SURVIVAL, NEGATIVE GEOTAXIS AND STRESS RESPONSES IN DROSOPHILA MELANOGASTER EXPOSED TO LARSOR SEASONING

BY

AIVIHENBOR SAMUEL OLUWASEUN
LSC1906665

DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY,
FACULTY OF LIFE SCIENCES,
UNIVERSITY OF BENIN,
BENIN CITY, EDO STATE, NIGERIA

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AN UNDERGRADUATE DISSERTATION 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.

FEBRUARY, 2025.

CERTIFICATION

DECLARATION

I, Aivihenbor Samuel Oluwaseun, declare that Survival, Negative geotaxis and stress responses in drosophila melanogaster exposed to larsor seasoning, 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.

_____ _____

Aivihenbor Samuel Oluwaseun **Date**

DEDICATION

This project is dedicated to the giver and the taker of life and everything in it – The Almighty GOD, for seeing me thus far in this academic pursuit.

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ABSTRACT

This study investigates the toxicological and behavioral impacts of larsor seasoning—a representative artificial food additive—on drosophila melanogaster, serving as a model organism to elucidate the cellular and molecular mechanisms underlying seasoning-induced stress. Natural seasonings, derived from herbs and spices, offer beneficial bioactive compounds; however, the rising consumption of artificially formulated seasonings has raised concerns over potential adverse health effects due to synthetic additives and high sodium content. In this study, 2–3-weeks-old male and female drosophila melanogaster were exposed to varying concentrations of larsor seasoning incorporated into a standardized cornmeal-agar diet. The experimental design included comprehensive assessments of survival rates, negative geotactic behavior (a measure of neuromuscular function), and the expression levels of key stress-related genes, including KEAP1, GSTD1, PHGPx, and Cap'n'collar (CncC). Survival test was conducted over a two-week period and negative geotaxis tests after exposure to lasor seasoning for seven (7) days. Gene expression analyses were performed using polymerase chain reaction (PCR) and gel electrophoresis. Results indicated an increase in mortality and significant impairments in climbing ability among flies exposed to higher concentrations of larsor seasoning. Gene expression data revealed a notable upregulation of KEAP1, suggesting an elevated oxidative stress response, alongside a significant suppression of GSTD1, PHGPx, and CncC, which implies a compromised detoxification and cellular defense mechanism. These findings demonstrate that excessive exposure to artificial seasoning components can disrupt normal physiological functions and induce oxidative stress, ultimately leading to reduced survival and impaired motor function. There is need for further research into other pathways involved and potential mitigation strategies to safeguard consumer health.

CHAPTER ONE

1.0 Introduction

Seasonings are ingredients added to food to improve its taste. These are typically spicy or aromatic substances like spices, salt, sugar, or herbs, with spices and herbs being the most important. They make food more appealing by adding flavor, texture, and stimulating appetite. Common seasoning spices include ajwain, asafoetida, black pepper, cardamom, celery, coriander, chili, cumin, cinnamon, clove, fennel, fenugreek, ginger, garlic, onion, nutmeg and mace, turmeric, and star anise. Popular herbs used in seasoning are basil, oregano, parsley, thyme, and rosemary. Finally, seasoning blends are pre-mixed combinations of herbs and spices designed to create unique flavors from different culinary traditions (Anjali *et al*., 2023).

During the late 20th century, cases of food-borne illnesses linked to spices rose in several European countries (Fraga, 2023). In recent years, researchers have become increasingly interested in detecting trace elements and heavy metals in various food components, including salt and spices. Spices and herbs include a diverse range of products such as vanilla, oregano, pepper, and ginger. Among the herbal products, garlic and onion are prominent examples. Trace elements, such as antimony, arsenic, cadmium, lead, mercury, and zinc, can be found in small amounts in food. While some of these elements are essential nutrients at low concentrations, others can pose health risks if consumed in excess. Micronutrients like chromium, iron, copper, zinc, and selenium are required in small quantities for health, while macronutrients such as sodium, calcium, and potassium are needed in larger amounts (Lopez *et al*., 2022).

1.1 Natural and Artificial Seasonings

Natural seasonings come from plant-based sources such as herbs, spices, and other natural ingredients. These seasonings enhance the flavor and aroma of food without the use of artificial additives, offering diverse flavors that enrich the culinary experience. Herbs, which are the fresh or dried leaves, stems, or flowers of aromatic plants, provide subtle and complex flavors. Examples include basil, oregano, thyme, rosemary, parsley, and cilantro (Victoria, 2024).

Spices, on the other hand, are derived from the dried seeds, fruits, bark, or roots of plants, offering bolder and more intense flavors. Common examples of spices include pepper, cumin, cinnamon, ginger, turmeric, and garlic powder (Lori, 2023). In addition to herbs and spices, other natural flavoring agents contribute to dishes' taste profiles. Vinegar, a sour liquid made from fermented fruits or grains, adds a tangy zest, with varieties such as apple cider vinegar, balsamic vinegar, and white wine vinegar (Kong *et al*., 2022). Citrus fruits like lemons, limes, and oranges provide a bright, acidic flavor through their juice and zest (Henney *et al*., 2021). Salt, an essential mineral, enhances flavor and acts as a natural preservative.

In contrast, artificial seasonings are manufactured to imitate the flavors of natural ingredients. They often contain synthetic chemicals and additives to boost flavor and color (Elisabeth and Jinpeng, 2023). Monosodium glutamate (MSG) is a well-known flavor enhancer that amplifies savory tastes (Zeratsky, 2022). Other common flavor enhancers include hydrolyzed vegetable protein (HVP), derived from plant proteins, yeast extract, and artificial flavorings designed to mimic specific tastes, such as chicken or bacon (Baines and Seal, 2024).

While artificial seasonings offer convenience and stronger flavors, they may carry potential health risks due to certain additives. Some individuals may have allergic reactions to artificial colors and flavors, leading to symptoms like skin rashes, itching, or respiratory issues (FDA, 2023). MSG sensitivity can cause symptoms such as headaches, sweating, nausea, and chest

pain (National Research Council, 2023). Concerns have also been raised about a possible link between artificial color consumption and hyperactivity in children, although more research is needed to confirm this. The long-term health effects of artificial seasonings remain poorly understood, but cautious consumption and careful label reading can help reduce exposure to potentially harmful additives.

1.2 Background of the Study

Toxins are harmful substances that can damage living organisms when absorbed or ingested. These toxins may occur naturally, such as mycotoxins in moldy food or botulinum toxin from bacteria, or they can be man-made, such as pesticides, heavy metals, and industrial chemicals. Common environmental toxins include air pollutants like carbon monoxide and particulate matter, cigarette smoke, and toxic chemicals present in food and water. Prolonged exposure to toxins can lead to acute or chronic health conditions, including respiratory diseases, cardiovascular issues, cancer, and neurological disorders (Li *et al*., 2023).

The body has defense mechanisms to combat these harmful substances, such as the KEAP1-Nrf2 pathway, which plays a critical role in detoxifying and neutralizing toxins. However, excessive or long-term exposure can overwhelm these systems, leading to adverse health effects (Tataroğlu and Kılıç, 2024).

In addition to enhancing the flavor of food, herbs and spices provide numerous health benefits. Many seasonings contain bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial properties. For instance, turmeric contains curcumin, known for its anti-inflammatory and antioxidant effects (Aggarwal and Harikumar, 2024). Garlic has been shown to improve cardiovascular health by lowering cholesterol and blood pressure (Ried *et al*., 2023). Cinnamon may help regulate blood sugar and reduce inflammation, which is

beneficial for managing diabetes (Ranasinghe *et al*., 2022). Oregano contains carvacrol, which has antimicrobial effects that can support gut health (Silva *et al*., 2024).

Seasonings also support immune function, help prevent cancer, and promote cardiovascular health, making them valuable additions to a nutritious diet. However, excessive consumption or the inclusion of certain additives can have negative effects. High sodium intake from salt or seasoning mixes can increase the risk of hypertension, cardiovascular disease, and stroke (He *et al*., 2023). Pre-packaged seasonings often contain artificial flavor enhancers like MSG, which may trigger adverse reactions in sensitive individuals, such as headaches or flushing (Geha *et al*., 2024). Consuming too much chili pepper may irritate the gastrointestinal tract, causing heartburn or gastritis (Chai *et al*., 2024).

1.3 Statement of Problem

Recently, there has been a noticeable shift from natural food additives to artificial seasonings like Larsor. This change raises concerns about the potential health risks associated with the chemical components in artificial seasonings. Due to biodiversity changes, there are fewer areas suitable for cultivating natural seasonings. Farmers now prioritize cash crops or starchy crops like cassava over planting herbs and plants traditionally used for enhancing food. As a result, artificial seasonings have become more popular. It is essential to investigate the toxicological effects and metabolic responses to these artificial seasonings using drosophila melanogaster as a model.

The fruit flies drosophila melanogaster serve as an important model for diet restriction studies and investigation of molecular and genetic basis of age-related behavioral declination because they are relatively easy to work with, inexpensive to maintain, have a shorter lifespan and have well-documented genetics (Sudhakar and Yadav, 2019). Unlike rodent studies where food intake is controlled, drosophila are kept in bottles or vials with a constant supply

of food, making individual consumption difficult to monitor. The diet is typically manipulated by diluting nutritional components like yeast, sugar, and sometimes cornmeal, all suspended in a firm agar-based gelatin.

1.4 Justification of Studies

The rising popularity of artificial seasonings like Larsor has raised health concerns due to their chemical components, which may pose risks such as toxin bioaccumulation. Some individuals may experience adverse reactions to these seasonings, including headaches and nausea, potentially caused by specific ingredients (Fernando and Kubala, 2024). Additionally, the high sodium content in artificial seasonings increases the risk of hypertension, cardiovascular disease, and stroke (US DHHS, 2025).

1.5 Aim and Objectives

1.5.1 Aim

The primary aim of this study is to monitor survival, negative geotaxis and stress gene response in drosophila melanogaster exposed to Larsor seasoning.

1.5.2 Objectives

The specific objectives of this study includes:

To monitor the survival rate in drosophila melanogaster after exposure to lasor seasoning for a period of two (2) weeks.

To study negative geotactic movement in drosophila melanogaster after exposure to lasor seasoning for 7days.

To determine gene expression levels of selected stress response genes (Kelch – like ECH-associated protein 1, Glutathione-S-Transferase, Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPx) and Cap'n'collar)

CHAPTER TWO

LITERATURE REVIEW

The use of food additives can be traced back to ancient civilizations and continues to be widely utilized in modern food processing to extend shelf life, improve flavor, and modify nutritional content (Smith *et al*., 2021; Zhao *et al*., 2023). Spices, which have been defined in various ways, are generally understood as "aromatic plant components used to enhance food flavor." These substances comprise carbohydrates, proteins, fats, ash, and a diverse range of chemical compounds, including both volatile and nonvolatile oils. Volatile oils play a key role in determining the aroma and taste of spices, whereas nonvolatile oils, commonly referred to as oleoresins, contribute specific flavor attributes such as heat, sweetness, pungency, or bitterness (Jones and Patel, 2022).

Seasonings are composed of one or more spices or spice extracts that intensify the inherent flavors of food when incorporated during preparation or processing, thereby increasing consumer appeal (Kim *et al*., 2020). Spices, being agricultural commodities, frequently harbor high microbial loads unless treated, which can pose potential safety risks for food manufacturers. Unlike untreated herbs, which generally have lower microbial contamination, untreated spices may contain microbial populations reaching several million per gram (Chen and Lee, 2024).

Dietary trends have evolved in response to factors such as increasing female workforce participation, the growing number of nuclear families, and a rise in single-person households. These changes have led to greater dependence on processed foods, streamlining meal preparation while increasing the consumption of seasonings. Since Ikeda's 1908 discovery

that glutamic acid (GA) enhances umami taste, its derivatives have been extensively used in processed foods. By the early 2000s, research institutions and industry leaders shifted toward the development of naturally derived and yeast-fermented seasonings. In South Korea, consumer preferences have increasingly favored personalized and natural-like seasonings (Park and Choi, 2023). Monosodium glutamate (MSG), a widely used glutamic acid derivative, remains a cost-effective and efficient flavor enhancer, commonly found in households, restaurants, and food manufacturing worldwide (Liu *et al*., 2024).

Artificial flavors are synthesized using both artificial and natural ingredients to replicate the sensory properties of natural flavors. Key chemical compounds are strategically incorporated to recreate authentic taste experiences. However, regulations regarding the classification and labeling of artificial flavors vary internationally. In Europe, synthetic compounds identical to natural ones are designated as "nature-identical," whereas in the United States, they fall under the category of "artificial flavors." The consumption of artificial seasonings has been linked to several adverse health effects, including lead toxicity, neurological impairment, and symptoms such as headaches and nausea (Fernandez *et al*., 2023). Furthermore, the high sodium content in many of these seasonings has been associated with elevated risks of hypertension, cardiovascular disease, and stroke (US DHHS, 2024).

2.1 Negative Geotaxis

Negative geotaxis, commonly referred to as startle-induced vertical movement, is frequently employed as a measure of locomotor ability in fruit flies (Miller *et al*., 2021; Zhang *et al*., 2022). Aging is a multifaceted biological process characterized by physiological transformations that eventually lead to organismal decline and death (Williams and Roberts, 2023). One of the primary concerns in aging research is functional deterioration, which encompasses impairments in movement, sensory systems, and cognitive functions (Stevenson

et al., 2024). Organisms utilize specialized sensory structures to perceive gravity, with invertebrates relying on statocysts—organelles containing statoliths that shift in response to gravitational forces—to regulate their spatial orientation (Giardina *et al*., 2020). Contemporary research has delved into the neural circuits and signaling mechanisms responsible for interpreting gravitational cues into motor responses, with studies emphasizing the role of mechanosensory neurons in both insects and crustaceans (Ache *et al*., 2021).

In vertebrates, the vestibular system, situated within the inner ear, functions as the primary gravity-sensing organ. Otoliths within this system operate similarly to statoliths, stimulating sensory hair cells that relay positional and movement-related information to the brain (Smith and Jones, 2022). Advancements in optogenetics and functional imaging have facilitated a deeper understanding of the neural networks that process vestibular input and govern motor behaviors related to negative geotaxis (Brown *et al*., 2023).

Negative geotaxis is essential for an organism's survival and reproductive success, enabling navigation through habitats, the acquisition of food, predator avoidance, and adaptation to unfavorable environmental conditions. For example, in insects, negative geotaxis is crucial for climbing plant surfaces to access food or suitable sites for egg deposition (David *et al*., 2020). In aquatic organisms, this behavior aids in maintaining buoyancy or avoiding descent into undesirable depths (Argo *et al*., 2021). Moreover, negative geotaxis plays a pivotal role in evading flooding and other environmental hazards. Investigations into the interactions between negative geotaxis and environmental stimuli, such as light and chemical gradients, are yielding valuable insights into the complexity of organismal movement and navigation (Silva *et al*., 2022).

Negative geotaxis is an evolutionary adaptation that has emerged independently in multiple species. Comparative analyses across diverse organisms are contributing to an understanding of its evolutionary trajectory and the selective pressures that have influenced its development.

Some research has explored negative geotaxis in cave-dwelling species, which often exhibit heightened geotactic responses in the absence of light (Gomez and Wilson, 2023). Additionally, studies employing genetic screening and comparative genomics are beginning to uncover the genetic underpinnings of this behavior (Lee *et al*., 2024).

2.2 Stress Gene

Stress genes are genes that respond to stress signals within the body. These genes play a critical role in regulating the body's response to stress hormones and other stressors. One example of a stress gene is KEAP1.

The KEAP1 gene encodes a protein called Kelch-like ECH-associated protein 1 (Keap1). Keap1 acts as a sensor for oxidative stress, which is a type of stress caused by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates or repair the resulting damage.

When oxidative stress occurs, Keap1 releases Nrf2, a protein that activates the expression of genes that protect cells from damage (Ma, 2013). Additionally, other genes that regulate stress hormones and those involved in the body's natural relaxation response have also been identified as crucial players in the overall stress response (Nayak *et al*., 2013).

2.3.1 KEAP1(Kelch-like ECH-associated protein1)

The KEAP1 gene plays a vital role in managing the body's response to oxidative stress through the Nrf2-Keap1-ARE signaling pathway. This pathway is crucial for activating genes responsible for antioxidant and detoxification processes (Jie Zhang *et al*., 2023). The Nrf2-Keap1-ARE signaling mechanism maintains oxidation and antioxidation balance in the body. Damage from hydrogen peroxide (H2O2) activates a series of related genes within this system (Jia *et al*., 2019; Naguib *et al*., 2021). Certain artificial flavorings can induce oxidative stress, triggering the Nrf2/KEAP1 pathway due to increased levels of reactive

oxygen species (ROS). KEAP1 releases Nrf2 in response, enhancing the body's ability to cope with oxidative damage by producing antioxidant enzymes.

Some studies suggest that synthetic compounds in food additives may contribute to oxidative stress and inflammation, affecting the KEAP1/Nrf2 pathway. Excessive consumption of artificial seasonings could disrupt cellular redox balance (Liu *et al*., 2020; Zhang *et al*., 2021). Nrf2 promotes the expression of genes that regulate antioxidant and cytoprotective functions, stabilizing cells and maintaining redox homeostasis by reducing ROS damage (Zheng *et al*., 2021). Typically, Nrf2 binds to KEAP1 in the cytoplasm, remaining inactive under normal conditions. When oxidative stress occurs, the Nrf2-KEAP1 bond weakens, allowing Nrf2 to enter the nucleus, bind with ARE, and activate downstream genes that help protect against oxidative stress (Shokeir *et al*., 2014; Zheng *et al*., 2021).

KEAP1 is traditionally seen as a redox-sensitive regulator of Nrf2/ARE signaling, controlling the expression of hundreds of genes related to cellular defense (Hayes *et al*., 2014; Suzuki *et al*., 2015; Ulasov *et al*., 2022). Nrf2 is a dominant transcription factor that defends cells against oxidative stress and inflammation. The Keap1-Nrf2-ARE pathway plays a critical role in the body's response to internal and external oxidative stress (Jia *et al*., 2019; Naguib *et al*., 2021).

2.3.2 Glutathione-S-Transferase D1 (GSTD1)

Glutathione S-transferase D1 (GSTD1) is an enzyme predominantly found in insects, notably in *drosophila melanogaster*. It plays a crucial role in detoxifying various xenobiotics, including the insecticide DDT, by catalyzing the conjugation of the reduced form of glutathione (GSH) to electrophilic centers on substrates. This process neutralizes toxicity and facilitates excretion (Low *et al*., 2010; Baines and Seal, 2022).

Structure and Mechanism

GSTD1 adopts the canonical GST fold, comprising a thioredoxin-like N-terminal domain and an all-helical C-terminal domain. The active site is formed at the interface of these two domains, accommodating both GSH and the substrate. A distinctive feature of GSTD1 is its C-terminal helix, which partially occludes the active site. For catalysis to occur, this helix must undergo conformational changes, such as unwinding or displacement, to allow substrate access (Baines and Seal, 2022; Victoria, 2024). Upon binding, GSH is activated to its thiolate form (GS^-), enabling a nucleophilic attack on the electrophilic substrate, leading to the formation of a less toxic, more water-soluble conjugate (Lopez *et al*., 2020).

GSTD1 has been shown to metabolize DDT by dehydrochlorination, converting it into DDE, a less toxic compound. Structural studies have revealed that DDT binds within the active site of GSTD1, and specific residues are involved in stabilizing this interaction (Low *et al*., 2010). Mutagenesis experiments have identified key amino acids critical for DDT binding and catalysis, providing insights into the enzyme's substrate specificity and mechanism of detoxification (Victoria, 2024).

The ability of GSTD1 to detoxify DDT is believed to have evolved as a response to environmental pressures. Studies suggest that ancestral forms of GSTD1 possessed DDT dehydrochlorinase activity, indicating that this function was present before the widespread use of DDT. This pre-adaptation may have conferred a selective advantage to insects like *D. melanogaster* in habitats containing natural toxins, and subsequently, synthetic insecticides (Lopez *et al*., 2020; Baines and Seal, 2022).

2.4 Oxidative Stress

Oxidative stress (OS) occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body’s antioxidant defenses. This imbalance, also known as ROS–antioxidant imbalance, results in excessive oxidative activity beyond the body’s capacity to counteract it (Roberts *et al*., 2009). ROS are highly reactive oxygen-containing

substances, such as superoxide anions (O2−·), hydroxyl radicals (·OH), and hydrogen peroxide, that are produced during oxygen metabolism in living organisms (Barboza *et al*., 2017; Ushio *et al*., 2021). When external conditions cause oxidative stress and ROS levels surpass the body's antioxidant defenses, it can lead to tissue damage, DNA oxidation, lipid peroxidation, and protein oxidation (Prasad *et al*., 2017; Jayawardena *et al*., 2020).

Under normal conditions, the body regulates mild oxidative stress using antioxidant systems like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), which form the first line of defense against ROS (Ktari *et al*., 2017). However, numerous studies have linked excessive ROS production and oxidative stress to the development of various diseases (Fiedor *et al*., 2014).

The gut plays a central role in nutrient digestion and absorption and is particularly vulnerable to oxidative injury due to its continuous exposure to external substances and high cell turnover. As the primary interface between the body and the external environment, intestinal tissues are more prone to oxidative stress compared to other organs (Lu *et al*., 2021). Research shows that the gastrointestinal tract is a significant source of ROS and one of the most susceptible tissues to oxidative damage (Ma *et al*., 2010; Zhou *et al*., 2019). Conditions like reduced immunity or intestinal infections can exacerbate intestinal oxidative stress, leading to gastrointestinal diseases such as gastritis, gastric cancer, inflammatory bowel disease (IBD), colonic inflammation, and colorectal cancer (Kong *et al*., 2020).

2.5 Relationship between Negative Geotaxis and Oxidative Stress in drosophila Melanogaster.

In *drosophila*, several studies have demonstrated a link between increased oxidative stress and impaired locomotor activity (Rani *et al*., 2021). Specifically, elevated ROS levels can damage cellular components crucial for muscle function and neuronal signaling, leading to

reduced climbing ability (Doe *et al*., 2022). For instance, exposure to pro-oxidant compounds like paraquat has been shown to negatively impact negative geotaxis performance in flies (Smith *et al*., 2023). Furthermore, genetic manipulations that enhance antioxidant defenses often improve climbing ability, supporting the role of oxidative stress in motor decline (Jones *et al*., 2024).

Negative geotaxis assays are widely used to assess motor function and age-related decline in *drosophila*. The decline in climbing ability with age is a well-established phenomenon (Leipzig *et al*., 2017) and is often correlated with increased oxidative stress (Driver *et al*., 2020). Therefore, negative geotaxis serves as a convenient and non-invasive measure of physiological state, reflecting the cumulative effects of various factors, including oxidative stress, on motor performance. However, it's crucial to acknowledge that negative geotaxis is a complex behavior influenced by multiple factors beyond oxidative stress, such as muscle integrity, neuronal function, and sensory perception (Yuan *et al*., 2021).

While oxidative stress can clearly impair negative geotaxis, the converse relationship, whether climbing itself influences oxidative stress is less well-understood. Physical activity, in general, can increase ROS production (Jackson *et al*., 2022). It is conceivable that the exertion involved in climbing could transiently elevate ROS levels in flies. However, this increase might also trigger adaptive responses, leading to enhanced antioxidant defenses and improved stress tolerance (Powers *et al*., 2020). Research is needed to determine the precise nature of this interaction. For example, studies comparing flies subjected to varying levels of climbing activity and measuring both ROS levels and antioxidant enzyme activity could provide valuable insights. Additionally, investigations into the role of specific signaling pathways, such as the JNK pathway, which is involved in both stress response and motor function (Terhzaz *et al*., 2023), could shed light on the molecular mechanisms underlying the relationship between negative geotaxis and oxidative stress.

2.6 drosophila

The term drosophila comes from the Greek word drósos, meaning "dew-loving." These flies belong to the drosophilidae family and are commonly known as fruit flies, vinegar flies, or wine flies due to their tendency to gather around ripe or decaying fruits. They are distinct from the Tephritidae family, often referred to as true fruit flies, which feed primarily on fresh fruits and can damage ripening crops (Green, 2002; Beers *et al*., 2010). The genus drosophila is considered paraphyletic and consists of over 1,500 species, varying significantly in appearance, behavior, and habitats (Markow, 2005).

drosophila melanogaster was introduced as a model species in genetics, developmental biology, signal transduction, and cell biology in the early 1900s. The D. melanogaster genome is only 5 % of the size of a typical mammalian genome, but most gene families and pathways are shared with mammals, as well as many tissues and organ systems. Aging research in D. melanogaster benefits from a comprehensive range of methods to perturb gene function, such as mutagenesis screens, RNA interference (RNAi), and transgenesis (Heidelberg, 2013).

drosophila melanogaster has been a key organism in genetic research due to its small size, short life cycle, high reproductive rate, and ease of genetic manipulation. This species has played a central role in advancing biology, genetics, medicine, and stem cell research. Its affordability and ease of cultivation make it a preferred model organism. drosophila has been extensively studied for over a century, providing valuable insights into genetic systems, human diseases, and developmental biology (Wolf and Rockman, 2009).

2.7 Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPx)

Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPx), also known as Glutathione Peroxidase 4 (GPx4), is a unique selenoenzyme that plays a pivotal role in cellular defense against oxidative damage by directly reducing lipid hydroperoxides within biomembranes (Borchert *et al*., 2018). This function is critical in maintaining cellular integrity and preventing oxidative stress-induced apoptosis (Forcina and Dixon, 2019).

PHGPx distinguishes itself from other glutathione peroxidases by its ability to directly reduce complex lipid hydroperoxides, including those integrated into phospholipids, cholesterol, and lipoproteins (Ran and Mozolewska, 2019). This direct reduction prevents the propagation of lipid peroxidation, thereby safeguarding cellular membranes from oxidative damage (Arevalo and Vázquez-Medina, 2018). Structurally, PHGPx is a monomeric enzyme containing a selenocysteine residue at its active site, which is essential for its peroxidase activity (Cao *et al*., 2024). The enzyme's unique structure allows it to interact intimately with hydrophobic membrane environments, facilitating the reduction of lipid hydroperoxides in situ (Chhillar *et al*., 2024).

PHGPx plays a crucial role in inhibiting apoptosis by preventing the peroxidation of cardiolipin, a mitochondrial phospholipid. Peroxidation of cardiolipin leads to the release of cytochrome c from mitochondria, a key event in the intrinsic pathway of apoptosis (Forcina and Dixon, 2019). By reducing cardiolipin hydroperoxides, PHGPx maintains mitochondrial integrity and prevents unwarranted cell death (Arevalo and Vázquez-Medina, 2018). PHGPx is vital during embryonic development. Knockout studies in mice have demonstrated that the absence of PHGPx leads to early embryonic lethality, underscoring its essential role in development (Ran and Mozolewska, 2019). PHGPx is abundantly expressed in testicular tissue and is essential for the maturation and structural integrity of spermatozoa. It functions as a structural protein in the mitochondrial sheath of sperm, contributing to sperm stability and functionality (Borchert *et al*., 2018).

PHGPx has been implicated in the protection of cancer cells from oxidative damage. Studies have shown that certain cancer cells upregulate PHGPx expression to mitigate oxidative stress, contributing to resistance against therapies that induce lipid peroxidation (Forcina and Dixon, 2019). Oxidative stress is a hallmark of many neurodegenerative disorders. Given PHGPx's role in reducing lipid hydroperoxides, it is considered a potential therapeutic target for conditions like Alzheimer's and Parkinson's diseases, where lipid peroxidation contributes to neuronal damage (Cao *et al*., 2024).

2.8 Cap'n'collar (Cnc)

Cap'n' Collar (Cnc) is a basic leucine zipper (bZIP) transcription factor in drosophila melanogaster that plays a crucial role in regulating the cellular response to oxidative stress and xenobiotic compounds (Sykiotis and Hayes, 2016). It is the functional homolog of the mammalian Nrf2 protein, which is a master regulator of antioxidant and detoxification genes (Nguyen *et al*., 2009). Cnc is essential for development, tissue homeostasis, and stress response in drosophila (Fröhlich *et al*., 2016).

Cnc Structure and Function

Cap 'n' Collar (Cnc) subfamily of bZIP transcription factors, also includes the mammalian Nrf2, Nrf1, and Nrf3 proteins (Itoh *et al*., 2017). Cnc contains a DNA-binding domain, a leucine zipper dimerization domain, and a transactivation domain (Zhang and Kensler, 2014). It functions by binding to the antioxidant response element (ARE) in the promoter region of target genes, leading to their transcriptional activation (Joiakim *et al*., 2010).

Cnc activity is tightly regulated by its interaction with Keap1, a cytosolic repressor protein (Kobayashi *et al*., 2018). Under normal conditions, Keap1 binds to Cnc and sequesters it in the cytoplasm, preventing its nuclear translocation and transcriptional activity. However, upon exposure to oxidative stress or electrophilic compounds, Keap1 undergoes conformational changes, releasing Cnc and allowing it to translocate to the nucleus, bind to

AREs, and activate the expression of target genes (Bellezza *et al*., 2018). Cnc regulates the expression of a wide array of genes involved in detoxification, antioxidant defense, and stress response. These include genes encoding enzymes involved in glutathione biosynthesis, NADPH regeneration, and xenobiotic metabolism (e.g., cytochrome P450s) (Misra *et al*., 2011). Cnc also regulates genes involved in other cellular processes, such as protein degradation, DNA repair, and inflammation (Lee and Johnson, 2024).

Cnc plays important roles in drosophila development and tissue homeostasis. It is required for proper wing development, gut homeostasis, and oogenesis (Lee *et al*., 2016). Cnc also contributes to the maintenance of redox balance and cellular integrity in various tissues, including the nervous system, muscles, and the immune system (Sánchez-Alonso *et al*., 2019). Cnc is a key mediator of the cellular response to oxidative stress and xenobiotic compounds. It plays a critical role in protecting cells from damage caused by reactive oxygen species (ROS) and electrophilic toxins (D'Autreaux *et al*., 2015). Cnc activation leads to the upregulation of detoxifying enzymes and antioxidant proteins, which help to neutralize harmful substances and maintain cellular redox balance.

The activity of Cnc declines with age in drosophila, contributing to the age-related decline in stress resistance and increased susceptibility to age-related diseases (Zhang *et al*., 2015). Restoring Cnc activity or enhancing its signaling pathway has been shown to extend lifespan and delay age-related decline in drosophila (Gao *et al*., 2017).Dysregulation of Cnc signaling has been implicated in various human diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases (Zhao *et al*., 2020). In drosophila, studies have shown that Cnc plays a role in tumor suppression, neuroprotection, and immune response (Wu *et al*., 2018).

CHAPTER THREE

MATERIAL AND METHODOLOGY

3.0 Materials

Materials used in this study includes 50ml falcon tubes, weighing balance, Larsor seasoning, 1000 microliter micropipette, foil paper, tissue paper, cotton wool, ice packs, small soft paint brushes, spatula, distilled water, a spoon, a cooking pot, electric cooker/gas cylinder, burner and a tripod, nipargin, ethanol, yeast, agar-agar, cornmeal, glucose, a cup, measuring cylinders, hand gloves, pen, masking tapes, and a notebook for recording data.

3.1 Sample Collection and Preparation

Larsor seasoning samples was purchased from Oba market in Benin City, Edo state, Nigeria. The coordinate of the market location is; 6.3406°N 5.6227°E.

3.1.1 Preparation of Treatment (Larsor seasoning)

One gram (1g) of Larsor powder was weighed on a balance using foil paper and transferred to a test container with a spatula. One milliliter (1ml) of distilled water was added using a micropipette, and the mixture was thoroughly shaken. Treatment vials (50ml Falcon tubes) were prepared by adding 9.8 grams of standard cornmeal diet to each tube, measured with a spatula and weighing balance. Then, 200 microliters of the prepared seasoning solution was added to each tube. The mixture was stirred with the bottom of a test tube to create a smooth surface and prevent flies from getting trapped. The final volume in each tube did not exceed 10ml. Each seasoning concentration was prepared in triplicates. Three additional tubes, containing only standard diet and distilled water, served as controls. Thirty flies were placed in each tube. Daily monitoring was conducted, and mortality was recorded.

3.2 Methodology

3.2.1 Study Design

Study Subjects included 2-3 weeks old male and female *drosophila melanogaster* fed with cornmeal-agar medium diet treated with larsor seasoning in separate vials. The control subjects were 2-3 weeks old male and female *drosophila melanogaster* fed with only cornmeal-agar medium diet.

The mixed gender pure *drosophila melanogaster* were initially obtained from the University of Ibadan's drosophila Laboratory stock.

The flies were placed in the jar containing cornmeal, and were exposed to a 12-hour light and 12-hour dark natural photoperiod daily at about 25 ± 2 degree Celsius. The jars were then placed in a small amount of water in a tray. These conditions were chosen to minimize contamination of any kind and environmental stress (including the creeping of ants into the meal due to the presence of sugar in the meal) on the flies and focus on the effects of the seasoning. Flies were collected and separated into four (4) groups. For each group, there were three replicates containing 30 flies in each vial. The flies were treated as stated below in the experimental design (Krugger and Denton, 2020).

Group	Treatment	Comparison
A	Control	distilled water + cornmeal
B	0.025g/ml	Larsor seasoning + cornmeal
C	0.05g/ml	Larsor seasoning + cornmeal
D	0.1g/ml	Larsor seasoning + cornmeal

3.2.2 *drosophila melanogaster* Culture and Maintenance

Male and female drosophila melanogaster flies of 2-3 weeks old were cultured in the *drosophila melanogaster* laboratory section of the BIOTOXCS Research Laboratory. The flies were placed inside solidified cornmeal-agar medium jars, where they fed and reproduced. Every 5-7 days, the meal was changed, to prevent the accumulation of waste products in the jars and thus prevent microbial growth, and to ensure nutrients don't become depleted leading to nutritional deficiencies in the flies. (Jiménez-Padilla *et al.,* 2024).

3.2.3 Diet Preparation Protocol

Table 3.2.3: Meal preparation protocol for *drosophila melanogaster*

INGREDIENTS	STANDARD MEAL	HALF STANDARD MEAL	QUARTER STANDARD MEAL
Corn meal	52 g	26 g	13 g
Agar – 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

The protocol for preparing the standard cornmeal (which is the same with that of the half and quarter meal is the same, except for the changes in the amount of ingredients) is:

1. 850ml of distilled water was measured.
2. 150ml of the measured water was set aside for later use with the cornmeal.
3. The remaining 700ml of distilled water was brought to a boil.
4. A small portion of the boiling water was removed and reserved for dissolving the brewer's yeast.
5. The agar-agar was added to the still-boiling water and stirred until completely dissolved.
6. The cornmeal, previously mixed with the reserved 150ml of water, was added to the agar solution and stirred thoroughly.
7. The glucose was then added and stirred into the mixture.
8. The combined ingredients were allowed to boil for two minutes.
9. The brewer's yeast, which had been dissolved in the small portion of reserved boiling water, was added to the mixture.
10. The nipagin was dissolved in the ethanol and subsequently added to the preparation.
11. The finished cornmeal medium was removed from the heat source and dispensed into prepared vials.

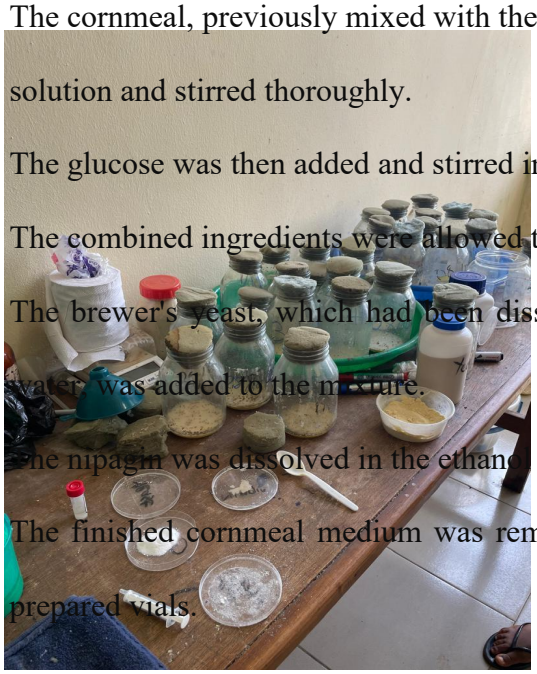

Plate 3.1 Standard measurement for meal preparation.

Plate 3.4a: Fly transfer

Plate 3.4b

First, flies were moved from cornmeal-filled breeding jars to empty ones. This was done by quickly and carefully tapping the breeding jars, removing the cotton wool, and inverting a funnel and empty jar over the breeding jar to prevent escape. The new jars of flies were then refrigerated for 4-5 minutes to slow them down. Next, the flies were poured onto foil paper atop an ice pack for counting with a soft paintbrush. Finally, the needed flies were moved to prepared experimental vials and plugged with cotton.

3.2.4 Survival rate assessment

Survival rate assessment is a technique used to measure the percentage of *drosophila melanogaster* that remain alive or active over a given period. This method is widely applied in various fields such as healthcare, ecology, and engineering to evaluate treatment effectiveness, population viability, or system reliability.

In this study, a survival test was conducted on experimental flies using lower treatment concentrations. The pilot study (acute toxicity test) had indicated that the flies could potentially survive at these lower concentrations, as they showed no observed adverse effect level (NOAEL). Different concentrations resulted in varying survival rates.

The main experiment involved four treatment groups: a Control, 0.025g/ml, 0.05g/ml, and 0.1g/ml. Each concentration had three replicates, totaling twelve tubes for analysis. Instead of counting the surviving flies, daily mortality was recorded, as it was a simpler and more reliable approach. A fly was considered dead if it failed to move upon gentle prodding.

The objective of this survival test was to determine how long the fruit flies could endure at reduced concentrations before proceeding to the main experiment. The meal preparation and fly transfer were performed three times: initially when the seasoning concentrations were prepared, and subsequently every 5-7 days. Each treatment tube initially contained thirty flies.

3.2.5 Main Experiment: Determination of the Survival and Negative Geotaxis of *drosophila melanogaster* Exposed to Larsor seasoning.

Group A: Control (distilled water + cornmeal)

Group B: 0.025g/ml (Larsor seasoning + cornmeal)

Group C: 0.05g/ml (Larsor seasoning + cornmeal)

Group D: 0.1g/ml (Larsor seasoning + cornmeal)

Each concentration had three replicates, making it a total of twelve treatment groups. The total number of flies placed per treatment tube were thirty, and this experiment was conducted for seven days.

3.2.6 Negative Geotaxis Assay:

This experiment was designed to determine the number of flies capable of moving against gravity within a specific time frame.

A 30ml (6cm) mark was drawn on the body of a Falcon tube. Ten immobilized flies were then placed into the marked tube following the standard fly transfer procedure. The tube was gently tapped to ensure all flies settled at the bottom before starting a 6-second timer. The number of flies that crossed the marked line within the time limit was recorded. Afterward, the flies were returned to their respective tubes, and the procedure was repeated for the other treatment groups.

Plate 3.5 Negative geotaxis in *drosophila melanogaster*

Three trials were conducted for each treatment group and the average count was noted.

Negative geotaxis can be calculated thus:

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

Negative geotaxis is expressed in percentage (%).

3.3 Gene expression Study

Isolation of Total RNA

Total RNA was isolated from whole drosophila 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).

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 (Olumegbon *et al*., 2020).

3.3.1 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:

Keap1, Forward primer: GAAGAGCGAGAAGTCCAAGTC Reverse primer: CAGGACGACACCAACGTTAT,

Glutathione transferase D1, Forward primer GCCGTTCACCAGGATCTATT Reverse primer AGCACAGGAAGTACGGATTG,

Cnc Forward primer GCGTCCCGTTAACTGTCTTTA Reverse primer CGTATCTGTAGCTGTGGCTTAG, >

PHGPx Forward primer GATACCCATGGCAACGATGT Reverse primer CCTTTAGATCCGTCAGCTTCTC,

Glycerol-3-phosphate dehydrogenase (Gpdh), Forward primer TCGGACTGCGTAGACACTAGA Reverse primer AGCGCCATCTATGTAAGGATGT.

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).

3.4 Data Collection

Data collected included the number of dead flies daily for the survival assay and the time taken to climb past a specific distance for the negative geotaxis assay.

3.5 Statistical Analysis

One way analysis of variance was used to check for significant differences between treatment and control groups. Where there is significant difference, I used a Turkey's HSD post hoc test to show specific significant difference. Alpha level of 0.05 was used (which means that $P < 0.05$ was considered statistically significant).

Statistical tool (software): All charts and analysis were made in R version 4.0.

3.6 Ethical Considerations

This study utilized *drosophila melanogaster*, an invertebrate organism. As such, it did not involve any procedures requiring formal ethical approval from an institutional review board. However, all experiments were conducted following standard laboratory practices for the handling and maintenance of drosophila.

CHAPTER FOUR

RESULT

4.1 Survival Study

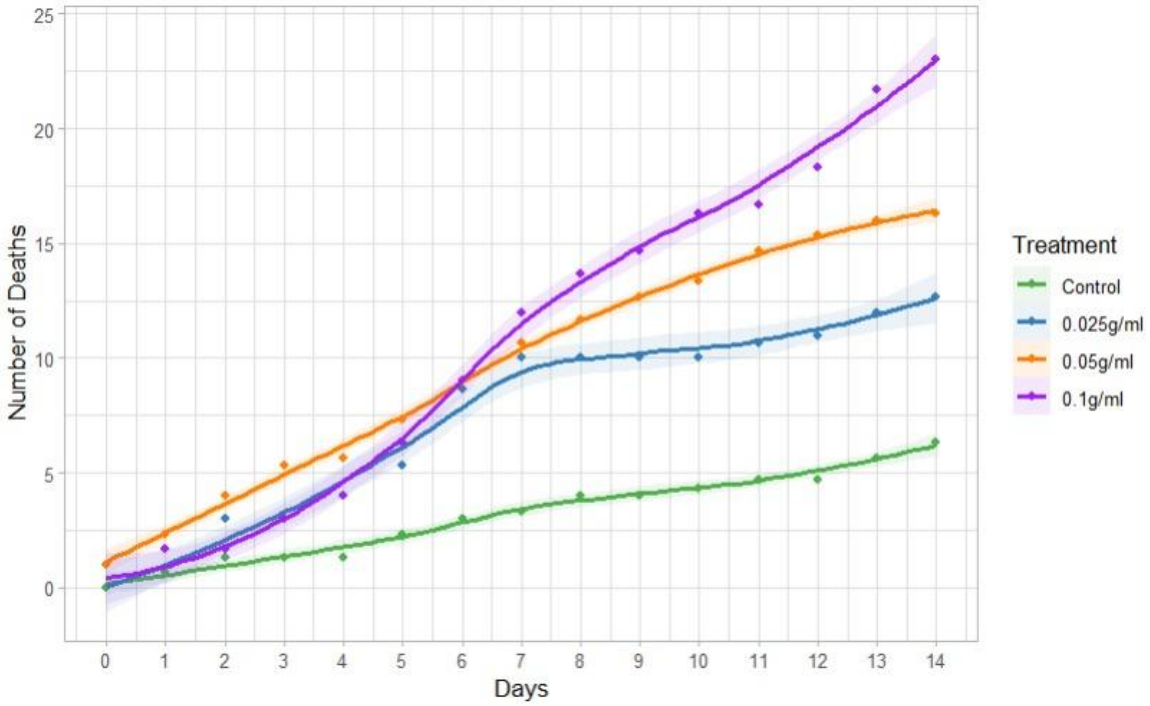

Figure 4.1: Mortality (death) pattern of *D. melanogaster* flies exposed to different concentrations of larsor over 14-day observation. *The distinct-coloured lines represent the number of survivors for each level of concentration.*

**Values are expressed as Mean ± SEM where n= 3, P-value <0.05

4.2 Negative geotactic responses

During the negative geotaxis assay, flies exhibited varied responses to the stimulus. While some flies crawled upwards along the container walls, others immediately took flight within the 6-second observation window. A subset of *drosophila melanogaster* displayed a contrasting behavior—initially moving upwards but quickly reversing direction and descending. The mean differences in level of response is shown in Figure 4.6.

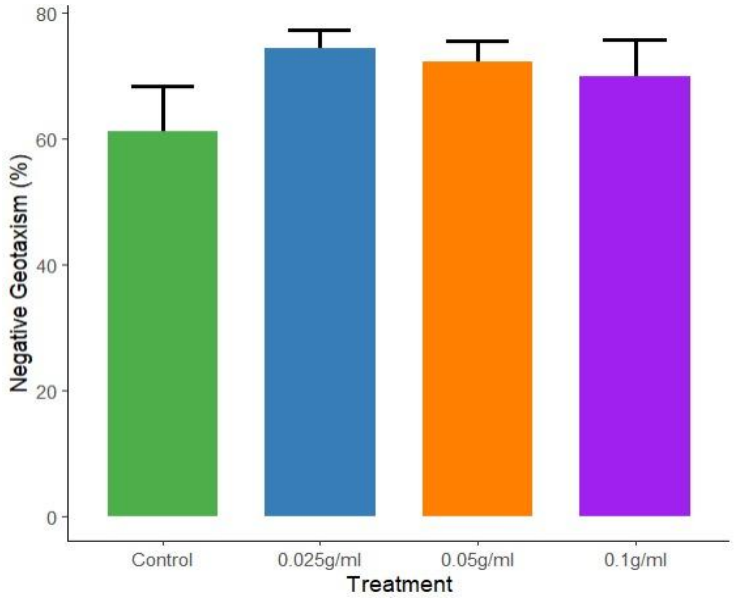

Figure 4.2: Negative geotaxis responses of *D. melanogaster* to exposure to varying concentrations of Larsor seasoning.

4.3 Kelch-like ECH- assoiciated protein (KEAP1)

A significant difference in KEAP1 gene expression was observed between the control and treatment groups ($F=29.77$, $p<0.05$). Specifically, no significant difference in KEAP1 gene expression was found between the control and 0.025g/ml groups (28.319 ± 1.03). However, KEAP1 gene expression was significantly increased in the 0.05g/ml (31.86 ± 3.85, $p<0.05$) and 0.1g/ml (43.206± 1.89426, $p<0.05$) groups compared to the control group."

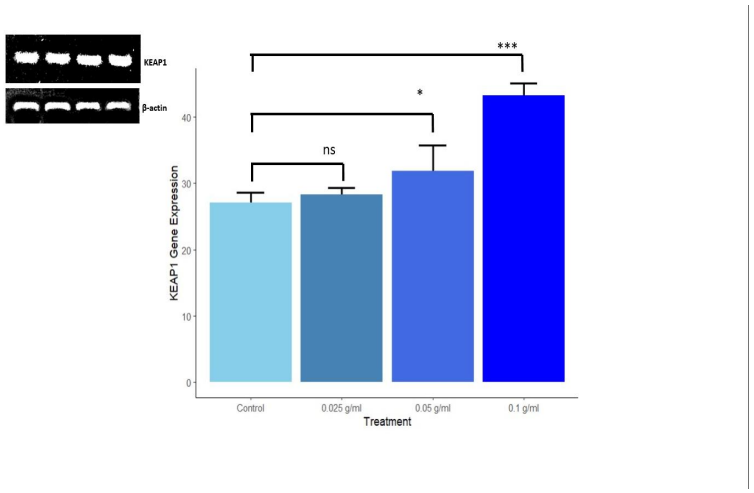

Figure 4.3. The KEAP1 activity in drosophila melanogaster exposed to different concentrations of larsor seasoning. Each bar represents the Mean ±SEM.

4.4 Glutathione-S-Transferase D1 (GSTD1)

A statistically significant difference in GSTD1 gene expression was observed between the control group and the treatment groups ($F=88.68$, $p<0.05$). GSTD1 gene expression was significantly decreased across the treatment group in a dose- dependent manner when treatments were compared with control.

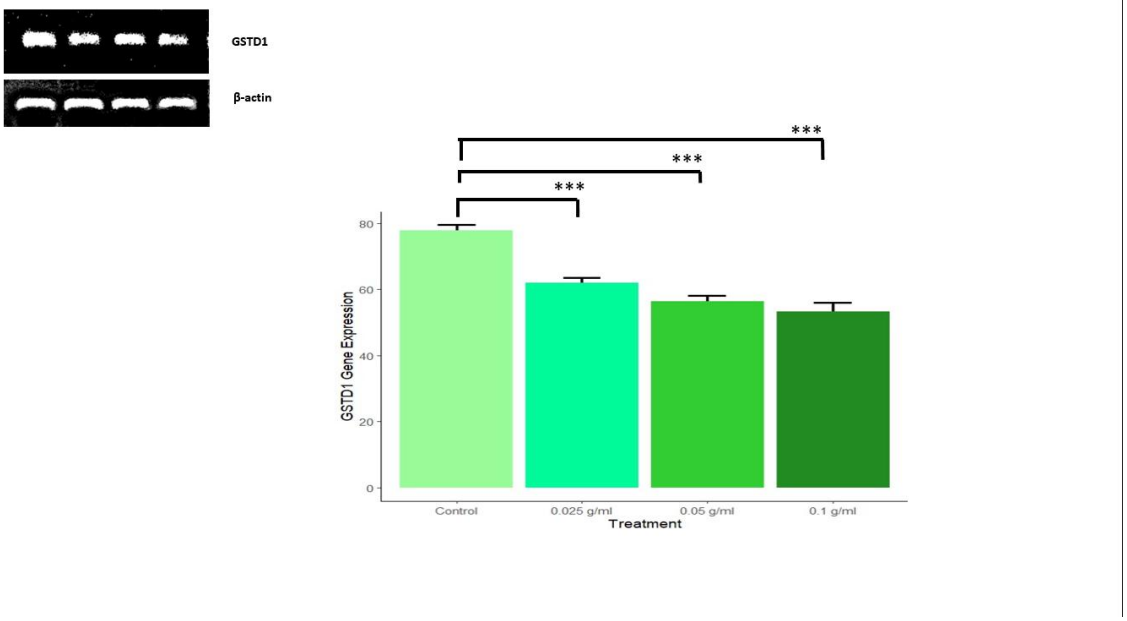

Figure 4.4 The GSTD1 activity in *drosophila melanogaster* exposed to different concentrations of larsor seasoning. Each bar represents the Mean ±SEM.

4.5 Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPx)

The result in figure 4.4 showed that there was no significant difference in PGHPx gene expression between the control group and the 0.025g/ml group (161.932 ± 1.89). However, PGHPx gene expression was significantly decreased in the 0.05g/ml (125.012 ± 2.55, $p<0.05$) and 0.1g/ml (70.587 ± 2.26, $p<0.05$) groups compared to the control group.

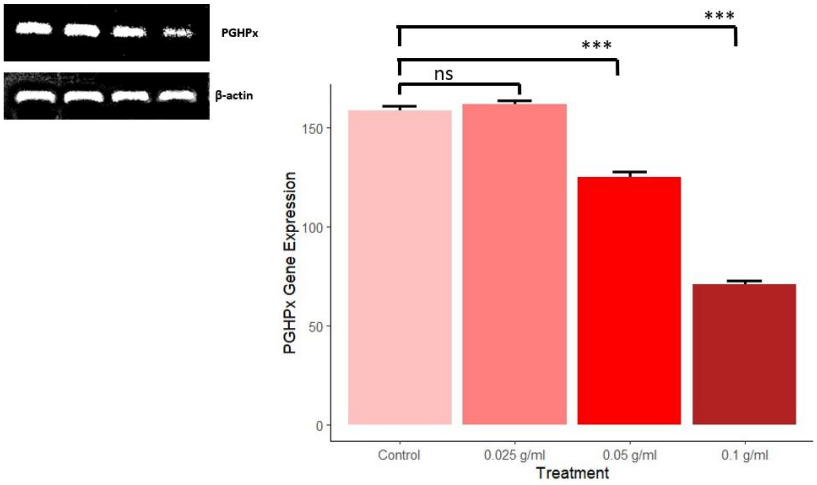

Figure 4.5 PGHPx activity in *drosophila melanogaster* exposed to different concentrations of larsor seasoning. Each bar represents the Mean ±SEM.

4.6 Cap'n'collar (CncC)

A statistically significant difference in CncC gene expression was observed between the control group and all the treatment groups in a dose-dependent manner as shown in Figure 4.4.

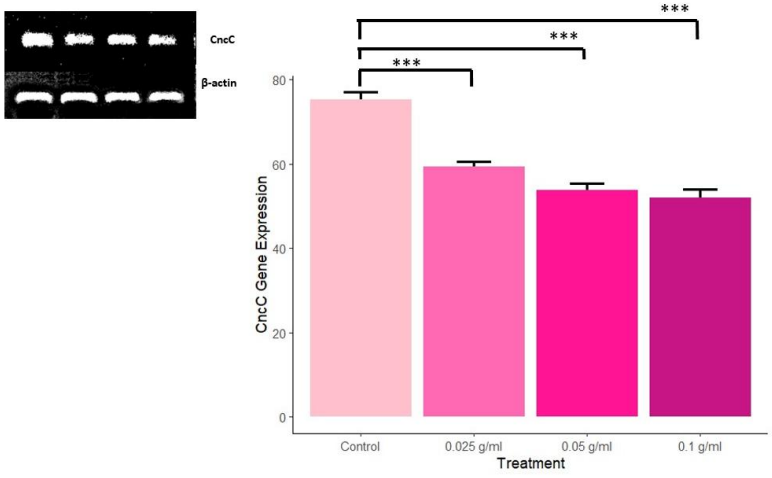

Figure 4.6 CncC activity in *drosophila melanogaster* exposed to different concentrations of larsor seasoning. Each bar represents the Mean ±SEM.

CHAPTER FIVE

5.1 Discussion of Results

The potential health risks associated with larsor Seasoning are a growing concern due to its increasing consumer use. This study evaluated the impact of larsor seasoning on the survival and negative geotaxis behavior of *drosophila melanogaster*. Flies exposed to larsor Seasoning exhibited significantly higher mortality rates and behavioral impairments compared to the control group, suggesting possible toxicological effects related to its physicochemical composition (Belyi *et al*., 2020).

The survival assay demonstrated that flies exposed to 0.1g/ml Larsor Seasoning experienced complete mortality by the 14th day of exposure, with a sharp increase in death rates observed as early as Day 5 (Figure 4.1). The accelerated mortality in the Larsor-exposed group, particularly at higher concentrations, is consistent with findings in previous toxicological studies (Belyi *et al*., 2020).

The result from the negative geotaxis test reveal that there was an increased in the climbing ability of drosophila M with increasing concentrations of larsor seasoning as shown in figure 4.2

Although was significant difference between 0.05g/ml and 0.1g/ml compared to the control. This upregulation of KEAP1, a key regulator of the Nrf2-mediated oxidative stress response, suggests a dose-dependent response to oxidative stress. Given KEAP1's role in facilitating Nrf2 degradation, this upregulation may indicate an increased oxidative burden, as a result of the inactivation of the antioxidant response element (ARE) (Kansanen *et al*., 2013). A similar

occurrence was noticed in toxic mechanism of the trichothecenes T-2 toxin and deoxynivalenol on protein synthesis (Jiefeng *et al*., 2022)

Conversely, GSTD1, a gene involved in detoxification, showed significant suppression across all treatment groups in a dose dependent manner. This suggests potential impairment in cellular detoxification pathways, possibly due to excessive oxidative stress that may have resulted from toxicant exposure (Li *et al*., 2020). GSTD1 is essential in protecting cells from oxidative stress, which arises from an imbalance between reactive oxygen species (ROS) production and the body's detoxification capacity.

Exposure to larsor Seasoning led to a statistically significant and dose-dependent decrease in GSTD1 gene expression. Figure 4.4 illustrates the relative GSTD1 mRNA levels in flies exposed to varying compound concentrations (Control, 0.025g/ml, 0.05g/ml, 0.1g/ml). The graph indicates a clear trend: as treatment concentration increases from control to 0.1g/ml, GSTD1 gene expression declines. This reduction is also evident in the gel images, where the bands corresponding to GSTD1 become progressively fainter with increasing treatment concentrations (Li *et al.,* 2020).

There was a dose- dependent decrease in PHGPx expression with increasing concentrations of lasor treatment. These changes were significant ($p>0.05$), in the groups administered 0.05g/ml and 0.1g/ml of lasor seasoning when compared with the control group as shown in Figure 4.5. PHGPx, an enzyme responsible for neutralizing lipid peroxides, exhibited reduced expression at higher concentrations of larsor seasoning. This pattern aligns with a previous studies demonstrating that food additives and ROS can compromise antioxidant enzyme function, leading to cellular vulnerability (Zhang *et al*., 2021).

Additionally, the downregulation of CncC suggests a broader disruption in stress-response pathways as shown in figure 4.6. larsor Seasoning exerts inhibitory effects on critical genes

involved in oxidative stress adaptation, similar to findings in prior research (Gunderson *et al*., 2020).

5.2 Conclusion

From this study we can conclude that larsor seasoning has significant negative impacts on *drosophila melanogaster*. Exposure to the seasoning resulted in increased mortality, impaired climbing ability, and significant alterations in the expression of key genes involved in oxidative stress response and detoxification. Specifically, higher concentrations of larsor seasoning led to complete mortality, indicating a potent toxic effect. While certain seasonings may offer beneficial properties at moderate concentrations, excessive exposure can lead to negative effects on survival and neuromuscular function. As observed in the case of larsor seasoning (Figure 4.2), some additives exhibit increased toxicity with prolonged consumption and higher doses. These findings suggest that moderate consumption of larsor seasoning may be preferable to higher concentrations, as excessive intake poses potential health risks. The observed decrease in climbing ability suggests neuromuscular dysfunction. Furthermore, the upregulation of KEAP1 suggests increased oxidative stress, while the downregulation of GSTD1, PGHPx, and CncC indicates a compromised detoxification system and broader disruption of cellular stress response pathways. These findings collectively demonstrate that Larsor Seasoning, particularly at higher concentrations, poses a significant threat to cellular function and survival, likely due to its induction of oxidative stress and impairment of crucial defense mechanisms. This raises concerns about the potential health risks associated with its consumption.

5.3 Recommendation

Further investigation is needed to elucidate the precise molecular mechanisms by which larsor Seasoning 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 seasoning induce oxidative stress and how they interfere with the Nrf2-mediated antioxidant response. Also investigating the toxicity of larsor seasoning 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* serves as a useful model, ultimately, research should investigate the potential health implications of larsor seasoning consumption in humans. This could involve epidemiological studies correlating seasoning intake with health outcomes, as well as in vitro studies using human cell lines to assess its toxicity. If larsor seasoning is shown to pose health risks, research should explore potential mitigation strategies, such as the use of antioxidants or other compounds that could counteract its toxic effects. This could lead to recommendations for reducing the risks associated with its consumption.

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APPENDIX

LASOR SEASONING											
Treatment Dose	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
0.1g/ml(T 1)	2	3	4	4	6	11	17	-	-	-	-
T2	0	2	2	3	5	3	8	-	-	-	-
T3	4	4	5	6	6	7	7 alive	5aliv e	5aliv e	-	-
0.05g/ml(T1)	5	5	5	5	5	7	13	3aliv e	3aliv e	-	-
T2	1	2	2	4	4	6	3aliv e	-	-	-	-
T3	0	0	2	4	5	7	1aliv e	-	-	-	-
1g/ml(T1)	1	2	3	14	16	18	All	-	-	-	-

							dead				
T2	1	1	3	3	6	6	1 alive	1alive	1alive	-	-
T3	0	0	0	3	6	6	4alive	3alive	4alive	1alive	-

LARSOR SEASONING (SURVIVAL TEST)														
Treatment	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
0.025 g/ml(T1)	0	0	4	4	5	7	11	13	13	13	13	14	14	15	15
T2	0	2	5	5	5	6	9	10	10	10	10	11	12	13	13
T3	0	0	0	0	2	3	6	7	7	7	7	7	7	8	10
0.05g/ml(T1)	0	0	1	1	1	1	3	5	8	8	8	10	10	12	12
T2	2	4	5	9	10	11	13	16	14	16	17	18	18	18	18
T3	1	3	6	6	6	10	11	11	13	14	15	16	18	18	19
0.1g/ml	0	1	1	3	3	7	9	12	12	14	17	17	18	22	22
T2	0	1	1	2	3	5	9	13	16	17	17	18	19	23	24
T3	0	3	3	4	6	7	9	11	13	13	15	15	18	20	23
Control (T1)	0	1	3	3	3	4	4	4	6	6	6	7	7	7	7
T2	0	0	0	0	0	0	1	2	2	2	2	2	2	3	5
T3	0	1	1	1	1	3	4	4	4	4	5	5	5	7	7