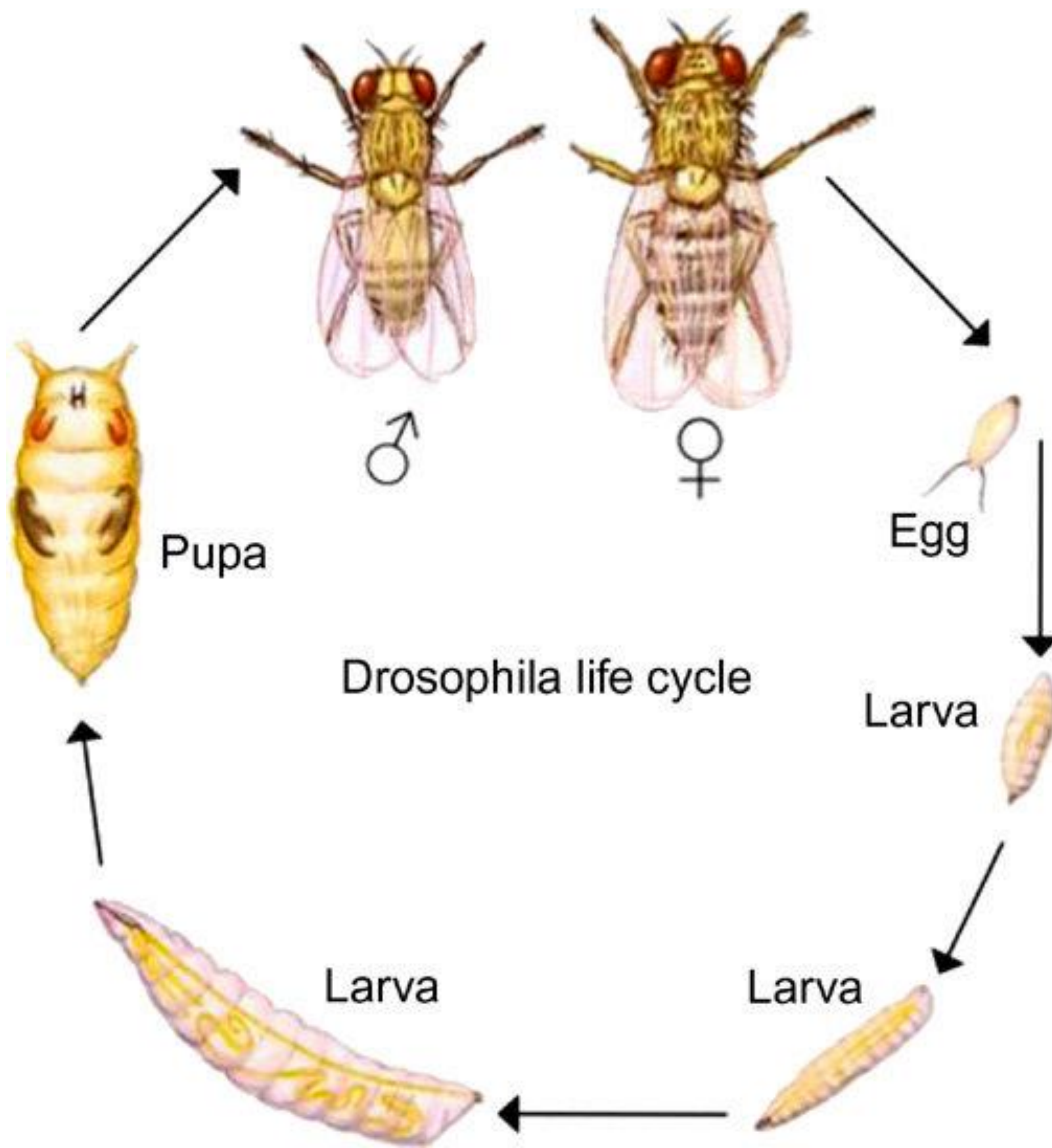


Figure 2.1. life cycle of *Drosophila melanogaster (*Lawson *et al.,* 2011).

2.2 X-ray and Therapeutic Radiation*:* Mechanisms of DNA Damage in *Drosophila melanogaster*

In the expanding domain of radiation biology, *Drosophila melanogaster* has become a key model organism for exploring how ionizing radiation especially X-rays and therapeutic forms impacts cellular and molecular processes (Toyoshima-Sasatani *et al*., 2023). Ionizing radiation, commonly encountered in clinical imaging and cancer treatment, inflicts genetic damage through ionization reactions and the production of reactive oxygen species (ROS), which compromise DNA stability and replication fidelity (Tanaka and Furuta, 2021). In multicellular systems like *Drosophila*, the genotoxic outcomes of such radiation are shaped by intertwined cellular responses, including DNA repair mechanisms, programmed cell death (apoptosis), and mutation fixation (Toyoshima-Sasatani *et al*., 2023). X-rays operate by releasing high-energy photons that penetrate tissues and disrupt the molecular bonds within DNA, resulting in critical lesions such as single-strand breaks (SSBs) and double-strand breaks (DSBs) (Nakajima *et al*., 2020). These breaks, especially DSBs, are highly cytotoxic because they affect both strands of the DNA double helix, posing a major challenge for precise repair (Sekelsky, 2017). To assess the mutagenic effects of such damage, researchers often employ the wing spot test in *Drosophila melanogaster*, a tool that reveals genetic alterations like recombination, chromosomal missegregation, and gene conversion all indicative of faulty or incomplete repair of radiation-induced damage (Toyoshima-Sasatani *et al*., 2023). Importantly, dose-dependent patterns have been observed between apoptosis rates and the emergence of mutation clones in irradiated wing discs, particularly following exposure to agents like methyl-nitrosourea (MNU), ultraviolet (UV) light, and X-rays. Using acridine orange staining and BrdU assays, Toyoshima-Sasatani *et al*. (2023) demonstrated that even low, sub-lethal doses of X-rays elevate both apoptosis and somatic mutation frequency. This co-occurrence suggests that cell death and mutagenesis are not

mutually exclusive processes, but rather are co-regulated as part of the organism's stress response. Moreover, the extent of this correlation appears to be influenced by genetic background. For instance, *Drosophila* mutants deficient in critical DNA repair pathways such as those involved in homologous recombination or base excision repair showed divergent relationships between cell death and mutation accumulation compared to wild-type strains (Toyoshima-Sasatani *et al*., 2023). This underscores the importance of intact repair systems not only in resolving damage but in determining whether a cell will self-destruct or continue proliferating with genomic alterations. Temporal dynamics also play a crucial role in radiation response. BrdU incorporation a marker of DNA synthesis was suppressed within six hours post-X-ray exposure, peaked by 12 hours, and showed partial recovery by 24 hours in wing imaginal discs (Toyoshima-Sasatani *et al*., 2023). These findings suggest that cells initially halt the cycle to facilitate DNA repair before reentering proliferation, potentially carrying unrepaired mutations forward. Interestingly, this temporal pattern was unique to X-ray exposure and was not mirrored following UV irradiation, implying radiation-type–specific cellular adaptations. One of the most striking discoveries in recent studies is the phenomenon described as "mutation-driven overgrowth following apoptosis." Under X-ray or MNU exposure, the size of mutant clones expanded in parallel with increased cell death, a pattern absent under UV treatment. This implies that when apoptotic cells are eliminated, nearby mutated cells with greater proliferative capacity can repopulate the tissue, resulting in clonal expansion (Toyoshima-Sasatani *et al*., 2023). Thus, a paradox emerges where apoptosis, typically a protective mechanism, may inadvertently enable the expansion of genomically compromised cells raising long-term risks for tissue integrity.

2.3 Role of the p53 Gene in DNA Damage Response in *Drosophila melanogaster*

In *Drosophila melanogaster*, the p53 gene encodes a transcription factor that is evolutionarily conserved and plays a pivotal role in orchestrating the cellular response to DNA damage. It is especially critical for promoting apoptosis and safeguarding genome stability following genotoxic stress, such as exposure to ionizing radiation (IR) (Brodsky *et al*., 2004). Upon encountering DNA damage, cells activate p53-dependent pathways that either facilitate DNA repair or trigger programmed cell death to prevent the proliferation of genetically compromised cells (Lee *et al*., 2003). Unlike mammals, which have multiple p53 family genes, *Drosophila* carries a single p53 gene that gives rise to distinct protein isoforms, most notably p53A and p53B, each with tissue-specific and functional specializations (Zhang *et al*., 2015). The p53A isoform is active mainly in somatic cells and is essential for responding to DNA damage in non-reproductive tissues, while p53B operates predominantly within the germline and regulates apoptosis during egg development (Park *et al*., 2019). Functional studies involving *Drosophila* mutants lacking p53 have revealed a failure to undergo apoptosis after IR exposure, emphasizing p53's essential role in mediating genotoxic stress responses (Lee *et al*., 2003). Upstream of p53, the checkpoint kinase Chk2 (also known as Loki) activates it upon DNA damage, forming a well-defined apoptotic signaling pathway: Chk2 → p53 → hid (Brodsky *et al*., 2004). This axis is especially prominent in dividing tissues like imaginal discs and germline stem cells, where the absence of either Chk2 or p53 leads to apoptosis resistance despite the presence of DNA lesions (Brodsky *et al*., 2004; Wichmann *et al*., 2006). Beyond its apoptotic role, *Drosophila* p53 contributes to preserving germline stem cell populations and ensuring reproductive capacity under stress. For example, after exposure to low-level radiation, flies deficient in p53 exhibit reduced egg production and a loss of stem cells, indicating a protective, non-lethal function of

p53 under moderate damage conditions (Park *et al*., 2019). The transcriptional targets of Drosophila p53 include critical pro-apoptotic genes such as *hid*, *reaper*, and *grim*. These effectors initiate apoptosis when DNA damage surpasses the cell's repair capacity (Brodsky *et al*., 2004; Liu *et al*., 2013). Although these targets are not sequence-identical to their mammalian counterparts, they fulfill comparable roles, illustrating the deep conservation of the p53-mediated death response across species (Song, 2005). Notably, the degree to which p53 drives apoptosis correlates with the severity of the DNA insult. Mild damage typically promotes repair mechanisms, while more severe damage activates caspase-dependent cell death pathways (Wichmann *et al*., 2006). Interestingly, extremely high radiation doses may trigger apoptosis that occurs independently of p53, suggesting the existence of alternative, compensatory pathways in the event of p53 dysfunction (Wichmann *et al*., 2006). More recent research has provided insights into isoform-specific functions. For instance, introducing p53A alone into p53-null flies restores egg-laying capability, while p53B fails to do so, pointing to a unique role for p53A in maintaining stem cell health that is distinct from its apoptotic activity (Park *et al*., 2019). These findings underscore that *Drosophila* p53 isoforms are functionally specialized and non-redundant. Additionally, p53 activity is modulated by various environmental stressors ranging from oxidative damage and nutrient stress to viral infections positioning it as a central integrator of stress-response networks (Liu *et al*., 2013). This regulatory flexibility makes *Drosophila* a powerful model organism for studying conserved pathways related to DNA repair, checkpoint regulation, apoptosis, and cellular resilience.

2.4 p53-Dependent and Independent Apoptotic Pathways p53-Dependent and Independent Apoptotic Pathways

2.4.1 p53-Dependent Apoptotic Pathways

In *Drosophila melanogaster*, apoptosis regulated by p53 plays a critical role in managing the cellular response to DNA damage, particularly after exposure to ionizing radiation (Brodsky *et al*., 2004). Following genotoxic stress, the p53 protein becomes activated via phosphorylation by the checkpoint kinase Chk2 (also known as *loki*), initiating a gene expression program that includes key pro-apoptotic genes such as *reaper*, *hid*, and *grim*. These genes function by inhibiting IAPs (Inhibitor of Apoptosis Proteins), leading to the activation of caspases and subsequent cell death (Akdemir *et al*., 2007). This mechanism closely parallels the apoptotic pathway controlled by p53 in vertebrates, though *Drosophila* possesses only a single p53 gene (Ingaramo *et al*., 2018). Further investigations have shown that p53-mediated apoptosis is tightly regulated in both space and time. As described by Park *et al*. (2019), specific developmental windows during oogenesis depend heavily on p53 signaling. Disruption of this pathway, such as through gene knockout, impairs the apoptotic response to IR and compromises the fidelity of germline development. In addition to responding to DNA damage, *Drosophila* p53 is also involved in broader stress response pathways, including reactions to oxidative stress and viral infection. These stimuli can likewise lead to the upregulation of *reaper* expression, further reinforcing p53's role in maintaining cellular integrity under various environmental conditions (Liu *et al*., 2013). Interestingly, recent studies suggest that p53 serves as a molecular decision-maker between apoptosis and autophagy, particularly in models of redox stress. In these contexts, p53 determines whether cells will initiate programmed death or enter a survival pathway via autophagy, demonstrating that its role extends beyond classical apoptotic functions

(Robin *et al*., 2019). This dual regulatory capacity reflects the gene's evolutionary adaptability in managing stress responses and preserving tissue homeostasis.

2.4.2 p53-Independent Apoptotic Pathways

While p53 serves as a key regulator of apoptosis in *Drosophila melanogaster*, evidence indicates that cell death can still proceed through p53-independent pathways, particularly under conditions of intense genotoxic stress or within specific cellular contexts. Notably, ionizing radiation has been shown to activate caspase-driven apoptosis even in mutant flies lacking functional p53 or Chk2, suggesting that alternative signaling routes are capable of compensating when the classical p53-dependent apoptotic cascade is disrupted (Wichmann *et al*., 2006). Genome-wide transcriptional analyses have uncovered distinct expression patterns associated with these non-canonical apoptotic responses, identifying sets of stress-inducible genes that are not under p53 regulation (van Bergeijk *et al*., 2012). This supports the notion that *Drosophila* possesses redundant apoptotic systems, an evolutionary strategy to maintain genome integrity particularly in cell types such as polyploid or endocycling cells, which bypass conventional mitotic checkpoints (Zhang *et al*., 2014). Earlier foundational research in mammalian models proposed that mitochondrial dysfunction, stress-activated kinases, and specific transcriptional repressors could facilitate p53-independent apoptosis (Shen and White, 2001; Benchimol, 2001). These insights have since been validated in *Drosophila*, where comparable alternative regulatory mechanisms have been documented, reinforcing the idea that multiple, overlapping pathways ensure the elimination of damaged cells even in the absence of p53 function.

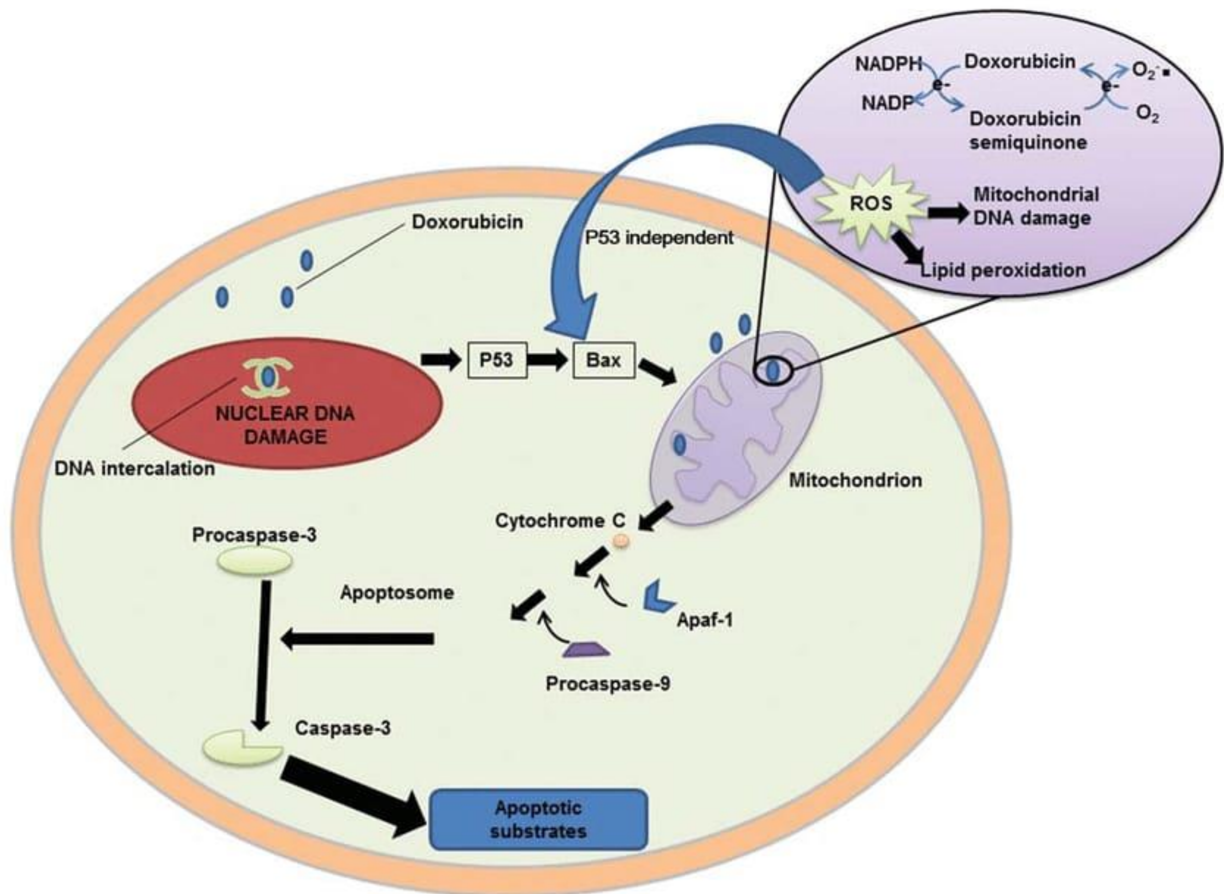


Figure. 2.2. Representation of p53 dependent and p53 independent apoptosis. (Spugnini *et al*., 2016)

2.5 Function and Regulation of DIAP1 in Apoptosis

Apoptosis in *Drosophila melanogaster* is tightly regulated by a network of molecular interactions that ensure proper development and homeostasis, with DIAP1 (Drosophila Inhibitor of Apoptosis Protein 1) playing a central role as a gatekeeper of caspase activation (Orme and Meier, 2009).

2.5.1 Function of DIAP1

In *Drosophila melanogaster*, DIAP1 functions as an E3 ubiquitin ligase that plays a central role in inhibiting apoptosis by directly binding to both initiator caspases like DRONC and effector caspases such as DRICE (Steller, 2008). This regulatory role is twofold DIAP1 not only physically blocks caspase activity but also enzymatically targets them for ubiquitination and subsequent degradation, effectively removing activated caspases before they can trigger downstream cell death processes (Hay, 2000). Without functional DIAP1, DRONC becomes active and initiates a proteolytic cascade that culminates in apoptosis (Meier *et al*., 2000). This caspase suppression is essential during development, where DIAP1 ensures cell survival. Notably, when DIAP1 is overexpressed, it can block apoptosis triggered by oxidative stress, such as that caused by the accumulation of reactive oxygen species (ROS), further emphasizing its protective function (Morey *et al*., 2003).

2.5.2 Regulation of DIAP1 by Pro-Apoptotic Proteins

The pro-apoptotic genes *reaper*, *hid*, and *grim* are central to *Drosophila*'s programmed cell death, functioning by counteracting the activity of DIAP1. These proteins bind to DIAP1 and facilitate its self-ubiquitination and degradation via the proteasome, thereby lifting the caspase inhibition that DIAP1 normally enforces and allowing apoptosis to proceed (Ryoo *et al*., 2002).

This regulatory mechanism represents a key post-translational control point in the fly's apoptotic pathway. The proteins encoded by the RHG gene cluster (Reaper, Hid, and Grim) share a conserved IAP-binding motif (IBM) that enables them to specifically engage with the BIR domains of DIAP1 a structural interaction essential for displacing its caspase-inhibitory effects (Hay, 2000). Crucially, the action of these RHG proteins is not constant but rather modulated in a spatially and temporally restricted manner, particularly during key developmental phases or in response to environmental insults such as ionizing radiation or oxidative stress (Khammari *et al*., 2011).

2.5.3 Ubiquitin-Proteasome Regulation of DIAP1 Stability

The stability of DIAP1 is tightly controlled by ubiquitination, which serves as a pivotal mechanism in regulating apoptosis in *Drosophila*. While DIAP1 promotes the ubiquitin-mediated degradation of caspases, it is also targeted for degradation through the same system when apoptotic signals are activated. Specifically, E3 ubiquitin ligases such as Ubr3 have been identified as factors that accelerate DIAP1 degradation, thereby promoting cell death when appropriate (Huang *et al*., 2014). Conversely, DIAP1 levels are preserved under normal physiological conditions by DUBAI, a deubiquitinating enzyme that removes polyubiquitin chains from DIAP1, preventing its breakdown and thus sustaining cell survival in the absence of stress (Yang *et al*., 2014). This interplay between ubiquitination and deubiquitination introduces a nuanced regulatory layer, fine-tuning apoptotic outcomes based on cellular context and environmental cues.

2.5.4 Post-Translational Modifications and Context-Dependent Roles

Post-translational regulation, including phosphorylation and proteolytic cleavage, plays a key role in modulating DIAP1 function. Under cellular stress, active caspases can cleave DIAP1, specifically targeting its RING finger domain, which impairs its E3 ubiquitin ligase activity and prevents it from marking caspases for degradation (Steller, 2008). This cleavage disrupts DIAP1's regulatory capacity and facilitates progression of the apoptotic pathway. Furthermore, DIAP1's activity is shaped by tissue-specific contexts. For example, within the anterior region of imaginal discs, ectopic expression of DIAP1 does not uniformly suppress apoptosis, indicating spatial variation in its functional impact (Morey *et al*., 2003). In some scenarios, DIAP1 can execute its anti-apoptotic role even in the absence of mitochondrial signaling, pointing to the limited involvement of cytochrome c in *Drosophila* apoptosis and reinforcing DIAP1’s central role in controlling cell death (Clavier *et al*., 2016).

2.6 Interaction Between p53 and DIAP1 Under Radiation Stress

The biological response to ionizing radiation is a finely tuned process that involves initiating cell cycle arrest, activating DNA repair pathways, and inducing apoptosis when the genetic damage exceeds the cell’s repair capacity (Colombani *et al*., 2006). In *Drosophila melanogaster*, two central regulators in this pathway are the transcription factor p53 and the apoptosis inhibitor DIAP1 (Drosophila Inhibitor of Apoptosis Protein 1), which functions to suppress caspase activity (Mollereau and Ma, 2014; Orme and Meier, 2009).

During exposure to cellular stressors like ionizing radiation, p53 and DIAP1 operate on opposite ends of the apoptotic spectrum. While p53 activates the transcription of genes that promote programmed cell death, DIAP1 works to restrain apoptosis by inhibiting caspase activation,

thereby ensuring that only cells with substantial, irreparable damage are eliminated (Hay, 2000). This dynamic interplay between p53 and DIAP1 becomes particularly vital under conditions of genotoxic stress, such as X-ray or gamma irradiation, where cells must swiftly and accurately determine whether to repair damage or undergo apoptosis (Colombani *et al*., 2006).

2.6.1 Molecular Overview of p53 and DIAP1

In *Drosophila melanogaster*, the Dmp53 gene encodes the p53 protein, which is swiftly activated in response to DNA damage particularly double-strand breaks caused by ionizing radiation (Mollereau and Ma, 2014). Upon activation, p53 binds to the promoter regions of several pro-apoptotic genes, most notably *reaper*, *hid*, and *grim* collectively known as the RHG genes which serve as powerful apoptosis inducers (Baonza *et al*., 2022). Rather than initiating cell death directly, the RHG proteins function by disabling DIAP1, an inhibitor of apoptosis, thereby releasing the suppression on caspases and allowing cell death to proceed (Steller, 2008). DIAP1, in contrast, plays a protective role under normal conditions by binding to and ubiquitinating key caspases like DRONC and DRICE, preventing their buildup and acting as a safeguard against untimely or excessive cell death (Hay, 2000; Yang *et al*., 2014). This regulatory interaction imposes a threshold mechanism, ensuring apoptosis is only triggered when genuinely needed.

The critical control point in this system is the dynamic equilibrium between p53 activation and DIAP1 availability. Under environmental stressors such as ionizing radiation, this balance shifts in favor of apoptosis, as p53 activity rises and DIAP1-mediated inhibition is overcome, directing damaged cells toward programmed cell death (Morey *et al*., 2003).

2.6.2 Radiation Stress and the DNA Damage Response

When *Drosophila* cells are exposed to ionizing radiation, they initiate a DNA Damage Response (DDR) that is evolutionarily conserved across species. This response involves the activation of ATM and ATR kinases, which phosphorylate and stabilize p53, enhancing its activity as a transcription factor (Liu *et al*., 2022). In its activated form, p53 upregulates pro-apoptotic genes such as *reaper*, *hid*, and *grim* (RHG), which in turn inhibit DIAP1, the key suppressor of caspase activity. This leads to the accumulation of cleaved caspases and the execution of apoptosis (Moon *et al*., 2008; Colombani *et al*., 2006). The sensitivity of this pathway to radiation dosage is a notable feature. At lower radiation doses, the amount of DIAP1 often remains adequate to suppress caspase activation, even if RHG genes are being transcribed at higher levels (Moskalev *et al*., 2011). However, at higher radiation intensities, RHG protein levels can surpass DIAP1's buffering capacity, promoting its degradation via the proteasome and allowing widespread caspase activation (Baonza *et al*., 2022; Ryoo *et al*., 2002). Moreover, p53 can also enhance its apoptotic signaling by downregulating DIAP1 expression or by promoting its destabilization, further tipping the balance toward cell death under genotoxic stress (Mollereau and Ma, 2014).

2.6.3 Crosstalk Between p53 and DIAP1

While the primary mediators of the antagonism between p53 and DIAP1 are the RHG proteins, emerging research suggests that additional layers of direct and indirect regulatory interaction exist between these two key apoptosis regulators. Experimental findings indicate that in *Drosophila* cells exhibiting elevated p53 activity following radiation exposure, both DIAP1 transcript and protein levels decline rapidly even before widespread caspase activation implying that p53 plays a priming role in sensitizing cells to apoptosis (Jassim *et al*., 2003; Betz *et al*., 2008). Moreover, the transcription factor STAT92E, which promotes DIAP1 expression under

normal conditions, is itself inhibited in response to ionizing radiation, shifting the cellular balance away from survival and toward programmed cell death (Betz *et al*., 2008). This positions DIAP1 at the intersection of pro-survival and pro-apoptotic signaling, acting as a central node where environmental cues are integrated. Additionally, post-translational mechanisms further refine this regulation. p53 activation enhances E3 ubiquitin ligase activity, leading to accelerated degradation of DIAP1, thereby amplifying the apoptotic response (Ryoo *et al*., 2002). These findings underscore the multifaceted control exerted by p53 over DIAP1, beyond simple RHG gene induction, and highlight DIAP1's role as a critical gatekeeper in the decision between survival and apoptosis.

2.6.4 Transcriptional and Post-Translational Regulation of p53 and DIAP1

In *Drosophila melanogaster*, the regulation of apoptosis following ionizing radiation (IR) is governed not just by the baseline levels of p53 and DIAP1, but also by rapid stress-induced shifts in gene expression and protein stability (Mollereau and Ma, 2014). When DNA damage occurs due to IR, p53 is activated via phosphorylation, a process mediated by upstream kinases such as ATM and Chk2 (also known as Loki). These phosphorylation events prevent p53 degradation by interfering with ubiquitin ligase pathways, allowing the protein to accumulate and become functionally active (Moon *et al*., 2008). Once stabilized, p53 promotes transcription of the pro-apoptotic RHG genes *reaper*, *hid*, and *grim* which play a pivotal role in overcoming DIAP1-mediated caspase inhibition (Ryoo *et al*., 2002). The RHG proteins engage DIAP1 and trigger its self-ubiquitination, targeting it for proteasomal degradation (Hay, 2000). This mechanism ensures that even modest increases in RHG expression can effectively dismantle DIAP1's protective role, permitting caspase activation and cell death (Baonza *et al*., 2022). Under normal physiological conditions, DIAP1 is tightly regulated at the protein level, undergoing a dynamic

cycle of synthesis and degradation to maintain sufficient levels for buffering against unintended apoptosis (Yang *et al*., 2014). However, radiation exposure alters this balance. RHG proteins bind to DIAP1's BIR domains through their conserved IAP-binding motifs (IBM), enhancing DIAP1's vulnerability to degradation by either self-ubiquitination or E3 ligases like Ubr3 (Orme and Meier, 2009; Huang *et al*., 2014). Interestingly, transcriptional downregulation of DIAP1 is minimal after IR exposure; mRNA levels remain relatively stable, indicating that the major regulatory shift occurs post-translationally (Ryoo *et al*., 2002). This underscores a key feature of the apoptotic response in *Drosophila* p53 primarily promotes apoptosis by accelerating DIAP1 degradation, rather than by repressing its gene expression.

2.6.5 Dose-Dependent Dynamics of Apoptotic Response

The interaction between p53 and DIAP1 is dynamic and context-sensitive, varying significantly with radiation dose, exposure time, and the type of tissue involved. When exposed to low radiation levels (typically less than 5 Gy), p53 is activated but does not strongly repress DIAP1, which permits cells to initiate DNA repair processes and recover without undergoing apoptosis (Moskalev *et al*., 2011). At moderate doses (around 10 Gy), p53-driven upregulation of RHG proteins begins to weaken DIAP1's caspase-inhibiting capacity, resulting in partial apoptosis, especially in proliferative tissues like the imaginal discs (Colombani *et al*., 2006). However, high radiation doses (20 Gy or more) provoke intense p53 activity, rapid induction of RHG expression, and swift DIAP1 degradation, leading to widespread apoptotic cell death (Betz *et al*., 2008). Importantly, different tissues exhibit variable sensitivity to DIAP1 degradation. For example, neuroblasts are relatively resistant to apoptosis and tend to preserve DIAP1 longer than more sensitive cells such as epithelial or germline stem cells (Baonza *et al*., 2022). These findings support the view that DIAP1 functions as a stress buffer, capable of maintaining cell

survival under low-level genotoxic stress but rapidly degraded when damage is extensive and beyond repair (Steller, 2008). This balance between p53-induced apoptotic signaling and DIAP1's anti-apoptotic buffering ultimately determines cellular fate following irradiation.

2.6.6 Feedback and Feedforward Mechanisms in p53–DIAP1 Control

A distinctive feature of the regulatory relationship between p53 and DIAP1 is the presence of feedback and feedforward loops, which ensure that once the apoptotic program begins, it becomes irreversible and self-reinforcing. For example, after initial activation, cleaved caspases like DRONC and DRICE contribute to the further breakdown of DIAP1, creating a positive feedback loop that magnifies apoptotic signaling once DIAP1 levels drop beneath a functional threshold (Meier *et al.*, 2000). Moreover, RHG proteins not only initiate DIAP1 degradation but also disrupt mitochondrial homeostasis, resulting in increased ROS (reactive oxygen species) production. These ROS molecules intensify the stress response, thereby enhancing p53 activation and strengthening the pro-apoptotic cascade (Morey *et al.*, 2003). This feedforward circuit, wherein ROS amplifies p53 signaling, ensures that once DIAP1 is sufficiently diminished, the cell becomes fully committed to death (Mollereau and Ma, 2014). Recent research has also demonstrated that p53 can transcriptionally activate components of the JNK signaling pathway, which further contributes to the stabilization of RHG proteins and promotes sustained suppression of DIAP1, adding a long-term amplification mechanism to the apoptotic program (Baonza *et al.*, 2022).

2.6.7 Developmental Stage–Specific Responses

The sensitivity of apoptosis to ionizing radiation (IR) in *Drosophila melanogaster* is highly dependent on the developmental stage, which directly influences the functional dynamics

between p53 and DIAP1 (van Bergeijk *et al*., 2012). During embryogenesis and early larval stages, the organism is particularly vulnerable to IR. This heightened susceptibility is largely due to the high proliferative activity in imaginal discs, where p53 is strongly expressed and readily activated in response to DNA damage (Mollereau and Ma, 2014). Upon irradiation, p53 rapidly triggers the expression of apoptotic genes such as *reaper* and *hid*, which neutralize DIAP1's inhibitory function and initiate caspase-mediated cell death (Ryoo *et al*., 2002).

In mid-larval development, imaginal tissues continue to exhibit a pronounced apoptotic response, whereas germline stem cells (GSCs) and certain neuronal populations are more resistant to apoptosis. This resistance is associated with tissue-specific elevations of DIAP1 and other anti-apoptotic factors that buffer these cells against stress-induced death signals (Hasan, 2017). In the developing nervous system, even in the presence of activated p53, neurons maintain elevated DIAP1 levels, which helps safeguard them from undergoing apoptosis. Only when exposed to very high doses of radiation do these neurons initiate cell death, indicating a high threshold for DIAP1 degradation in neural tissues (Zhang, 2016). This developmental and tissue-specific regulation reflects a finely tuned apoptotic network that adapts to both cellular context and environmental stressors.

2.6.8 Tissue-Specific Sensitivity to p53–DIAP1 Modulation

Tissue-specific variation in DIAP1 expression plays a critical role in determining how different cell types in *Drosophila* respond to ionizing radiation-induced apoptosis (Proshkina *et al*., 2021). For instance, epidermal cells are particularly sensitive to IR because they maintain relatively low baseline levels of DIAP1. In contrast, muscle cells and gut epithelial tissues exhibit significant resistance, even at high radiation doses, which is partly attributed to the upregulation of DIAP1

and its modulation by STAT-dependent signaling pathways (Betz *et al*., 2008). Functional studies involving tissue-specific knockdown of DIAP1 have further confirmed this regulation. Notably, suppressing DIAP1 in muscle tissue dramatically increases radiation-induced apoptosis, whereas similar interventions in neuronal tissues have minimal impact (Vishal *et al*., 2018). These findings reinforce the concept that p53-driven expression of RHG genes, while essential, is not sufficient to induce apoptosis unless DIAP1 concentrations drop below a critical threshold. Thus, tissue-specific expression of DIAP1 finely tunes the cellular response to stress, illustrating how local regulatory environments shape the ultimate outcome of p53 activation.

2.6.9 Hormonal and Environmental Influences on DIAP1-p53 Dynamics

The regulatory interaction between p53 and DIAP1 in *Drosophila melanogaster* is influenced not only by genetic and developmental factors but also by external environmental conditions such as nutrient status and oxidative stress, which vary across tissues and developmental stages. For example, under conditions of nutrient scarcity, the modulation of DIAP1 by p53 is further shaped by microRNA activity and systemic hormonal cues like insulin/IGF signaling, particularly impacting tissues such as the fat body and ovaries in divergent ways (Guerrero, 2014). These signals can either amplify or suppress apoptotic responses independent of direct DNA damage, adding further complexity to the regulation of cell death. Genetic investigations using *Drosophila* as a model for radiation exposure have demonstrated how variation within the p53–DIAP1 axis can drive distinct radiation resistance phenotypes (Wichmann *et al*., 2006). For instance, mutant or truncated p53 variants often fail to trigger effective apoptosis, even when the RHG genes are transcriptionally activated, indicating that RHG induction alone is not sufficient without functional p53 signaling (Baonza *et al*., 2022). Conversely, genetic overexpression of DIAP1 has been shown to protect tissues from apoptosis and even promote tissue regeneration

following high-dose radiation, highlighting its critical survival function (Simón *et al.*, 2014). However, when DIAP1 is knocked down, even sub-lethal doses of ionizing radiation can cause widespread cell death and developmental abnormalities, demonstrating how sensitive and tightly regulated its protective role must be (Lopes *et al.*, 2019). This dynamic balance between pro-survival and pro-death signals plays a central role in organism-wide radiation tolerance, especially during key phases of larval development and tissue remodeling. What makes *Drosophila* an especially effective model for studying these pathways is the deep evolutionary conservation of both p53 and DIAP1 orthologs across the metazoan lineage (Ingaramo *et al.*, 2018). The p53 family of proteins is present in nearly all multicellular animals, from insects and nematodes to vertebrates, and retains its core function in coordinating DNA damage responses (Mota *et al.*, 2019). Similarly, DIAP1 belongs to the IAP (Inhibitor of Apoptosis Protein) family, with BIR domains structurally analogous to those found in mammalian proteins like XIAP, c-IAP1, and c-IAP2 (Ribeiro Lopes *et al.*, 2019). This molecular conservation underscores the relevance of *Drosophila* findings to understanding apoptotic regulation in higher organisms.

2.7 LOH and Genomic Instability in Irradiated Systems

Ionizing radiation is a well-known contributor to genomic instability, particularly in tissues with high cellular proliferation rates (Vijg and Suh, 2013). One of the most critical manifestations of this instability is Loss of Heterozygosity (LOH) a chromosomal alteration where one allele at a heterozygous site is lost, potentially unmasking harmful recessive mutations that can lead to tumor development or cell death (Gerlach and Herranz, 2020). In model organisms like *Drosophila melanogaster*, LOH has proven to be a highly sensitive indicator of genomic damage following radiation exposure, as well as a marker for ineffective DNA repair processes (Brown *et al.*, 2020). Various forms of radiation-induced genomic alterations including somatic

recombination, chromosomal deletions, gene conversion, and mis-segregation during mitosis can result in LOH, particularly in rapidly dividing tissues such as the developing wing discs (Sekelsky, 2017). These events create genetically altered clonal populations, serving as a measurable outcome of radiation-induced genomic disruption.

2.7.1 Mechanisms Leading to LOH After Irradiation

Loss of heterozygosity (LOH) in cells exposed to ionizing radiation can result from several underlying mechanisms, most notably DNA double-strand breaks (DSBs) that are incorrectly repaired through either non-homologous end joining (NHEJ) or faulty homologous recombination (HR) pathways (Karotki and Baverstock, 2012). Improper resolution of DSBs can lead to the loss of large chromosomal segments or unequal recombination events, which may expose harmful recessive mutations (Gerlach and Herranz, 2020). Studies using *Drosophila melanogaster* have revealed that cells undergoing LOH are frequently identified and removed through apoptosis, involving both p53-dependent and alternative apoptotic pathways, particularly during larval development, to preserve tissue homeostasis (Brown *et al.*, 2020). However, in cases where such genetically compromised cells escape programmed cell death, they can undergo clonal expansion, thereby sustaining and spreading genomic instability into adult tissues (Kronenberg, 1994).

2.7.2 Detection and Visualization of LOH in Drosophila Models

Advancements in transgenic technology in *Drosophila melanogaster* have enabled real-time detection of Loss of Heterozygosity (LOH) through systems like Mosaic Analysis with a Repressible Cell Marker (MARCM) and various fluorescent reporter constructs designed to highlight cells lacking heterozygous loci (Brown *et al.*, 2020). These approaches have shown that

even low doses of X-ray radiation can induce a considerable number of LOH events, especially within imaginal disc tissues, and this effect is amplified in genetic backgrounds where DNA repair mechanisms are compromised (Koval *et al*., 2020). The frequency of LOH is not uniform; it varies based on cell type and developmental timing, and it is particularly elevated in highly proliferative cells that exhibit weakened cell cycle checkpoints or impaired apoptotic responses (Yushkova and Bashlykova, 2021). Remarkably, elevated LOH rates have been observed not only in irradiated individuals but also in their offspring, suggesting that radiation-induced genomic instability can persist across generations (Yushkova, 2020).

2.8 The Modulatory Role of E2F1 in Radiation-Induced Apoptosis in *Drosophila melanogaster*

Ionizing radiation (IR) is a powerful trigger of apoptosis in multicellular organisms. In *Drosophila melanogaster*, which serves as a genetically accessible model system, the transcription factor E2F1 has emerged as a key regulator of radiation-induced apoptosis, functioning both in parallel with and independently of p53 (Wichmann *et al*., 2010). Although traditionally known for promoting the G1–S phase transition, E2F1 has now been shown to operate in dual roles advancing both cell division and programmed cell death, depending on the stress conditions and tissue context. E2F1's pro-apoptotic capability is largely due to its transcriptional activation of genes such as *hid* and *reaper*, which it upregulates by binding directly to their promoter sequences, especially following DNA damage caused by ionizing radiation (Skorobagatko and Mazilov, 2020). This apoptotic function persists even in p53-deficient backgrounds, as demonstrated in *Drosophila* mutants where E2F1 overexpression is sufficient to trigger apoptosis despite the absence of functional p53 (Wichmann, 2008). These

observations indicate that E2F1 can drive a p53-independent apoptotic cascade, adding redundancy to the cell's defense mechanisms against genome instability. Genetic studies further highlight the interplay between E2F1 and its paralog E2F2, revealing that while E2F1 promotes cell death post-irradiation, E2F2 suppresses it (Wichmann *et al*., 2010). Mutants lacking E2F1 show reduced apoptotic responses after radiation, whereas loss of E2F2 enhances apoptosis, indicating that the balance between these two factors is critical for modulating tissue-specific stress responses (van Bergeijk *et al*., 2012). At a mechanistic level, E2F1 activity is regulated by the DNA damage response kinase ATM, which is activated by double-strand breaks. ATM enhances E2F1's function both by upregulating its target genes and stabilizing the E2F1 protein, placing it as a central link between DNA damage detection and execution of the apoptotic program (Liu *et al*., 2022). This pathway is especially crucial in proliferative tissues such as imaginal discs, where damaged cells must be rapidly eliminated to preserve developmental integrity. Genome-wide transcriptional profiling has further confirmed E2F1's significance. In irradiated *Drosophila*, E2F1 was found to regulate a gene set that partially overlaps with p53 targets, but also includes E2F1-specific genes, highlighting its unique and shared roles in the apoptotic transcriptional network (van Bergeijk *et al*., 2012). Beyond its role in gene activation, recent work suggests E2F1 may intersect with other pathways, such as JNK signaling which is involved in both apoptosis and stress adaptation and may even influence mitochondrial stability under genotoxic stress (Baonza *et al*., 2022).

Further support comes from functional genomics and RNA interference experiments, which show that E2F1 knockdown via RNAi reduces apoptosis following IR, while overexpression dramatically increases cell death even when p53 is non-functional (Bilak *et al*., 2014). These findings position E2F1 as a crucial effector of p53-independent cell death and suggest its

therapeutic potential in targeting radiation-resistant malignancies. Recent work by Ruiz-Losada *et al*. (2022) expands this understanding by showing how E2F1 coordinates cellular proliferation and apoptotic elimination, helping tissues make rapid decisions during recovery from DNA damage. This balance ensures that genetically compromised cells are removed while preserving regenerative potential an essential function for genomic maintenance and organismal survival under stress.

2.8.1 Involvement of JNK Signaling Pathway in DIAP1 Regulation

The c-Jun N-terminal kinase (JNK) pathway plays a pivotal role in orchestrating cellular stress responses and apoptosis in *Drosophila melanogaster*, particularly after ionizing radiation exposure. Upon DNA damage, JNK is swiftly activated, influencing both cell death and tissue regeneration, depending on the biological context (Fogarty *et al*., 2016). One of the principal downstream targets of this pathway is DIAP1 (Drosophila Inhibitor of Apoptosis Protein 1), which functions to inhibit caspase activity and support cell viability (Ryoo *et al*., 2004). However, in irradiated tissues where JNK signaling is upregulated, it triggers the transcriptional activation of pro-apoptotic genes, such as *reaper* and *hid*. These gene products neutralize DIAP1, leading to caspase activation and apoptotic progression (McEwen and Peifer, 2005). Genetic studies have demonstrated that loss of JNK function significantly reduces radiation-induced apoptosis, underscoring JNK's importance in promoting DIAP1 degradation during stress responses (Kanda and Miura, 2004). In contrast, ectopic or overactive JNK signaling results in widespread cell death and developmental abnormalities, further confirming its direct involvement in the apoptotic machinery via its impact on DIAP1.

2.8.2 Chk2 and ATM/ATR Pathways in Radiation Sensing in *Drosophila*

Ionizing radiation (IR) primarily induces DNA double-strand breaks (DSBs), which in turn activate a conserved network of DNA damage response (DDR) kinases, most notably ATM (Ataxia Telangiectasia Mutated) and ATR (ATM and Rad3-related). These kinases subsequently activate Checkpoint kinase 2 (Chk2), known in *Drosophila* as the mnk gene product (Wichmann *et al*., 2006). In the fruit fly model, these signaling molecules initiate apoptotic responses by phosphorylating p53 and additional nuclear substrates, modulating the transcription of pro-apoptotic genes such as *reaper*, *hid*, and *grim* (Brodsky *et al*., 2004). Loss-of-function mutations in mnk/Chk2 reduce p53 phosphorylation and hinder apoptosis after radiation exposure, underscoring Chk2's upstream regulatory function in this stress-response cascade (Xu *et al*., 2001). Beyond apoptosis, ATM and ATR also play essential roles in orchestrating DNA repair, and when these pathways are impaired, unresolved genomic lesions accumulate, escalating the activation of death signals. Interestingly, Chk2 also interfaces with the transcription factor E2F1, forming a regulatory axis that enables both p53-dependent and independent apoptotic pathways to function in tandem (Wichmann *et al*., 2010). This molecular network not only detects DNA damage but also calibrates the response based on the extent of genomic injury and the developmental status of the tissue. Ultimately, these kinases act as primary responders to radiation, setting the stage for DIAP1 inhibition, which accelerates caspase activation and commits the cell to apoptosis.

2.8.3 Mitochondrial Involvement in Radiation-Induced Apoptosis

Although the mitochondrial role in apoptosis is broadly conserved across species, *Drosophila melanogaster* exhibits unique features compared to mammalian systems. In flies, mitochondrial stability is governed by Bcl-2 homologs Debcl and Buffy, which modulate the release of cytochrome c and the subsequent activation of caspases (Igaki *et al*., 2000). Following exposure to ionizing radiation, mitochondrial outer membrane permeabilization (MOMP) facilitates the activation of Apaf-1, which functions downstream of DIAP1 inactivation to initiate the apoptotic cascade (Zimmermann *et al*., 2002). Interestingly, DIAP1 has been partially localized to mitochondria, hinting at a role in attenuating death signals derived from these organelles (Hay *et al*., 1995). Under irradiation stress, the production of reactive oxygen species (ROS) exacerbates mitochondrial injury, reinforcing the apoptotic signal and pushing the cell past a point of no return. This amplification loop, mediated through mitochondrial damage, ensures the full activation of caspases and secures irreversible cell death once apoptotic signaling intensifies. Additionally, mitochondrial dynamics, governed by key regulators such as Drp1 (dynamin-related protein 1) and Marf (mitofusin), are disrupted by radiation exposure, affecting a cell's susceptibility to apoptosis (Roy *et al*., 2022). Overall, mitochondria in *Drosophila* not only act as effectors of apoptosis but also serve as amplifiers of IR-induced cell death, operating in tandem with JNK, Chk2, and DIAP1 signaling networks.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The research was conducted at the University of Benin, Edo State, Nigeria. The University of Benin (UNIBEN) established in 1970. has gained recognition across the country as a prominent public research institution,situated in Benin city, Edo state.

3.2 Study Location

The study was conducted in a dedicated laboratory facility equipped with appropriate infrastructure and equipment for rearing and manipulating *Drosophila melanogaster*. The laboratory, Biomedical Toxicology Chemicals Safety (BIOTOXCS) Research Laboratory, Central Biomedical Research, was located at University of Benin, Benin City, Edo State, Nigeria. The controlled environment of the laboratory provided the necessary conditions for maintaining the flies and conducting the experiments.

3.3 Study Population

In this study, *Drosophila melanogaster* was used as a model. *Drosophilia melanogaster* was obtained from the Drosophila Laboratory, Department of Biochemistry, University of Ibadan, Oyo State, Nigeria. The flies were allowed to acclimatize before the commencement of feeding and transfer procedures. The flies were maintained on the standard cornmeal diet, formulated with the following components: cornmeal (52 g), brewer's yeast (5 g), glucose (3.5g), agar (7.9 g), nipargin (lg), ethanol (2mL) and Distilled water (850ml).

3.4 Procedure for Feed Preparation and Handling of Drosophila

Distilled water (850ml) was measured, and 150 mL was removed to mix the cornmeal. The remaining 700 mL of distilled water was brought to a boil. A small portion of the boiling water was set aside to dissolve the yeast. Agar-agar was added to the boiling water and stirred until completely dissolved. The pre-mixed cornmeal was then incorporated into the boiling solution, followed by glucose, with constant stirring. The mixture was allowed to boil for approximately two minutes. Subsequently, the dissolved yeast was added to the boiling mixture. Finally , the mixture was removed from heat, then nipargin dissolved in ethanol was added. The meal was then transferred into different vials.

3.5 Experimental Design

The study aimed to evaluate the impact of diagnostic radiation exposure on mRNA expression profiles in *Drosophila melanogaster*. A controlled laboratory-based experimental design was adopted, using defined radiation doses and standardized exposure conditions. Healthy, laboratory-reared *Drosophila melanogaster* (Harwich strain) were randomly assigned into experimental and control groups. Each group contained an equal distribution of male and female flies, aged 3–5 days post-eclosion. Flies were maintained in vials (100 flies per vial) under standard culture conditions throughout the study.

3.5.1 Experimental Groups

The flies were divided into carefully structured groups as follows:

1. Control Group A -- Flies were handled and placed under identical conditions as the experimental groups but were not exposed to radiation. This group served as the baseline reference for mRNA expression levels.

2. Treatment Groups (Radiation-Exposed) - Flies were exposed to diagnostic radiation delivered through (X-ray and CT)**.** The groups were stratified according to the absorbed radiation dose:

Group B (X-ray, 7 days): Flies in this group were exposed to X-ray irradiation at a dose of 0.1 Gy daily for 7 consecutive days. This group was designed to evaluate the short-term effects of direct X-ray exposure.

Group C (X-ray, 14 days): Flies were exposed to X-ray irradiation at a dose of 0.1 Gy daily for a prolonged duration of 14 consecutive days. This group was intended to assess the cumulative biological effects of extended exposure to X-ray radiation.

Group D (CT scan, 7 days): Flies in this group were subjected to computed tomography (CT) radiation at a dose of 0.1 Gy daily for 7 consecutive days**.** This group allowed comparison between short-term effects of CT and X-ray exposures at the same dose.

Group E (CT scan, 14 days): Flies were exposed to CT radiation at a dose of 0.1 Gy daily **for** 14 consecutive days. This group provided data on the long-term effects of CT radiation relative to X-ray exposure of the same intensity and duration.

Each experimental group was maintained under identical environmental conditions (temperature, humidity, and feeding regimen), with the only variable being the radiation source and duration of exposure**.** Vials containing flies were positioned at a standardized location relative to the radiation source to ensure uniform dose distribution. Exposure parameters (kVp, mA, time, or CTDIvol for CT scans) were recorded for each session.

3.5.2 Radiation Source and Dosimetry

Two different sources of ionizing radiation were employed in this study: X-ray irradiation and computed tomography (CT) radiation. These sources were selected to enable comparison between direct X-ray exposure and medical diagnostic-type exposure under controlled laboratory conditions.

3.5.3 X-ray Source

The X-ray exposures were performed using **a** Siemens Multix Pro X-ray system (Siemens Healthcare GmbH, Erlangen, Germany), operating **at** 100 kVp and 5 mA, with a calibrated output to deliver a dose of 0.1 Gray (Gy) per exposure cycle. The machine was routinely calibrated and maintained by the hospital's Department of Medical Physics, ensuring accurate and reproducible dose delivery. Flies assigned to Groups B and C were placed in ventilated plastic containers at a standardized distance of 1 meter from the X-ray tube focus, ensuring uniform irradiation across the group.

3.5.4 CT Radiation Source

CT exposures were carried out using a GE BrightSpeed 16-slice CT scanner (GE Healthcare, Milwaukee, WI, USA)**.** The scanner was configured to deliver an equivalent absorbed dose of 0.1 Gy, based on dose-length product (DLP) measurements and conversion factors recommended by the International Commission on Radiological Protection (ICRP, 2007)**.** Flies in Groups C and E were positioned in sealed, ventilated containers within the CT gantry and subjected to daily exposures according to the experimental schedule.

3.5.5 Dosimetry and Dose Verification

The absorbed dose of **0.1 Gy** was selected as it represents a low-dose ionizing radiation level relevant to diagnostic exposures and sub-lethal biological effects (Brenner and Hall, 2007). Dosimetry was verified using a thermoluminescent dosimeter (TLD-100, Harshaw/Bicron, Solon, OH, USA) placed adjacent to the fly containers during exposure. Dose calibration was cross-checked against the machine's internal dose monitoring system and validated by a qualified medical physicist to ensure reproducibility and consistency across all exposures.

3.5.6 Exposure Protocol

Radiation was delivered once daily for 7 consecutive days (Groups B and D) and 14 consecutive days (Groups C and E)**.** Each exposure session lasted approximately 3–5 minutes, during which flies were immobilized in perforated containers to minimize movement and ensure uniform exposure. Control flies (Group A) were maintained under identical environmental conditions without radiation exposure.

Preservation of Flies

After the period of exposure, the flies were transferred into a container of trizol reagent to preserve the RNA, then taken for gene analysis

3.7 p53 and DIAP1 mRNA Assay

3.7.1 RNA Extraction and semi-Quantitative Polymerase Chain Reaction (PCR)

RNA was isolated from *Drosophila melanogaster* with Trizol reagent (ThermoFisher Scientific) in an eppendorf tube converted to cDNA using ProtoScript II first – strand technology (New England BioLabs). PCR amplification of p53 and DIAP1 was done using one Tap®2X Master Mix (NEB).

3.7.2 Isolation of Total RNA

Total RNA was Isolated from whole drosophila samples with Quick – RNA MiniPrep™ Kit (Zymo Research). The DNA was removed following DNAse 1 (NEB, Cat: M030S) treatment. The RNA was quantified at 260nm and the purity confirmed at 260nm ans 280nm using A and E Spectrophotometer (AandE Lab. UK.).

3.7.3 Complementary DNA (cDNA) Conversion

One (1µg) of DNA – free RNA was converted to cDNA by reverse transcriptase reaction with the aid of cDNA synthesis kit based on ProtoScript II first – strand technology (New England BioLabs) in a condition of 3 – step reaction: 65^0C for 5min, 42^0C for 1 hour and 80^0C for 5 minutes (Olumegbon *et al.,* 2020).

PCR amplification and agarose gel electrophoresis

Polymerase chain reaction (PCR) for the amplification of gene of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa):set:> PCR amplification was performed in a total of 25 μl volume reaction mixture containing cDNA, primer (forward and reverse) and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95 ◦C for 5 min, followed by 30 cycles of amplification (denaturation at 95 ◦C for 30 s, annealing for 30 s and extension at 72 ◦C for 60 s) and ending with final extension at 72 ◦C for 10 min. The amplicons were resolved on 1.0% agarose gel. The GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using "image J" software (Elekofehinti *et al*., 2020).

3.7.4. Primers

p53

Forward primer: TGATTAATGAATGAGTTCGGGC

Reverse primer: TGCTCAGGAAACTTGACTGTTT

DIAP1

Forward primer: TGATTAATGAATGAGTTCGGGC

Reverse primer: TGCTCAGGAAACTTGACTGTTT

3.8. Statistical Analysis

Data generated from this study were analyzed using GraphPad Prism version 8.0 (California, USA). Differences among treatment groups for continuous variables were assessed using

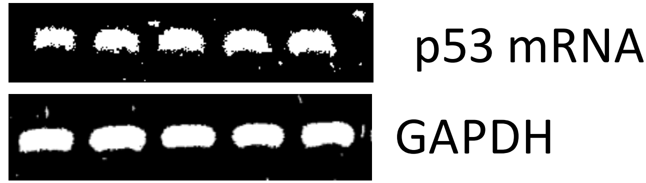
Analysis of Variance (ANOVA). Where ANOVA indicated statistical significance, Tukey's Honestly Significant Difference (HSD) post hoc test was performed. mRNA gene expression profiles were illustrated using bar charts. A p value of ≤ 0.05 was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Results

Figure 4.1 shows the expression of genes as represented by gel electrophoresis picture and internal control (Glycealdehyde-3-Phosphate Dehydrogenase {GADPH}) of mRNA expression of p53 of groups A, B, C, D and E, representing Group A (Control), Group B (X-ray Exposure for 7 days), Group C (X-ray Exposure for 14 days), and Groups D, E (CT scan Exposure for 7 days**,** CT scan Exposure for 14 days, respectively) represented on different bars on the bar chart. There was a significant increase in the mRNA expression of p53 of group B, C, D and E when compared to group A (*$p<0.05$, **$p<0.01$, ***$p<0.001$).



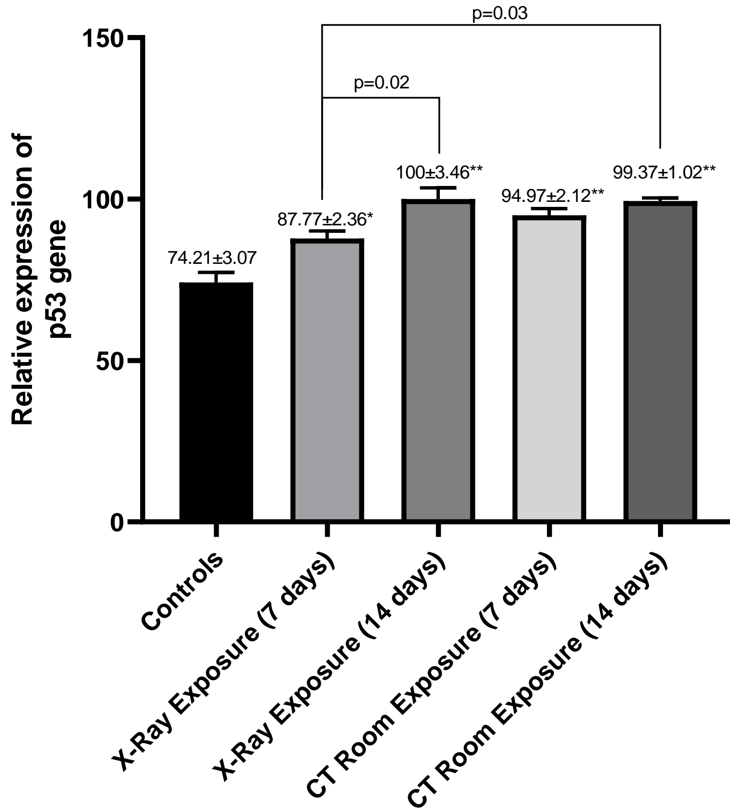


Figure 4.1**:** PCR and agarose gel analysis of p53**.**

Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, **p<0.01, ***p<0.001)

Key: GADPH=Glyceraldehyde-3-Phosphate Dehydrogenase

Figure 4.2 shows the expression of genes as represented by gel electrophoresis picture and internal control (Glycealdehyde-3-Phosphate Dehydrogenase {GADPH}) of mRNA expression of DIAP1 of groups A, B, C, D and E, representing Group A (Control), Group B (X-ray Exposure for 7 days), Group C (X-ray Exposure for 14 days), and Groups D, E (CT scan Exposure for 7 days**,** CT scan Exposure for 14 days, respectively) represented on different bars on the bar chart. There was a significant increase in the mRNA expression of DIAP1 of group B, C, D and E when compared to group A (*p<0.05, **p<0.01, ***p<0.001).

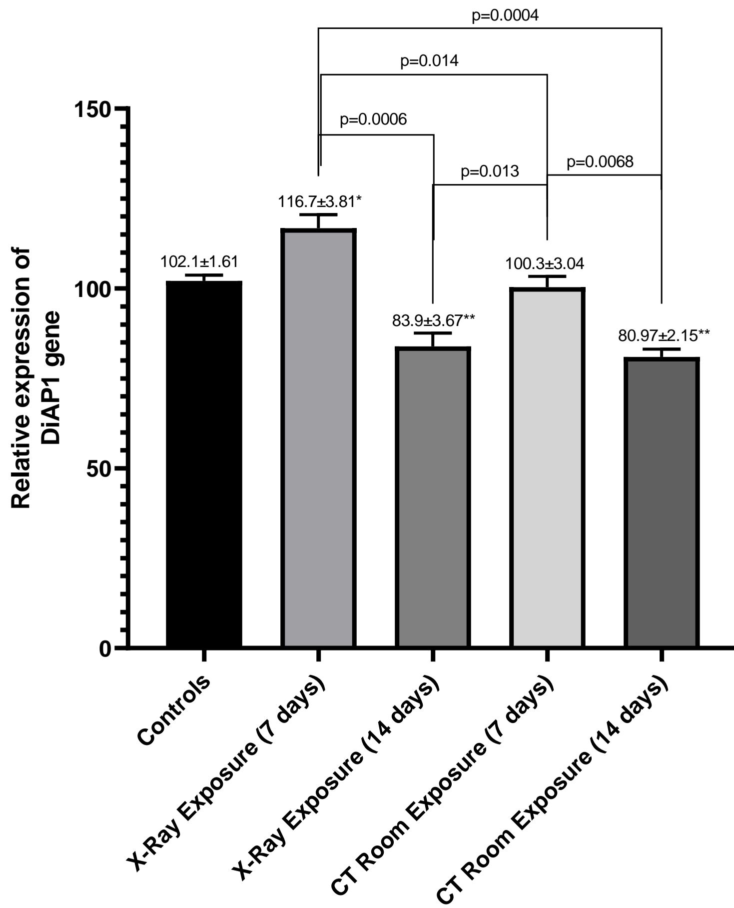


Figure 4.2: PCR and agarose gel analysis of DIAP1.

Error bar represents mean±SEM. Statistical significance represented by (*p<0.05, **p<0.01, ***p<0.001)

Key: GADPH=Glyceraldehyde-3-Phosphate Dehydrogenase

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

This study examined the molecular effects of diagnostic radiation exposure (X-ray and CT scan) on *Drosophila melanogaster*, focusing on the expression of p53 **and** DIAP1 mRNA. Results demonstrated a significant upregulation of p53 expression in all radiation-exposed groups relative to controls, with the highest induction occurring after 14 days of exposure. Conversely, DIAP1 expression displayed a biphasic response**:** initial upregulation after 7 days of X-ray exposure, followed by downregulation after 14 days of X-ray or CT exposure. These findings suggest that diagnostic radiation activates both pro-apoptotic and anti-apoptotic pathways in *Drosophila*, with the balance between them depending on exposure duration. The upregulation of p53 observed in this study is consistent with its well-established role as a "genomic gatekeeper," mediating DNA damage responses by activating cell cycle arrest, repair, or apoptosis. In *Drosophila*, Dmp53 acts similarly to mammalian p53, activating apoptotic genes such as *reaper* and *hid* after ionizing radiation (van Bergeijk *et al*., 2012). Jassim *et al*. (2003) further demonstrated that p53 protects retinal tissue in *Drosophila* following DNA damage, highlighting its tissue-conserving role during stress (Jassim *et al*., 2003).

Studies in mammalian systems echo this finding, where diagnostic radiation has been shown to induce dose-dependent increases in p53 expression and activity, serving as an early biomarker of genomic instability (Brenner and Hall, 2007). Collectively, these findings reinforce that p53 is a sensitive marker of low-dose radiation exposure across species. In contrast to p53, DIAP1 expression followed a dynamic pattern. The transient upregulation after 7 days suggests a protective mechanism, where DIAP1 prevents premature apoptosis to allow cells time to repair

DNA. This agrees with the findings of Brown and Su (2024), who reported that DIAP1, together with E2F1, promotes genomic stability after X-ray exposure by suppressing excessive apoptosis in *Drosophila* (Brown and Su, 2024).

However, prolonged exposure resulted in DIAP1 downregulation, possibly reflecting an adaptive shift where apoptotic pathways are allowed to proceed once cellular damage becomes irreparable. Baonza *et al*. (2022) described this regulatory switch, emphasizing that suppression of DIAP1 allows caspase activation and apoptotic clearance of damaged cells (Baonza *et al*., 2022). Similarly, Martín *et al*. (2008) linked DIAP1 inhibition with compensatory proliferation, where cell death stimulates regenerative responses (Martín *et al*., 2008).

The inverse trends between p53 and DIAP1 observed in this study underline their antagonistic roles in cell fate decisions. Whereas p53 promotes apoptosis, DIAP1 inhibits it by directly binding and suppressing caspases. This balance ensures that only cells with excessive DNA damage undergo apoptosis, while mildly affected cells are preserved. Garcia-Arias *et al*. (2023) emphasized the importance of this balance, showing that prolonged DIAP1 suppression without compensatory responses can drive senescence and tumorigenesis in epithelial tissues (Garcia-Arias *et al*., 2023).

The findings from this study indicate that short-term exposure promotes survival responses (DIAP1 upregulation), while long-term exposure triggers apoptotic clearance (p53 dominance over DIAP1)**.** This dynamic resembles the “two-phase” DNA damage response described by Wichmann (2008), where early anti-apoptotic defenses give way to apoptosis when damage accumulates (Wichmann, 2008).

These findings carry important implications for understanding the biological risks of diagnostic radiation. Although doses used in medical imaging are considered safe, the activation of p53 and modulation of DIAP1 in this model suggest that even low-dose exposures can alter apoptosis regulation. In humans, cumulative diagnostic exposures have been associated with increased cancer risks over time (Brenner and Hall, 2007). Thus, *Drosophila melanogaster* provides a cost-effective, genetically tractable model for investigating radiation-induced molecular pathways relevant to human health.

Furthermore, the differential responses between X-ray and CT exposures suggest subtle variations in biological impact despite equivalent doses. This aligns with studies showing that CT radiation, due to higher energy delivery and fractionated dosing, may cause more persistent DNA damage compared to conventional X-ray (Baonza *et al*., 2022).

5.2 Conclusion

This study provides evidence that diagnostic radiation, even at low doses relevant to medical imaging, significantly alters the molecular balance between survival and apoptosis in *Drosophila melanogaster*. p53 was consistently increased in all groups exposed to radiation, showing that it helps detect DNA damage and trigger cell death. DIAP1 increased at first to help the cells survive, but decreased during longer exposure, allowing cell death to occur when the damage could no longer be repaired. This shows that the two proteins work together to maintain a balance between cell survival and cell death. These findings are in agreement with previous studies in both *Drosophila* and mammalian systems, reinforcing the value of *Drosophila melanogaster* as a model organism for radiation biology. In conclusion, this work demonstrates that diagnostic radiation, though considered low risk, can induce measurable genetic responses

that may accumulate over time. Understanding these molecular dynamics provides a foundation for further studies on the long-term implications of diagnostic exposures, bridging insights between experimental models and human health.

5.3 Recommendations

Based on the findings of this study, the following recommendations are proposed. Since even low-dose diagnostic radiation induced measurable molecular changes in *Drosophila melanogaster*, caution should be exercised in human medical practice. Clinicians should adopt the ALARA principle (As Low As Reasonably Achievable) when prescribing diagnostic imaging procedures, particularly in vulnerable populations such as children and patients requiring repeated scans. Health authorities and regulatory agencies should strengthen radiation protection guidelines, especially for computed tomography (CT), which delivers higher doses compared to conventional X-rays. Standardized protocols should be developed to limit unnecessary repeat exposures. Future research should extend beyond p53 and DIAP1 to include a broader range of DNA damage and repair genes (e.g., *atm, chk2, rad51*) and apoptotic regulators (*reaper, hid, grim*). Awareness campaigns should be introduced to educate healthcare workers and patients about the potential risks of cumulative diagnostic radiation, emphasizing alternatives such as ultrasound or MRI where appropriate. Longitudinal studies should assess whether repeated low-dose exposures contribute to adverse phenotypic outcomes such as reduced lifespan, fertility impairment, or tumorigenesis in *Drosophila*, thereby offering predictive insights into possible human risks.

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APPENDIX I

Drosophila *CULTURE VIAL*