SURVIVAL, LOCOMOTOR BEHAVIOUR AND NEUROTOXIC GENE EXPRESSION IN *Drosophila melanogaster* EXPOSED TO HUMAN HAIR DYE

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this research titled **Survival, Locomotor Behaviour and Neurotoxic Gene Expression in *Drosophila melanogaster* exposed to Human hair dye** was carried out by **Osahenrhumwen Miracle AMADIN** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfilment of the requirement for award of Bachelor of Science (B.Sc.) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

______________________________ ______________________________

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(PROJECT SUPERVISOR)

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PROF. (MRS.) E. T. AISIEN **DATE**
(HEAD OF DEPARTMENT)

DECLARATION

I, Osahenrhumwen Miracle AMADIN declare that "Survival, Locomotor Behaviour and Neurotoxic Gene Expression in *Drosophila melanogaster* exposed to Human hair dye" is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

______________________________ ______________________________

Osahenrhumwen Miracle AMADIN Date

DEDICATION

This project is dedicated to God Almighty for seeing me through the entire process of this project, my parents and siblings for their love and support, and my friends for the amazing time.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to God Almighty, who sustains all life, for giving me the knowledge, wisdom and understanding during this time.

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ABSTRACT

Given the widespread global use of hair dyes, which contain chemicals like aromatic amines and oxidative agents, understanding their toxic potential is a critical component of assessing human safety. *Drosophila melanogaster* served as a model organism due to its well-characterized genome, conserved neurological pathways, and ease of genetic tractability. The flies were exposed to three treatment concentrations of the hair dye (0.05%, 0.5%, and 2.0%). The study assessed overall survival rates and observed climbing ability of the flies using the negative geotaxis assay. Flies were exposed to various concentrations of the dye through dietary administration, and survival was monitored over a 7-day period for the pilot study and a 5-day period for the main study. The relative expression of several key genes including Spitz(Spi), Wingless(Wnt), Eiger(egr), Forkhead box(FOXO), Hedgehog(HH), and Kelch-like ECH-associate protein 1(Keap1) was analyzed. Results demonstrated a dose-dependent response of the organism. The locomotor behavior analysis showed declining climbing ability of the flies at increasing concentrations. The Spi gene, associated with the EGFR pathway, showed a significant increase at the highest (2.0%) concentration. Wnt gene was significantly decreased at the 0.5% and 2.0% concentrations while Eiger gene was increased. The transcription factor FOXO was decreased while the signaling gene HH was significantly increased at high concentrations, indicating the activation of stress-induced and developmental response mechanisms. Conversely, the stress regulator Keap1, involved in the Nrf2 pathway was decreased at the 0.5% and 2.0% concentrations. These findings collectively establish that exposure to the hair dye induces significant neurotoxicity and disturbs fundamental regulatory mechanisms in *Drosophila melanogaster*, highlighting the need for accurate toxicological evaluation of these cosmetic products.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The increasing use of personal care products, such as hair dyes, has raised concerns about their potential toxicological effects on human health and the environment. Hair dyes contain a complex mixture of chemicals, including aromatic amines, oxidative agents, and other compounds, some of which have been associated with cytotoxicity, genotoxicity, and neurotoxicity in various biological systems. Understanding the impact of these chemicals is crucial for assessing their safety and informing regulatory guidelines. Human hair dyes represent one of the most widely used cosmetic products globally, with over 70% of women and 10% of men in developed countries applying them regularly (Nohynek *et al.,* 2004). Permanent hair dyes, which dominate 80% of the market, rely on oxidative chemistry involving aromatic amines such as para-phenylenediamine (PPD) and resorcinol. During application, these compounds are oxidized by hydrogen peroxide to form reactive quinone intermediates that penetrate the hair shaft but also generate reactive oxygen species (ROS) capable of damaging cellular components, including DNA and proteins. While dermal sensitization and allergic reactions are well-established risks (Basketter *et al.,* 2010), growing evidence indicates systemic absorption through the scalp, with potential neurotoxic effects via blood-brain barrier penetration and neuronal protein adduct formation. Despite their ubiquity, the neurological implications of chronic low-level exposure remain underexplored.

The fruit fly, *Drosophila melanogaster*, is an exemplary model organism for neurotoxicity research due to its short lifespan (60 days), genetic tractability, and 60-

75% homology of neurodegeneration related genes with humans (Bellen *et al.,* 2010). Conserved neural circuits enable precise assessment of locomotor behavior, while advanced genomic tools facilitate real-time monitoring of neurotoxic gene expression. Prior *Drosophila melanogaster* studies have demonstrated mutagenicity of hair dye components and behavioral deficits from structurally similar azo dyes.

1.2 Justification

With yearly global hair dye sales reaching $30 billion and increasing use among younger populations, identifying subtle neurotoxic risks is critical for public health. *Drosophila melanogaster* provides a high-throughput, ethically sound platform for testing dye formulations, bridging gaps between in vitro experiments and mammalian models. This study addresses a critical knowledge gap by evaluating dose-dependent effects on fly survival, negative geotaxis (a proxy for motor coordination), and the expression of key neurotoxic genes, providing mechanistic insights into ROS-mediated neuronal damage. *Drosophila melanogaster*, the common fruit fly, serves as an excellent model organism for toxicological studies due to its genetic similarity with humans, rapid life cycle, and well-characterized genome.

1.3 Aim of study

This study is aimed at assessing toxicological responses in *Drosophila melanogaster* exposed to different concentrations of human hair dye

1.4 Specific Objectives

1. To determine survival rate in *Drosophila melanogaster* exposed to human hair dye at different concentration(0.05%, 0.5%, 2.0%)

2. To assess behavioral responses in exposed *Drosophila melanogaster* using negative geotaxis assays (climbing assays).

3. To assess neurotoxic effects in exposed *Drosophila melanogaster* using specific genetic markers of neurotoxicity (Wingless, Eiger, Spitz, Forkhead box, Hedgehog, Kelch-like ECH-associate protein 1).

CHAPTER TWO

LITERATURE REVIEW

2.1 *Drosophila melanogaster* as a biological model organism

Humans are continually exposed to chemicals from a range of sources, including air, water, medication, industrial chemicals, personal care products, and so on (Holsopple *et al.* 2023). Thousands of new products are introduced each year, many of which have not been adequately tested to assure their safety for human health and the environment (Holsopple *et al*. 2023). Most of these products contain chemicals that may be harmful to human health. To ensure their safety for human use, these products must first be tested on biological models, which are typically rats and mice. While these rodents are instructive, using them for chemical safety assessment can be costly, time-consuming, and frequently results in the test animal experiencing intolerable levels of suffering (Holsopple *et al*. 2023).

The fruit fly (*Drosophila melanogaster*) is a versatile model organism in biology because of its short life cycle, ease of breeding, genetic tractability, and genes that are conserved in humans. *Drosophila* and humans are 60% genetically related, with around 75% of the genes that cause human illnesses having fly homologs (Allocca *et al*., 2018). It is used to investigate a variety of biological processes, including genetics, developmental biology, ageing, and illness (Tolwinski, 2017; Ugur *et al*., 2016). It can also be utilised to explore complex processes related to scientific research, such as cancer and other toxicological characteristics (Atoki *et al*., 2024).

Drosophila melanogaster shares a significant number of genes with humans, many of which are involved in disease. It has a short generation time (about 10 days from egg

to reproductive adult) and lifespan, making it appropriate for genetic and ageing study. They are inexpensive to culture and have a high reproduction rate. The enormous quantity of embryos deposited externally makes them simple to study. There are numerous genetic tools available for manipulation, enabling for targeted gene modification to research specific functions or develop disease models (Atoki *et al*., 2024).

2.1.1 History of *Drosophila melanogaster* as a model organism

From its first use in the laboratory in the early 1900s until the present day, *Drosophila melanogaster* has been central to major breakthroughs in genetics. The use of this fruit fly as a model organism began with the pioneering work of Thomas Hunt Morgan, who used the fruit fly to establish the 'chromosome theory of inheritance'. Morgan, utilized the fruit fly to define genes and prove that they were located within chromosomes (much before it was ever proven that DNA is the genetic material), this significantly improved the idea of heredity initially put forth by Gregor Mendel (Giansanti *et al.,* 2025). He was awarded the 1933 Nobel Prize in Physiology or Medicine for his discoveries. He is considered the "father" of *Drosophila* research. The fly's history as a model is marked by several Nobel Prizes awarded for work on sex-linked inheritance, genetic mutations, embryonic development, and innate immunity (Giansanti *et al.,* 2025).

In 1910, Morgan's laboratory found sex-linked inheritance while examining an eye color abnormality. His student, Herman J. Muller, later won the prize in 1946 'for the discovery of the creation of mutations by means of X-ray irradiation' (Popis *et al*., 2018). In the 1920s, Muller used *Drosophila* to demonstrate that x-rays could actually damage chromosomes and dramatically speed gene mutation (Atoki *et al*., 2024). In

Muller's experiment, irradiated flies appeared normal, but mutation was frequently observed in their offspring (Schaeffer, 2018). When man first attempted to harness and exploit nuclear fission, it was discovered that radiation causes harmful genetic defects in the progeny of exposed persons (Atoki *et al*., 2024).

In 1995, *Drosophila* researchers Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric F. Wieschaus shared the prize 'for their discoveries concerning the genetic control of early embryonic development' (Giansanti *et al.*, 2025). It has recently been established that many of the genes identified as critical for fly development are also required for the development of all other animals, including humans (Giansanti *et al*., 2025). Many of the essential building blocks and engineering processes have survived evolution and are quite similar, despite considerable physiological and toxonomical variations between the final design of *Drosophila melanogaster* and *Homo sapiens* (Wang *et al*., 2025).

In 1999, Craig Venter and colleagues established the practicality of the "shot-gun" method for sequencing the human genome using the *Drosophila melanogaster* genome as a test bed. This strategy worked, and the *Drosophila* genome sequence was made public in March 2000, barely eleven months before the human genome was sequenced. Everyone gets free access to the *Drosophila* genome sequencing and annotation via "Flybase," an excellent online database dedicated to *Drosophila* (Gramates *et al.*, 2022). The legitimacy of fruit flies as a model organism for medical research was reinforced by comparisons between the fully sequenced genomes of *Drosophila* and humans, which showed that over 75% of known human disease genes have an identifiable match in the fruit fly genome. Approximately 14,000 genes are thought to

exist in *Drosophila* at this time, and each one has its own Flybase page with links to almost all of the information thatis currently available about it, including the gene's sequence, gene product sequence, known mutations, and associated (Jenkins *et al.,* 2022).

Most recently, Jules Hoffman shared the 2011 prize for 'discoveries concerning the activation of innate immunity' in *Drosophila melanogaster* (Giansanti *et al.,* 2025).

How did one species of *Drosophila melanogaster*, come to be a model system? Harvard entomologist Charles Woodworth was the first to rear *Drosophila melanogaster*, just after the turn of the 20th century (Markow, 2015). It is not clear why or how he came to breed them, but their short generation time and ease of rearing were probably very appealing attributes (Markow, 2015). Woodworth then recommended them to his colleague William Castle, who initially worked on mammals but utilized the flies to study inbreeding (Markow, 2015). During this same period, another entomologist, Frank Lutz at the American Museum of Natural History, also began studying this fly's basic biology, publishing more than a dozen papers about them (Carson, 2013). It was from Lutz that Thomas Hunt Morgan introduced them into his laboratory at Columbia University. At the time Morgan began his work, the basic principles of heredity were still under debate (Markow, 2015). Morgan's discoveries and the fact that he attracted a highly talented group of graduate students no doubt fuelled the use of *Drosophila melanogaster* as a model system (Markow, 2015).

Today, the fly is used to study a range of human diseases, including cancer, Alzheimer's, and Parkinson's, due to the high degree of gene conservation between flies

and humans. It's also used for drug screening and has been the first animal to be sent into space (Giansanti *et al.,* 2025).

2.1.2 Normal development of *Drosophila melanogaster*

The development of *Drosophila melanogaster*, commonly known as the fruit fly, is a well-studied model for understanding animal development due to its short life cycle, genetic tractability, and conserved developmental processes. The fruit fly *Drosophila melanogaster* has been extensively studied for over a century as a model organism for genetic investigations, It also has many characteristics that make it an ideal organism for the study of animal development and behavior, neurobiology, and human genetic diseases and conditions (Sharma, 2023). The normal development of *Drosophila melanogaster* is a process of complete metamorphosis: egg, larva, pupa, and adult. It's life cycle consist of six stages: embryo, first-instar larva, second-instar larva, third-instar larva, pupa, and adult (Nishihara, 2007). A *Drosophila* egg is about a half millimeter in length. The time taken for embryogenesis and subsequent development varies with temperature. At 25 °C, an embryo takes about 1 day after fertilization to develop and hatch into a worm-like larva (Nishihara, 2007). The larva grows continuously and undergoes three molts: the first after 1 day, the second after 2 days, and the third after 4 days. The final molt produces an immobile pupa. The pupal stage lasts about 4 days, during which the body is completely remodeled to give the adult winged form. The adult hatches from the pupal case and is fertile within about 12 h. Overall, it takes *Drosophila* approximately 10 days, at 25 °C, to develop from an egg to an adult fly (Nishihara, 2007). The females adult weigh about 1.4 mg and male 0.8 weigh mg, adult female flies are typically larger than adult male flies. Females can deposit up to 100 eggs a day and are ready to mate less than 24 hours after eclosion

(emergence from the pupa case). Adult flies typically live about two months after eclosion (Nishihara, 2007).

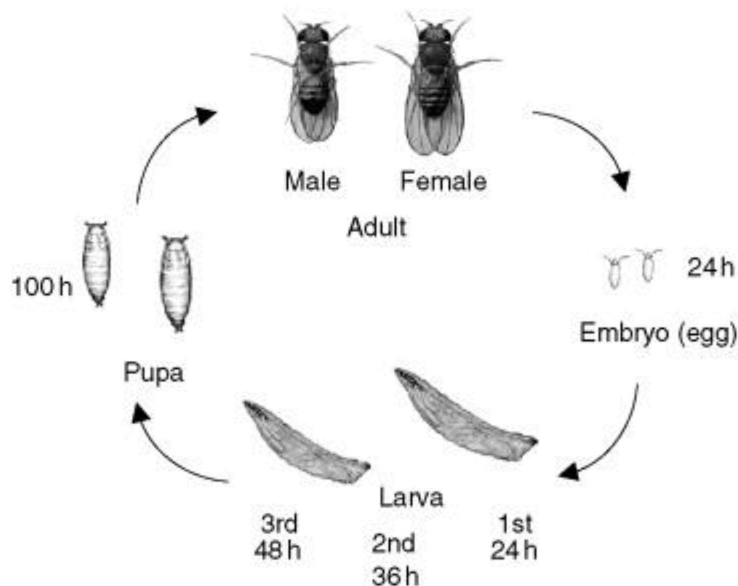


Figure 2.1 Life cycle of *Drosophila melanogaster* (Nishihara, 2007).

2.1.3 Biological Relevance of using *Drosophila melanogaster* as a model organism

Drosophila is a genus of fly that belongs to the Drosophilidae family. Members of this family are typically referred to as 'small fruit flies', pomace flies, vinegar flies, or wine flies, because they are frequently observed lingering about decaying or too ripe fruit (Atoki *et al*., 2024). They are distinct from the Tephritidae, a related family of insects commonly known as fruit flies (also known as 'real fruit flies'); tephritids feed primarily on ripe or unripe fruit, and many species, particularly the Mediterranean fruit fly, are considered harmful agricultural pests (Atoki *et al*., 2024). Since its introduction more than a century ago, *Drosophila melanogaster,* one of the many species of *Drosophila*, has been extensively used in genetic studies and serves as a prominent model organism

in biomedical research and developmental biology, particularly genetics and molecular biology (Atoki *et al*., 2024).

The similarities in molecular processes involved in the management of longevity and aging between *Drosophila melanogaster* and humans, combined with a high degree of genetic homology between the two species, make *Drosophila melanogaster* an appealing model system for toxicologists. *Drosophila melanogaster* has more than 65-70% of human illness genes (Atoki *et al*., 2024), making it a valuable model for understanding not only how genes cause diseases, but also the identification of the relationship between such genes and diseases. Compared with other models, *Drosophila melanogaster* offers rapid generation time, ease of use, and easy to maintain in the laboratory in a large quantity due to its tiny body size and short lifespan. *Drosophila melanogaster* is a commonly used model organism in genetics, biochemistry, cell biology, and developmental biology. It has been utilized as a model to understand human disorders in recent decades, as well as in preliminary toxicological research (Atoki *et al*., 2024). Some of the importance of using *Drosophila melanogaster* as model organism are;

- Genetic similarity to humans: Approximately 75% of the genes linked to human diseases have functional homologs in *Drosophila*. Fruit flies and humans share many basic biological processes, including cell signaling, development, and metabolism (Atoki *et al*., 2024). By adding human disease-associated mutations or examining similar fly genes, *Drosophila melanogaster* is used to research diseases such as cancer, neurological disorders (such as Alzheimer's and Parkinson's), diabetes, and cardiovascular diseases.

- Short generation time and high reproductive rate: Fruit flies have a rapid life cycle (about 10–14 days at 25°C), allowing researchers to study multiple generations in a short period. Females lay hundreds of eggs, providing large sample sizes for experiments, which is critical for statistical analysis and studying rare genetic events (Tolwinski, 2017).
- Simple and Well-Characterized Genome: *Drosophila* has a relatively small genome (180 Mb, 13,600 genes) compared to humans (3,200 Mb, 20,000–25,000 genes), making it easier to manipulate and study. Its genome was fully sequenced in 2000, providing a wealth of genetic information (Atoki *et al.,* 2024). Fewer genetic redundancies (e.g., fewer paralogs) simplify the study of gene function compared to vertebrates (Atoki *et al.,* 2024).
- Ease of Maintenance and Cost-Effectiveness: Fruit flies are small, easy to maintain, and inexpensive to culture in the lab, requiring minimal space and resources compared to vertebrate models like mice. Their simple dietary needs (e.g., cornmeal-based media) and robust nature make them ideal for large-scale experiments (Tolwinski, 2017).
- Complex yet Accessible Biology: Despite their simplicity, *Drosophila* have complex organ systems, including the brain system, muscles, gut, heart, and immune system, that function similarly to human organs (Ugur *et al*., 2016). They show complex activities such as learning, memory, courtship, and circadian rhythms, making them ideal candidates for neurological and behavioral studies. *Drosophila's* well-characterized embryonic development has helped researchers understand developmental processes such as segmentation, organogenesis, and tissue patterning (Ugur *et al.,* 2016).

2.2 Use of Hair dye

Hair is a symbol of attractiveness, femininity, masculinity, health, and beauty. As society places a greater emphasis on youth and beauty, hair dying has become popular among both men and women who appreciate such qualities or follow fashion trends. Irrespective of economic and education status, people dye their hair to emphasize the importance given to appearance (Kim *et al.,* 2016). Despite adverse reactions, many people continue dyeing mainly for cosmetic purposes. Hair dyes are a cosmetic product category that can be traced back thousands of years. Globally, hair colorants are a rapidly growing industry of over $7 billion (Kim *et al.,* 2016). Hair dyeing involves treatment of the hair with various natural and/or artificial chemical compounds mainly for cosmetic purposes (e.g., to cover gray hair, to change to a color regarded as more fashionable or desirable at a given time, etc.) (Kim *et al.,* 2016). Natural hair color comes in a wide range of colors and tones, from light blonde and warm brown to fiery red and deep black. Hair dyes can either partially erase natural hair color or add new artificial color, or both (Rust and Schlatter, 2022).

Hair dyes come in two forms: (i) oxidative (permanent) and (ii) non-oxidative (semi-permanent and temporary). Permanent hair dye, which is the most commonly used product type, is formed by an oxidative process involving arylamines to bring about concerns with long-term exposure (Kim *et al.,* 2016). Hence, significant efforts have been put to understand the possible side effects of such exposure including cancer risk. Semi-permanent hair dyes use a combination of preformed dyes to obtain results that last up to six to eight shampoos (Rust and Schlatter, 2022). However, hair dyes and their ingredients are mainly identified to have moderate to low acute toxicity such as the cause of allergic contact dermatitis (Kim *et al.,* 2016). Although some hair dye components are reported to be carcinogenic in animals, such evidence is not consistent

enough in the case of human studies. Consequently, further research is desirable to critically address the significance of this issue, especially with respect to the safety of hair dye ingredients (Kim *et al.,* 2016).

2.2.1 Chemical Components of Hair dyes

Human hair dyes are complicated compositions that modify the natural color of hair by interacting with its keratin structure and melanin pigments. They are composed of a variety of chemical components that differ depending on the type of dye. These components combine in an alkaline environment, usually provided by an alkalizer like ammonia, to penetrate the hair shaft and form larger, permanent color molecules. Modern hair dyes are grouped into three types: oxidative (permanent), direct (temporary or semi-permanent), and natural dyes, with permanent colors being the most frequent and chemically intensive, necessitating precursors, couplers, and oxidizers (He *et al.*, 2022).

Oxidative hair dyes: Oxidative hair dyes are the most important group and have a market share of approximately 80%. (Da França, *et al.,* 2015). Oxidative (permanent) hair dyes differ from other dye categories since they consist of two components that are mixed before use and generate the dye on/in the hair by chemical reactions (Da França, *et al.,* 2015). Their chemistry and use has recently been reviewed. Oxidative dyes contain several ingredients with different functions as follows:

1. Primary intermediates (precursors or color precursors): These are small, colorless molecules that act as building blocks for the finished dye. They penetrate the hair cortex and oxidize, resulting in colorful chemicals. They include arylamines (para-phenylenediamine (PPD), para-toluenediamine (PTD), modified para-diamines), para-aminophenols (4-amino-meta-cresol), 4,5-diaminopyrazole, and pyrimidines (He *et al.,* 2022). The oxidation of these compounds, followed by a reaction with couplers, yields

colored reaction products. The proportion of primary intermediates in on-head hair dye formulations ranges from 0.05% (light hues) to 2.0% (dark shades).

2. Couplers or modifiers: Examples include meta-substituted arylamines or their derivatives (m-phenylenediamines, meta-aminophenols, resorcinol), pyridines, and naphthols (He *et al.,* 2022). Couplers create the final shade by reacting with the oxidized version of initial intermediates, followed by additional oxidative coupling processes. They aid in the creation of specific hues as well as color stability.

3. Oxidants or oxidizing agents: They include hydrogen peroxide, urea peroxide, sodium percarbonate, and perborate (Da França *et al.*, 2015). They serve two functions: bleaching the hair's natural melanin to allow dye penetration, and initiating an oxidation reaction between the precursors and coupling agents, leading them to unite and produce bigger pigment polymers.

4. Alkalinizing agents: such as ammonia, monoethanolamine or aminomethylpropanol is added to raise the pH of the mixture (Sankar *et al.,* 2017). This swells the hair's outer cuticle layer, allowing the small dye molecules to penetrate to the hair's cortex

Direct Hair dyes: Direct hair dyes represent the second category of economically important hair colorants and they include semi-permanent (resisting several shampooing processes) and temporary (resisting one or few shampooing processes) hair dyes

Semi-permanent colouring agents contain low-molecular-weight dye molecules, such as nitro-phenylenediamines, nitro-aminophenols, and some azo or anthraquinone dyes. These dyes may be used on their own in semi-permanent hair dyes or in combination with oxidative hair dyes in permanent hair-dye products to improve the tone of the final colour on the hair (Handa *et al.,* 2012).

Temporary colouring agents are relatively large molecules and include azo-, triphenylmethane-, indophenol- or indamine-type dyes that are less resistant to washing and may be rinsed off by a single or a few shampooing processes (He *et al.,* 2022).

Natural hair dyes

Natural hair dyes are hair coloring options made from plant-based and natural ingredients like henna, indigo, coffee and chamomile tea (Cui *et al.,* 2022). Natural hair dyes are safer option of hair dyes to use as they do not contain harsh chemicals.

2.2.2 Toxicological effects of Hair dyes

Hair dyes can have a variety of toxicological effects since they contain dangerous chemical compounds such as para-phenylenediamine (PPD), ammonia, resorcinol, toluene, and others. These consequences vary from acute allergic responses to severe systemic poisoning and organ damage. Some of the Toxicological effects of Hair dyes include;

Allergic Reactions: The most prevalent toxicological consequence of hair dyes is allergic contact dermatitis, which is predominantly caused by paraphenylenediamine (PPD). PPD accounts for over 75% of all hair dye allergy reactions, causing significant skin irritation, redness, swelling, and itching, particularly on the scalp, face, and neck. Respiratory discomfort might also result from inhaling PPD fumes during application (Nwose *et al.,* 2022).

Acute Poisoning: Oral ingestion or severe exposure to PPD can lead to life-threatening conditions like airway obstruction, asphyxia, and respiratory distress. It can cause rhabdomyolysis (muscle breakdown), acute kidney injury, pneumothorax (lung collapse), and even death if untreated (He *et al.,* 2022).

Skin Irritation: Ammonia, hydrogen peroxide, and resorcinol may cause scalp burns, dryness, or irritation (Nwose *et al.,* 2022).

Respiratory Issues: Ammonia, a common alkalizing agent in hair dyes, can cause respiratory irritation, coughing, and throat burning. Salon workers may face chronic respiratory effects from repeated exposure to ammonia vapors (He *et al.,* 2022).

Endocrine Disruption and Organ Toxicity: Chemicals like resorcinol can disrupt thyroid and reproductive hormones. Toxic effects on the liver, blood, renal, and muscular systems have also been reported from frequent exposure to hair dye chemicals (Nwose *et al.,* 2022).

Carcinogenic Potential: Long-term exposure to aromatic amines in some dyes may increase risks of bladder cancer, non-hodgkin lymphoma, or leukemia, especially in hairdressers (He *et al.,* 2022).

Neurotoxicity: Ingredients such as toluene and Lead acetate (in some progressive dyes) can affect the nervous system with chronic exposure.

Given these risks, patch tests should be done before dyeing hair, exposure duration should be minimal, use of PPD-free or natural alternatives, and following safety guidelines are advised to reduce toxicological harm from hair dyes (Jairoun *et al.,* 2024).

2.3 Survival and locomotor behavior in *Drosophila melanogaster*

2.3.1 Survival studies

Survival, measured as lifespan, mortality rates, or reproductive success, is an important endpoint in toxicological investigations involving *Drosophila melanogaster.* The fruit fly's short lifetime (60-80 days under optimum conditions) and susceptibility to environmental stressors make it an attractive model for examining the impact of toxicants (Lingford *et al.,* 2013). According to research, insecticides, heavy metals, and oxidative agents all drastically limit *Drosophila* longevity. However, few research have

investigated the effect of beauty compounds, such as those found in human hair dyes, on *Drosophila* survival. Hair dye components such as paraphenylenediamine (PPD) and hydrogen peroxide have been shown to cause oxidative stress in mammalian models (Nohynek *et al.,* 2004), but their effects on *Drosophila* survival are mainly unknown.

2.3.2 Locomotor Behavior as an Indicator of Neurological Health

Locomotor behavior in *Drosophila melanogaster* is a well-established indicator of neurological function and fitness (Tomkielska, 2025). Assays like the negative geotaxis (climbing) assay evaluate the fly's capacity to move against gravity, which is susceptible to neurotoxic assaults. Impaired locomotor activity often indicates changes in neural signaling or motor coordination. Locomotor behavior is especially important for researching neurotoxicity since *Drosophila* has conserved neurological pathways with humans, including genes such as para (sodium channel) and Ddc (dopamine synthesis) (Tomkielska, 2025).

2.3.3 Relevance to Hair Dye Exposure

The chemical composition of human hair dyes, which includes oxidative agents and aromatic amines, suggests that they may be toxic to *Drosophila*, affecting survival and locomotion. Other oxidative stressors, such as hydrogen peroxide, have been found to reduce *Drosophila* lifespan and motor activity in a dose-dependent manner. These findings lay the groundwork for hypotheses that hair color exposure may impact survival and motility in comparable ways, possibly through mechanisms such as oxidative stress or neural injury. Investigating these endpoints in *Drosophila* can shed light on the systemic and neurological effects of hair dyes, providing a cost-effective model for determining their safety.

2.4 Neurotoxicity and Gene Expression

Neurotoxic gene expression in *Drosophila melanogaster* is a key indication of cellular and molecular responses to toxicant exposure. *Drosophila* is an ideal model for researching neurotoxicity because of its conserved neurological pathways and well characterized genome, which contains homologs to around 60% of human genes (Mishra *et al.,* 2023). Neurotoxicants change the expression of genes involved in neuronal function, oxidative stress response, and apoptosis, offering insights into toxicity mechanisms (Mishra *et al.,* 2023).

2.4.1 Relevance to Hair Dye Exposure

Human hair dyes contain compounds such as paraphenylenediamine (PPD), hydrogen peroxide, and ammonia, which are known to cause oxidative stress and neurotoxicity in mammalian models (Nohynek *et al.,* 2004). Oxidative stresses have been proven in *Drosophila* to modulate neurotoxic gene expression. Given their neurotoxic potential, hair dye chemicals may affect genes involved in neural signaling or stress responses, potentially contributing to the reported impacts on survival and locomotor behavior.

2.4.2 Genetic markers of neurotoxicity

Genetic markers of neurotoxicity are specific genes (or their expression changes) that reliably indicate neuronal damage, dysfunction, or death in response to toxic insults. Some genetic markers of neurotoxicity include;

1. Wingless(Wnt)

Wnt signaling pathway genes serve as genetic markers of neurotoxicity, particularly in Alzheimer's disease (AD). Amyloid-β (Aβ) induced toxicity disrupts canonical Wnt/β-catenin signaling, reducing β-catenin levels, increasing tau hyperphosphorylation, and promoting neuronal apoptosis ((inestrosa and Toledo, 2008). Dysregulation is evident in AD models, with Wnt activation offering neuroprotection against Aβ effects.

2. Eiger(egr)

Eiger, the Drosophila homolog of TNF superfamily ligands, acts as a genetic marker for neurotoxicity by triggering JNK-mediated neuronal apoptosis in response to cellular stress and damage . Expressed in embryonic neurons and glia, Eiger triggers damage-induced cell death via upregulation of pro-apoptotic gene hid, independent of caspases, but is not required for developmental apoptosis (Shklover *et al.,* 2015). This pathway highlights Eiger's role in stress-induced neuronal loss, and mirrors excitotoxic damage in mammalian systems.

3. Forkhead box(FOXO)

FOXO transcription factors, particularly FOXO3a, function as genetic markers of neurotoxicity in AD and Parkinson's disease. dephosphorylation of FOXO3a activates nuclear translocation, promoting BIM expression and Aβ1-42-mediated neuronal apoptosis in response to β-amyloid oligomers (Sanphui and Biswas, 2013). In Parkinson's, FOXO3 determines dopaminergic neuron fate in the substantia nigra, initially protective via stress resistance genes, but becoming neurotoxic with prolonged α-synuclein accumulation. Genetic variations in FOXO genes are implicated in age-related neuropathies, modulating autophagy and oxidative stress in neuronal survival (Webb and Brunet, 2014).

4. Kelch-like ECH-associate protein 1(Keap1)

KEAP1, a negative regulator of Nrf2, serves as a key genetic marker of neurotoxicity in oxidative stress-related disorders like ALS. KEAP1 dysfunction impairs Nrf2-mediated antioxidant defenses, leading to motor neuron degeneration (Corbo *et al.,* 2021). In keap1-knockout models, constitutive Nrf2 activation enhances neuronal resistance to glutamate- and rotenone-induced oxidative damage via upregulation of phase 2 detoxifying enzymes. Mutations in KEAP1 are therapeutic targets for Nrf2-activation to mitigate reactive oxygen species (ROS)-mediated neuronal loss.

5. Spitz(Spi)

Spi gene enncodes an EGF (Epidermal Growth Factor)-like transmembrane protein that acts as the primary activating ligand for the single *Drosophila* EGF receptor (DER/EGFR). (Pasnuri *et al.,* 2023).

6. Hedgehog(HH)

The hedgehog gene encodes a signallin pathway that transmit information to embryonic cells required for proper cell differentiation. It is secreted and act as morphogen, forming concentration gradients that influences cell differentiation and proliferation (Zhang and Beachy, 2023).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Study area

This experiment focuses on the survival rates, locomotor behaviour (negative geotaxis), and neurotoxic gene expression of *Drosophila melanogaster* exposed to a human hair dye. The experiment was conducted at the Biomedical Toxicology and Chemistry Research (Biotoxcs) Laboratory, located at the University of Benin Central Research Laboratory (CRL), Benin city, Edo state, Nigeria. The human hair dye used for the experiment was gotten from a hair salon.

3.2 Materials used

The materials usec were: falcon tubes, weighing balance, hair dye, 1000 microlitre micropipette, 100 microlitre micropipette, cotton wool, tissue paper, centrifuge, glass jar, foil paper, paint brush, homogenizing stick, eppendorf tubes, polyurethane foam, phosphate buffer, ice pack, distilled water, corn meal, agar, yeast, glucose, nipargin, ethanol, test tubes, beakers, pot, electric cooker, gas cylinder, bunsen burner, hand gloves, masking tape, pen and notebook for recording.

3.2.1 Model organism

The organism used for this study is *Drosophila melanogaster*, it was provided and breeded at the Biomedical Toxicology and Chemistry Research (Biotoxcs)Laboratory, located at the University of Benin Central Research Laboratory (CRL), Benin city, Edo state, Nigeria.

3.3 Methods

3.3.1 Preparation of treatment Concentration

Three (3) treatment concentrations were prepared 0.05%, 0.5%, 2.0%.

To make 5 ml of 0.05% concentration

Volume of dye(µl) = (0.05 x 5 ml) / 100 = 0.0025 ml

Volume of dye(µl) = 0.0025 ml x(1000 ml / 1 ml) = 2.5 µl

Pipette 2.5 µl of dye into a container and add 4.9975 ml of water to make 5 ml of 0.05% concentration, (4997.5 µl).

To make 5 ml of 0.5% concentration

Volume of dye(µl) = (0.5 x 5 ml) / 100 = 0.025 ml

Volume of dye(µl) = 0.025 ml x(1000 ml / 1 ml) = 25 µl

Pipette 25 µl of dye into a container and add 4.975 ml of water to make 5 ml of 0.5% concentration, (4975 µl).

To make 5 ml of 2.0% concentration

Volume of dye(µl) = (2.0 x 5 ml) / 100 = 0.1 ml

Volume of dye(µl) = 0.1 ml x(1000 ml / 1 ml) = 100 µl

Pipette 100 µl of dye into a container and add 4.9 ml of water to make 5 ml of 2.0% concentration, (4900 µl).

3.3.2 Preparation of treatment set up

Two hundred (200) micro-litre of prepared concentration (0.05%, 0.5%, 2.0%) was taken using a micro-pipette and added to 9.8 grams of solidified prepared meal (standard, half standard or quarter standard) in a falcon tube, this mixture was then crushed into the bottom of the tube, and the side of the tube is cleaned with tissue paper, thirty flies were added to the tube and covered with cotton wool.

Table 3.1: Shows the experimental set-up for exposure of *Drosophila melanogaster* to hair dye

Control (A)	200µl of distilled water + 9.8g of meal

Control (B)	200µl of distilled water + 9.8g of meal
Control (C)	200µl of distilled water + 9.8g of meal
Treatment 1 (A)	200µl of 0.05% (Dye) + 9.8g of meal
Treatment 1 (B)	200µl of 0.05% (Dye) + 9.8g of meal
Treatment 1 (C)	200µl of 0.05% (Dye) + 9.8g of meal
Treatment 2 (A)	200µl of 0.5% (Dye) + 9.8g of meal
Treatment 2 (B)	200µl of 0.5% (Dye) + 9.8g of meal
Treatment 2 (C)	200µl of 0.5% (Dye) + 9.8g of meal
Treatment 3 (A)	200µl of 2.0% (Dye)+ 9.8g of meal
Treatment 3 (B)	200µl of 2.0% (Dye)+ 9.8g of meal
Treatment 3 (C)	200µl of 2.0% (Dye)+ 9.8g of meal

Plate 3.1: Shows the experimental set-up for exposure of *Drosophila melanogaster* to hair dye.

3.3.3 Meal preparation

The meal was composed of corn meal, agar, glucose, nipargine, ethanol, yeast, and distilled water at quantities depending on the volume of meal needed. Cornmeal was dissolved in 0.15L of water while the rest of the water is allowed to boil, little hot water was taken and used to dissolve the yeast. The agar was added to the boiling water and left for 10mins, the dissolved cornmeal was then added to the boiling water and stirred as it is added for about 10mins. The yeast dissolved in hot water was then added and stirring continues for about 10-15mins, after which the glucose was added. The nipargin was dissolved in ethanol and then added to the pot. The pot was brought down and allowed to cool and the meal was then distributed to glass jars where the flies will be breeded

Table 3.2: Shows the measurement of the various ingredients needed for meal preparation.

INGREDIENTS	STANDARD MEASUREMENT (g)	HALF STANDARD MEASUREMENT (g)	QUARTER STANDARD MEASUREMENT (g)
Corn meal	52 (g)	26 (g)	13 (g)
Agar	7.9 (g)	3.95 (g)	1.975 (g)
Glucose	3.5 (g)	1.75 (g)	0.875 (g)
Yeast	5 (g)	2.5 (g)	1.25 (g)
Nipargin	1 (g)	0.5 (g)	0.25 (g)
Ethanol	1-2 (mL)	0.5 (mL)	0.25 (mL)
Water	850 (mL)	425 (mL)	212.5 (mL)

3.4 Breeding of flies

The prepared meal was poured into glass jars to cool down and solidified. Then flies were then transferred into the glass jar and covered with polyurethane foam that was pre-cut to fit the jar opening perfectly and securely to prevent escape as well as allowing adequate aeration. Polyurethane form is used in order to allow aeration in the jar. The setup was maintainedx under standard laboratory conditions to facilitate breeding. After approximately 8-10 days, successful reproduction was observed, evidenced by the

presence of larvae and pupae. The breeding procedure was carried out at least three times per week to obtain sufficientnumber of flies for experimental use.

3.4.1 Transfer of Flies into Experimental Vials

Flies were first transferred from the breeding jars into clean, empty jars, this was achieved by gently tapping the jars on a ftat surface to dislodge the flies, after which the polyurethane foam cover was carefully removed. An inverted funnel was then placed over the jar opening, and an empty jar was positioned on top to allow the flies to move upward into the new jar. This process was carried out swifty to minimize fly escape. The jars containing the transferred flies were then placed in a refrigerator for 4-5 minutes to temporarily immobilize the lies. The immobilized flies were carefully poured onto a foil paper placed over an ice packto maintain low temperature during counting. Using a soft paintbrush, the required number of flies were counted and then tranferred into the prepared experimental tubes.

3.4.2 Groupings

Thirty (30) flies were counted and then transferred into the prepared experimental tubes and the opening was securely sealed with cotton wool to prevent escape while allowing proper ventilation. The tubes were observed daily and mortality counts were conducted and recorded.

3.4.3 Homogenization and extraction of supernatant

Appropriately labeled eppendorf tubes were first weighed using a weighing balance, then flies from each experimental groups were then added into their corresponding labeled tubes and weighed in order to determine the exact weight of the flies in each group. Following the weight determination, the flies were homogenized directly inside the eppendorf tubes using a sterile homogenizing stick. Phosphate buffer(PO_4) was then

added in microlitres at a ratio of ten(10) times the calculated weight of flies (in milligrams) to ensure adequate homogenization The eppendorf tubes were then placed inside a centrifuge and set to run at 4000 rpm for seven (7) minutes. Then the supernantant were carefully collected from the samples using a micro-pipette into clean labeled eppendorf tubes and stored in a freezer before analysis.

3.5 Survival study

A survival study was conducted to determine the number of flies that remained alive and active after a certain period of exposure to the treatment.

3.5.1 Pilot study

A pilot study was first conducted to assess the feasibility of the survival study as well as to obtain preliminary data on the survival response of the flies exposed to the dye. For this study, varying concentration of the dye were incorporated into a tube containing cornmeal. The experiment consisted of four(4) treatment groups as outlined below;

Group 1 (Control): Distilled water + cornmeal

Group 2: 0.05% dye + cornmeal

Group 3: 0.5% dye + cornmeal

Group 4: 2.0% dye + cornmeal

Each treatment group was prepared in triplicate, giving a total of twelve(12) experimental tubes. Thirty(30) flies were introduced into each tubes at the beginning of the experiment, the flies were maintained under standard laboratory conditions. Mortality was recorded daily for seven(7) consecutive days, flies were considered dead when they exhibit no movement. The daily counts of dead and surviving flies were used to get the preliminary survival data.

3.5.2 Main survival study

The main survival study was carried out to determine the survival rate of the flies foillowing exposure to hair dye. The experiment also consisted of four(4) treatment groups as outlined below;

Group 1: (Control): Distilled water + cornmeal

Group 2: 0.05% dye + cornmeal

Group 3: 0.5% dye + cornmeal

Group 4: 2.0% dye + cornmeal

Each treatment group was prepared in triplicate, giving a total of twelve(12) experimental tubes. A total of forty three(43) flies were introduced into each treatment tubes at the beginning of the experiment. The flies were maintained under standard laboratory conditions and observed daily for five(5) consecutive days. Mortality was recorded each day and the number of surviving flies per tube was documented to determine the survival rate across all treatments.

3.6 Locomotor behaviour (climbing assay)

Climbing assay also known as negative geotaxis assay is used to assess the locomotor behaviour of the fruit fly. A climbing assay was conducted to determine the number of flies capable of moving against gravity within a given period of time.

A 30 mL (6 cm) mark was drawn on a falcon tube. Ten(10) immobilized flies were then placed into the marked tube following standard fly transfer procedure. The tube was tapped gently to allow the flies settle at the bottom before starting a 6-second timer. The number of flies that crossed the mark within the 6-second time limit were recorded

as successful climbers. Afterward the flies were returned to their respective tubes. The procedure was repeated in triplicate for each treatment group.

Negative geotaxis can thus be calculated as:

Number of flies to cross the mark/ the total number of flies placed in the tube x 100

Negative geotaxix is expressed in percentage (%)

3.7 Gene expression study

Gene expression study in *Drosophila melanogaster* often explores how signaling pathways respond to developmental cues, environmental stresses(e.g., oxidative damage, nutrient availability) and aging. It involves the analysis of patterns and levels of gene transcription throughout its development, from embryo to adult, to understand gene regulation, developmental processes and response to environmental stimuli. The specific genes used in this experiment are;

Spitz(Spi) a ligand of the epidermal growth factor receptor(EGFR) pathway.

Wingless(Wnt) a signaling pathway

Eiger(egr) a TNF superfamily ligand

Forkhead box(FOXO) a Transcription factor

Hedgehog(HH) a Signaling pathway

Kelch-like ECH-associate protein 1(Keap1) a regulator of the Nrf2 homolog CncC

3.7.1 Isolation of Total RNA

Total RNA was isolated from the tissue samples with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNAse I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity confirmed at 260 nm and 280 nm using A&E Spectrophotometer (A&E Lab. UK).

3.7.2 cDNA conversion

One (1 µg) 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 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min (Elekofehinti *et al*., 2020).

3.7.3 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). 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 (Olumegbon *et al*., 2022).

3.7.4 Primer sequences

CNC

Forward GCGTCCCGTTAACTGTCTTTA

Reverse CGTATCTGTAGCTGTGGCTTAG

Wingless

Forward: CAGTTAGTCCGAATGCAGCC

Reverse: GTTCGGGTGATGGATCTTGC

Eiger

Forward: TCGATAATCTCCAGCAGCGT

Reverse: CGCCAACATCATCCACAGAG

Hh droso

Forward CCAGTTGCTATGCGGTGATC

Reverse: GACCTTGTAGAGCGCATTGG

KEAP1

Forward: ATTTATCAGCCGGCGTGTTC

Reverse: CCGTACGTGTTTTGTGGTGT

FOXO

Forward: CAACACAAACGGCTACCACA

Reverse: CACACACAACAACGACGACT

Spi

Forward: GCTGGAGCTGATCGAGAAGA

Reverse: TCGATGATGGCGAAGATGAC

CHAPTER FOUR

RESULT

4.1 Survival study

Table 4.1: Shows the result of the survival study of *Drosophila melanogatser* exposed to various concentration of human hair dye

Dye	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control A	0	0	5	5	6	6
Control B	0	1	3	5	6	6
Control C	0	4	5	5	5	5
0.05% A	0	3	8	10	10	10
0.05% B	0	2	4	4	4	5
0.05% C	0	3	3	7	7	7
0.5% A	0	1	2	3	3	3
0.5% B	0	2	2	3	4	5
0.5% C	0	1	4	6	6	6
2.0% A	0	0	0	1	3	3
2.0% B	0	1	1	2	7	7
2.0% C	0	0	4	9	9	9

4.2 Lomotor behaviour(climbing assay)

During the climbing assay, the flies exhibited varied responses against gravity. While some flies took flight immediately within the 6-second time period, others crawled upwards along the walls of the tubes. Some of the flies also showed contracdictory behaviour, initially moving upwards but later descending down the tube. The assay was conducted in triplicates for each concentration. The mean differences in levels of responses is shown in figure 4.1

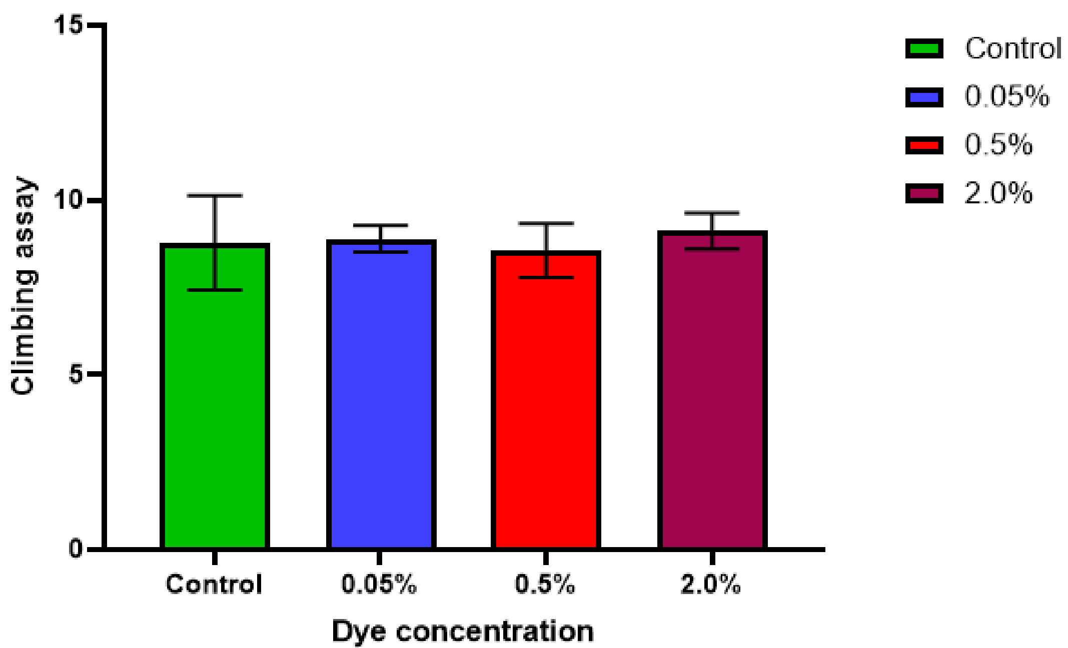
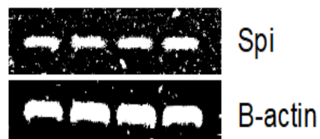


Figure 4.1: Climbing assay responses of *Drosophila melanogaster* exposed to various concentrations of hair dye.

4.3 Spitz(Spi) - (EGFR) pathway

A significant difference in Spi gene expression was observed between the control and the various treatment groups (F=20.75, $p<0.05$). Specifically there was no significant difference in Spi gene between control and 0.05% and 0.5 %. However Spi gene was significantly increased between control and 2.0% group.



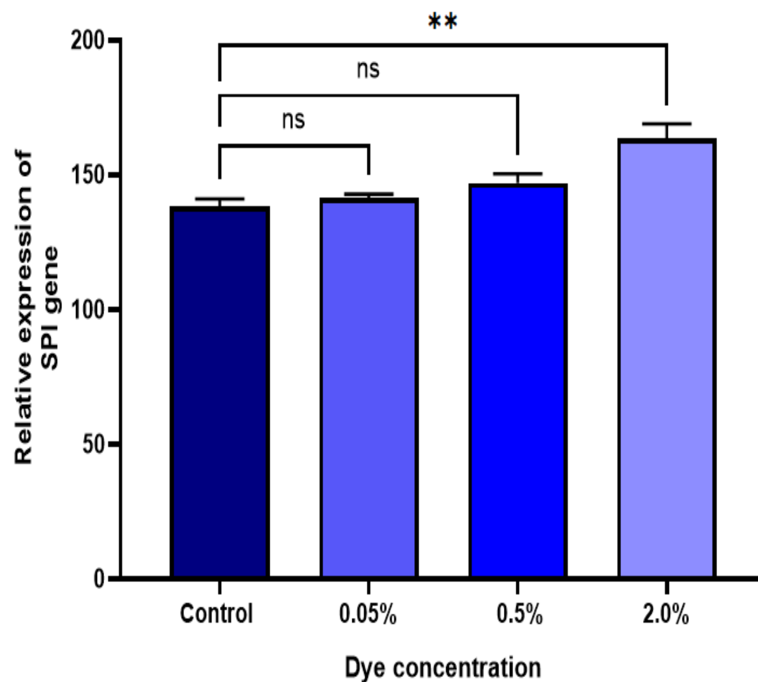


Figure 4.2: The Spi activity in *Drosophila melanogaster* exposed to various concentration of hair dye. Each bar represent the Mean ±SEM, where p<0.05.

4.4 Wingless(Wnt) - Signaling pathway

A significant difference in Wingless gene expression was observed between the control group and the various treatment groups ($F=52.56$, $p<0.05$). Specifically there was no significant difference in Wingless gene between control and 0.05%. However Wingless gene was significantly decreased between control and 0.5% and 2.0% group.

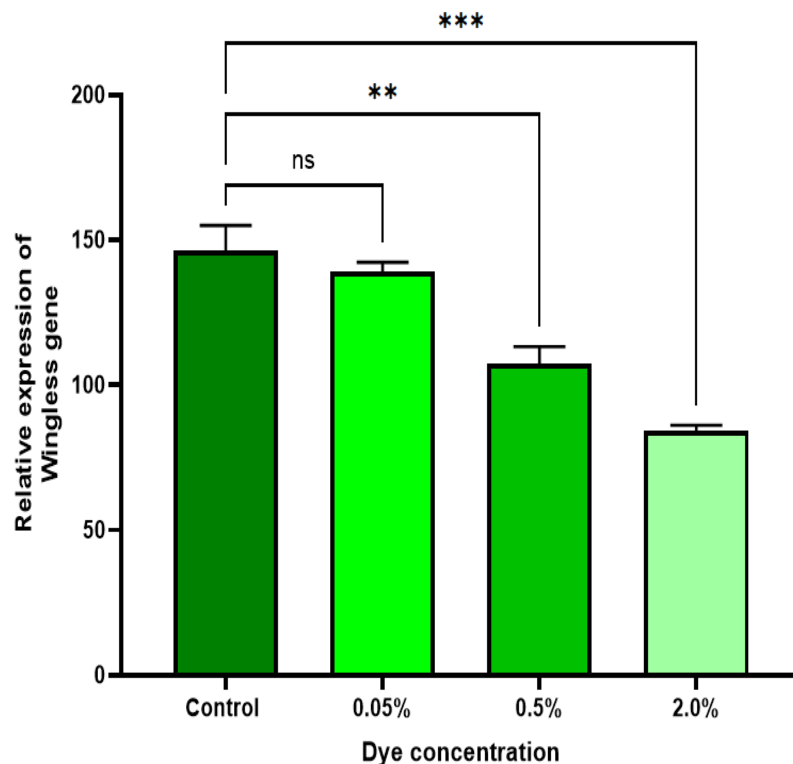


Figure 4.3: The Wingless activity in *Drosophila melanogaster* exposed to various concentration of hair dye. Each bar represent the Mean ±SEM, where p<0.05.

4.5 Eiger(egr) - TNF superfamily ligand

A significant difference in Eiger gene expression was observed between the control and the various treatment groups ($F=42.04$, $p<0.05$). Specifically there was no significant difference in Eiger gene between control and 0.05%. However Eiger gene was significantly increased between control and 0.5% and 2.0% group.

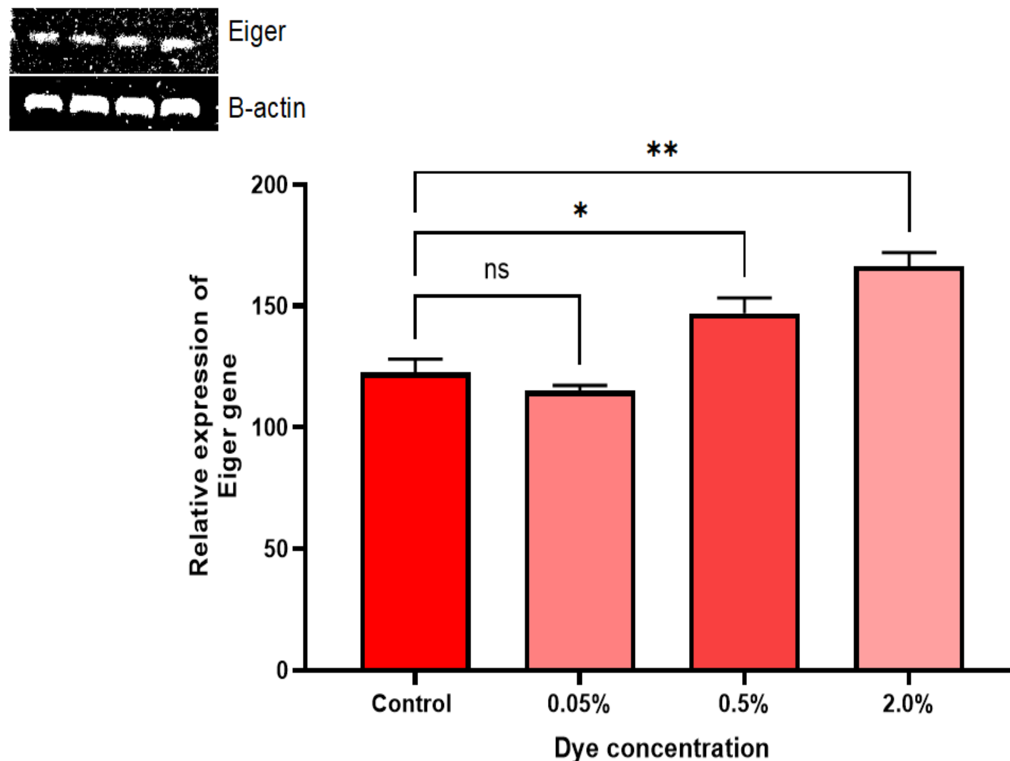


Figure 4.4: The Eiger activity in *Drosophila melanogaster* exposed to various concentration of hair dye. Each bar represent the Mean±SEM, where p<0.05.

4.6 Forkhead box(FOXO) - Transcription factor

A stastitically significant difference in Foxo gene expression was observed between the control and the various treatment groups (F=168.1, $p<0.05$). Foxo gene was significantly decreased across the various treatment groups.

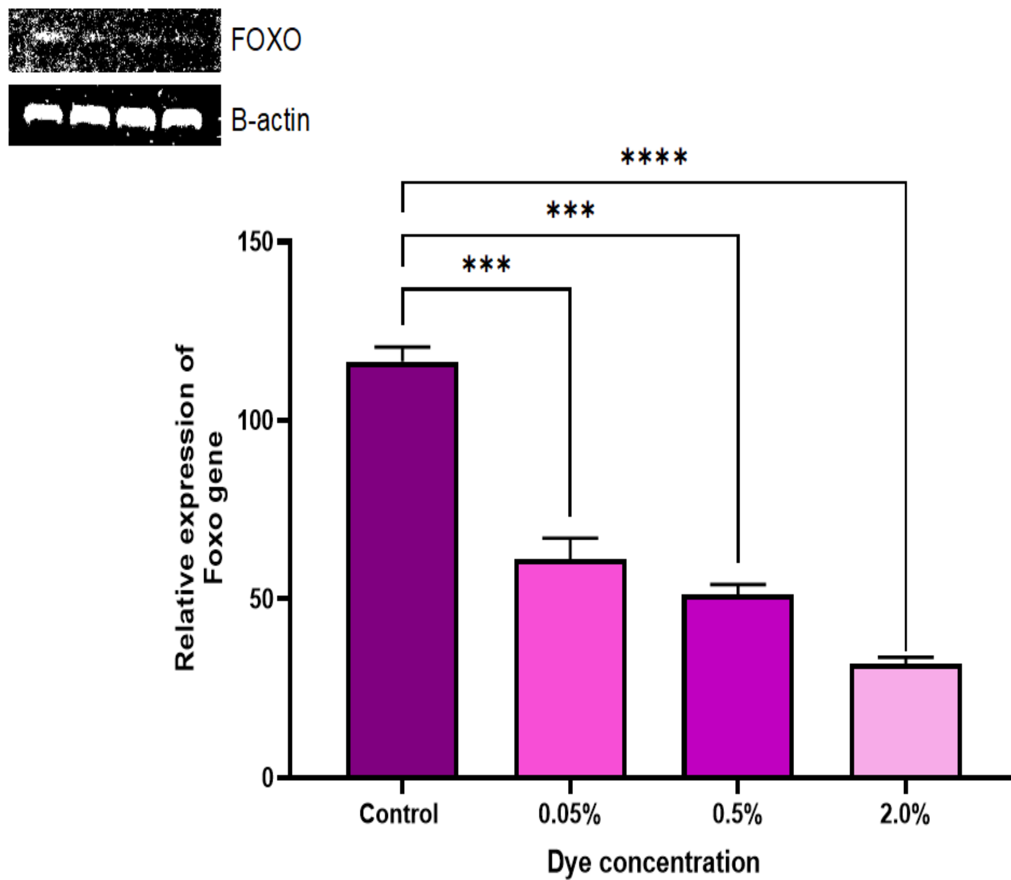


Figure 4.5: The FOXO activity in *Drosophila melanogaster* exposed to various concentration of hair dye. Each bar represent the Mean ±SEM, where p<0.05.

4.7 Hedgehog(HH) - Signaling pathway

A stastistically significant difference in HH gene expression was observed between the control and the various treatment groups (F=62.21, p<0.05). Specifically HH gene was significantly increased across the various treatment groups.

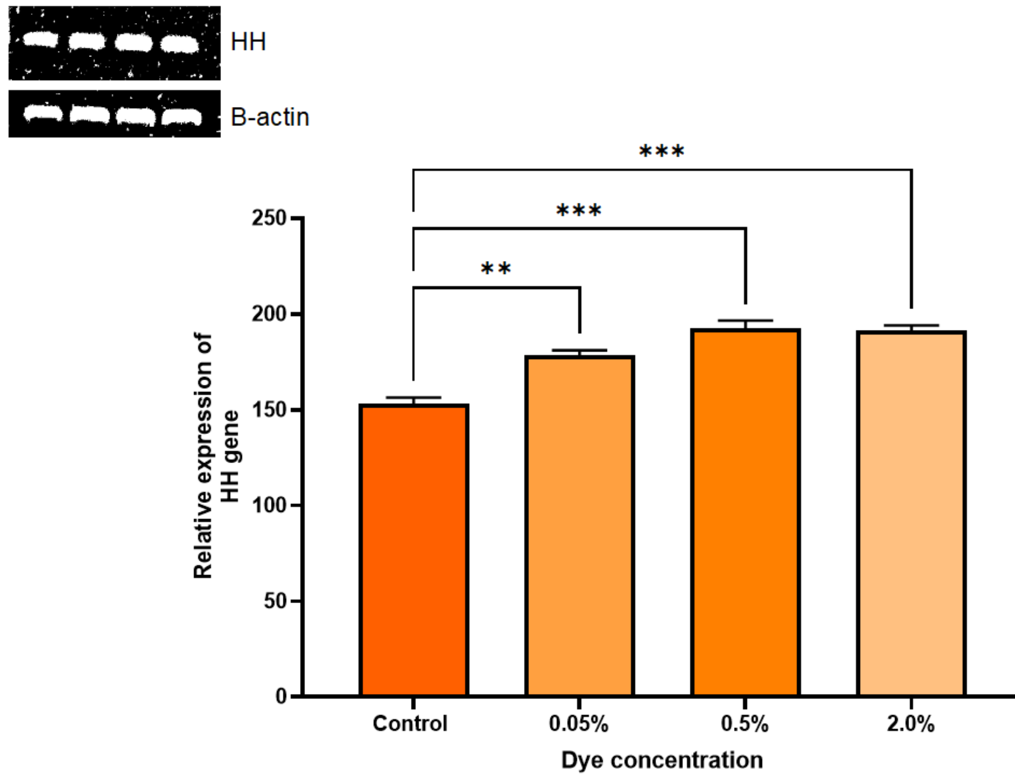


Figure 4.6: The HH activity in *Drosophila melanogaster* exposed to various concentration of hair dye. Each bar represent the Mean ±SEM, where p<0.05.

4.8 Kelch-like ECH-associate protein 1(Keap1) - Nrf2 regulator

A significant difference was observed between the control and the various treatment groups (F=73.14, $p<0.05$). Specifically there was no significant difference in Keap1 gene between control and 0.05%. However Keap1 gene was significantly decreased between control and 0.5% and 2.0% group.

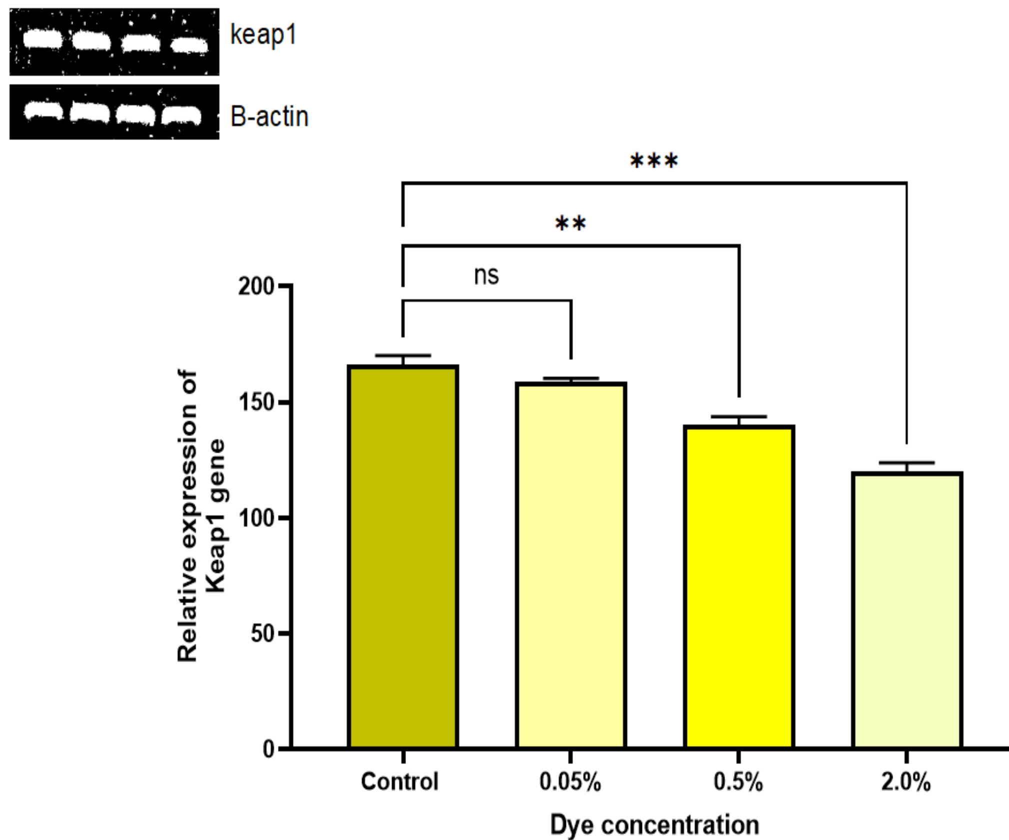


Figure 4.7: The Keap1 activity in *Drosophila melanogaster* exposed to various concentration of hair dye. Each bar represent the Mean ±SEM, where $p<0.05$.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The potential health effects associated with the use of hair dye is a growing concern due to increasing consumer use. This study evaluated the impact of hair dye on the Survival, locomotor behavior and neurotoxic gene expression of *Drosophila melanogaster.* Flies exposed to hair dye exhibited significantly high mortality rates and behavioral impairments compared to the control group, suggesting possible toxicological effects related to its chemical composition.

The survival assay results shows that *Drosophila melanogaster* survival rate decreseases with increasing concentrations, with the number of death increasing each day, this indicate that high concentration of hair dye (2.0%) poses high toxicity while lower doses (0.05%, 0.5%) are less detrimental.

The results from the climbing assay reveal that there was a decrease in the climbing ability of *Drosophila melanogaster* with increasing dye concentration as shown in figure 4.1. The control with no dye exposure shows a high average climbing score indicating normal mobility, as the concentration increases (0.05%, 0.5%, 2.0%), the average climbing score decreases implying that hair dye has an effect on the flies locomotor function becoming more pronounced at higher concentrations.

There was a dose-dependent response of hair dye exposure on Spi gene expression in *Drosophila melanogaster*. Although there was no significant difference in Spi gene expression between 0.05% and 0.5% dye concentrations compared with the control, a

significant increase in Spi gene expression was observed at the 2.0% dye concentration (figure 4.2). This suggests that while low concentrations of hair dye do not appear to affect the gene's expression, a high concentration leads to a significant increase which indicates that high levels of hair dye can activate the EGFR pathway by upregulating the Spi gene, Potentially affecting related biological processes in *Drosophila.* Buchon *et al.* (2010), reported similar EGFR pathway activation via Spi upregulation in *Drosophila* gut remodeling under toxin stress, indicating a conserved response.

Exposure to hair dye significantly affects Wingless gene expression in *Drosophila melanogaster* in a dose-dependent manner. Figure 4.3 shows that no significant change was observed at 0.05% dye concentration compared with control suggesting a threshold effect. However, a significant decrease in Wingless gene expression was observed at 0.5% and 2.0% dye concentrations compared with control, indicating a dose-dependent inhibitory effect where higher concentrations of hair dye lead to a significant decrease in the expression of the Wingless gene. The Wingless signaling pathway is crucial for many developmental processes, cell proliferation, and differentiation. A decrease in its expression could have significant biological consequences for the organism, potentially leading to developmental defects or other health issues. Similar findings were reported by Zou *et al*. (2000), who identified a suppression of Wnt/Wg pathway under oxidative stress in *Drosophila* exposed to paraquat.

A dose-dependent increase was observed in Eiger gene expression in *Drosophila melanogaster* when exposed to varying concentrations of hair dye. Figure 4.4 shows a significant difference in Eiger gene expression across the treatment groups with no significant change between control and 0.05% dye concentration, but a notable increase at 0.5% and 2.0% concentrations compared with control. This suggests that higher dye concentrations may upregulate Eiger expression, a TNF superfamily ligand, potentially

indicating a stress response or toxicity effect in the flies. Similarly. Kodra *et al.* (2020), observed Eiger upregulation in response to metabolic stress fat body tissue.

Figure 4.5 illustrates the impact of hair dye concentrations on Foxo gene expression in *Drosophila melanogaster*. The dose-dependent decrease in Foxo expression implies that hair dye components likely interfere with the transcriptional activity or expression of this gene, which is critical for stress resistance and longevity. Barretto *et al.* (2020), reported similar findings, where hypoxia exposure lead to induced Foxo transcriptional activity in *Drosophila melanogaster.*

There was a statistically significant dose-dependent increase in HH gene expression across the treatment groups compared to the control (figure 4.6) This suggests that hair dye components may act as environmental stressors or signaling modulators, upregulating the Hedgehog signaling pathway in *Drosophila melanogaster*. Similar findings were reported by Lu *et al.* (2017), who found enhanced HH expression in *Drosophila* wing pouch cells under survival stress, where pathway activation promotes cell viability against environmental perturbations.

Additionally, the Keap1 gene associated with the Nrf2 regulator (a key player in oxidative stress response), shows a dose-dependent decrease in gene expression with increasing hair dye concentration (Figure 4.7). At low concentration (0.05%), the effect is negligible, suggesting a threshold that hair dye does not significantly impact Keap1 expression. However At higher concentrations (0.5% and 2.0%), there is a statistically significant decrease implying that hair dye exposure may significantly downregulates Keap1 expression in *Drosophila melanogaster*, potentially affecting the organism's ability to handle oxidative stress. Sykiotis and Bohmann (2008), reported similar findings where Keap1 loss-of-function mutations affects oxidative stress tolerance in *Drosophila* exposed to environmental xenobiotics.

5.2 Conclusion

From this study, we can conclude that human hair dye has significant negative impacts on *Drosophila melanogaster*. Exposure to the dye resulted in increased mortality, impaired climbing ability, and notable alterations in the expression of key neurotoxic genes. Higher concentrations of hair dye led to complete mortality, indicating a potent toxic effect. While certain exposure levels may have minimal effects, excessive concentrations can lead to negative outcomes on survival and neurological functions. As observed in the case of hair dye, some components exhibit increased toxicity with prolonged exposure and higher doses. These findings suggest that moderate exposure to hair dye may be preferable to higher concentrations, as excessive exposure, poses potential health risks. The observed decrease in climbing ability suggests neurological dysfunction. Specifically, the findings reveal that high concentrations of hair dye can disrupt critical molecular mechanisms such as cell signaling (Spi, Wnt, HH), stress response (FOXO, Keap1), and apoptosis (Eiger), indicating potential oxidative and genotoxic consequences.

5.3 Recommendations

Further investigation is needed to elucidate the precise molecular mechanisms by which human hair dye exerts its toxicity. This could involve studying its interaction with specific cellular targets, signaling pathways, and metabolic processes. Focus should be placed on how the components of the dye induce neurotoxic stress and how they interfere with the neurotoxic response. Regulatory agencies should promote awareness of the potential health hazards associated with excessive or frequent hair dye use. Additionally, investigating the toxicity of human hair dye in other organisms, including other insect species and mammalian cell lines, would provide a broader understanding

of its potential health risks. This would help determine if the observed effects are specific to *Drosophila* or if they translate to other species, including humans. While *Drosophila melanogaster* serves as a useful model, ultimately, research should investigate the potential health implications of hair dye use in humans. This could involve epidemiological studies correlating dye exposure with health outcomes, as well as in vitro studies using human cell lines to assess its toxicity. If hair dye is shown to pose health risks, research should explore potential mitigation strategies such as the use of plant-based or natural dye alternatives to reduce chemical exposure risk

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APPENDIX

Climbing assay

Dye	T 1	T 2	T 3	Mean
Control A	10	10	10	10
Control B	7	8	7	7.33
Control C	9	9	9	9
0.05% A	8	9	9	8.67
0.05% B	10	9	9	9.33
0.05% C	8	8	10	8.67
0.5% A	8	10	9	9
0.5% B	9	9	9	9
0.5% C	8	7	8	7.67
2.0% A	8	9	9	8.67
2.0% B	9	8	10	9
2.0% C	10	9	10	9.67

Pilot Survival study

Dye	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control A	0	1	2	2	2	3	3	4
Control B	0	0	1	1	2	2	2	2
Control C	0	0	0	2	2	2	2	3
0.05% A	0	0	3	6	7	11	18	20
0.05% B	0	0	5	5	5	11	18	20
0.05% C	0	0	4	8	8	12	20	22
0.5% A	0	3	4	5	6	8	16	20
0.5% B	0	1	5	8	10	13	22	23
0.5% C	0	1	1	7	8	11	22	23
2.0% A	0	1	1	2	5	8	23	25
2.0% B	0	3	5	5	5	8	15	18
2.0% C	0	1	3	5	6	12	18	18