INVESTIGATING THE MODULATORY ROLE OF COMMONLY USED FOOD SEASONING[TASTY CUBE CHICKEN FLAVOR]ON SURVIVAL RATE AND SELECTED ANTIOXIDANTS IN *Drosophilia melanogaster.*

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BENIN CITY.

OCTOBER, 2025.

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE

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SUPERVISED BY

PROF.O.G. IGHARO

OCTOBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by **JOHNSON VICTORY EVESHOMHEOREMAH** with matriculation number **BMS2001176** in partial fulfilment of the requirements for the award of Bachelor of Medical Laboratory Science (BMLS) from the University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This work is dedicated to my Heavenly Father who is the source of all knowledge and wisdom and to my wonderful parents for their unwavering love and support.

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ABSTRACT

Seasoning cubes such as Tasty Cube (Chicken Flavour) are widely consumed in African households for flavor enhancement. While generally regarded as safe, their content of monosodium glutamate (MSG), sodium, and preservatives has raised concerns about potential biochemical effects, particularly oxidative stress. Previous studies have implicated MSG and sodium-rich additives in the alteration of antioxidant enzyme activity and lifespan reduction in animal models.The aim of this study was to investigate the modulatory effects of Tasty Cube on survival rate and selected antioxidant enzymes Sodiundismutase,Catalase and Gluthathione Peroxidase (SOD, CAT, GPx) in *Drosophila melanogaster*. Adult flies of about 525 in total were divided into four (5) groups;A,B,C,D, and E representing Control,0.3g,0.15g,0.10g,and 0.05g group and fed diets supplemented with these concentrations of Tasty Cube alongside a control diet. Survival assays were conducted over a defined period, while biochemical analysis of antioxidant enzyme activities was carried out using standard spectrophotometric methods.The results showed a mild reduction in survival rate, with flies exposed to higher concentrations of Tasty Cube exhibiting significantly reduced lifespan compared to controls ($p<0.05$). Antioxidant assays revealed that while low to moderate doses caused a mild increase in CAT activity ($p<0.05$), higher doses significantly suppressed SOD and GPx activities ($p<0.05$), indicating oxidative imbalance.In conclusion, Tasty Cube seasoning exerts measurable toxicological effects in *Drosophila melanogaster*, characterized by mildly reduced survival and disruption of antioxidant defense mechanisms. These findings highlight the potential risks associated with chronic seasoning cube consumption and underscore the need for public health awareness regarding moderated dietary use.

CHAPTER ONE

INTRODUCTION

1.1 Background Of Study

In the past few years,the use of artificial food enhancers and consumption of processed food has been recorded to increase significantly overtime,especially in developing countries and urban areas(Hess *et al.,* 2013).Historically, industrialisation in high-income nations has prompted rural-to-urban migration and a transition in nutrition from predominantly plant-based foods (vegetables, fruits, whole grains, legumes) to hyper-caloric diets characterised by high levels of total and saturated fats, cholesterol, animal protein, added salt, and sugar, while being deficient in fibre(Baker *et al.,* 2020).In numerous low and middle income nations, dietary habits are transitioning from traditional meals abundant in fresh produce to highly processed, calorie-dense items.In sub-Saharan Africa (SSA), food systems and diets are rapidly transforming as is the epidemiology of the burden of malnutrition (Reardon *et al.,* 2020).The Food and Agriculture Organization (FAO, 2023) notes a 45% rise in processed food sales in sub-Saharan Africa over the past two decades, largely driven by urban migration and changing lifestyles(“The State of Food Security and Nutrition in the World 2023,” 2023)The safety of these processed foods is an issue of public health importance, especially in Africa as it leads to excessive consumption of various novel 'chemicals'(food enhancers) which poses significant health threats(Ibrahim *et al.,* 2021).The "nutrition transition" has elicited apprehensions regarding prolonged exposure to food additives, such as monosodium glutamate (MSG) and sodium-laden seasoning products, which are routinely ingested in numerous households. Seasoning cubes and other seasoning products as well as food additives such as Tasty Cube chicken flavor have been used to add extra flavors to meals and improve the taste of meals,but concerns have been raised in regards to their safety especially in its

continuous consumption particularly due to their high content of Monosodium Glutamate[MSG] and Sodium(Afolabi and Olagoke, 2020).A number of causes, including modern lifestyle changes and different marketing methods, led to a rise in the intake of processed foods, a behaviour that has been connected to a number of negative health effects. This can be linked to the overuse of flavour enhancers in food processing, such as monosodium glutamate ($C_{15}H_8NO_4Na$, MSG) (Onyesife *et al.,* 2022).Although these additives are generally recognized as safe,the European Food Safety Authority (EFSA) re-evaluated the safety of MSG in 2017 and set an "acceptable daily intake" of 30 mg/kg BW[Body weight] (Abdou *et al.,* 2025).Sodium on the other hand is a critical nutrient for controlling extracellular fluid and moving molecules around cell membranes for vital processes but excessive salt consumption is linked to the occurrence of certain illnesses,therefore sodium level of meals is therefore still a specific issue for the food business especially in food seasonings (Nurmilah *et al.,* 2022).Continuous intake and excessive consumption of these ingredients in food seasonings may lead to biochemical changes in the body,including the generation of reactive oxygen specie[ROS]and disruption of antioxidant defence systems (Abolaji *et al.,* 2017),neurotoxic, metabolic, and cardiovascular effects. Oxidative stress occurs when there is an imbalance between free radicals and antioxidants in the body.The German physician Helmut Sies originally used the phrase "oxidative stress" in 1985 to describe an imbalance between the generation of oxidants and antioxidant defences that could harm biological systems(Forman and Zhang, 2021).It is described as disturbed oxidative balance between increased reactive oxygen species such as hydroxyl radical (OH•), superoxide radical (O2), hydrogen peroxide (H2O2) which occurs during normal cellular metabolism and decreased antioxidants which have scavenging effects on free

radicals and can lead to damage of cellular protein,lipids and DNA (Özcan *et al.,* 2015).The key physiological roles of ROS include the regulation of cellular homeostasis via the mediation of processes including redox signaling, defense against pathogens and protein folding (Olufunmilayo *et al.,* 2023).Many cellular organelles have an intrinsic ability to scavenge and 'mop up' ROS Hansen *et al.* (2017) so therefore,the antioxidant defense system based predominantly on the enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GSR) protects the body and its tissues from ROS-induced cellular damage(Deponte, 2012). However, when reactive species reach critical quantities within cells, their negative consequences quickly outweigh the advantages, and antioxidant responses are overcome. Oxidative stress results from the inability to maintain redox equilibrium, which can be caused by overproduction or reduced/impaired removal of reactive species.Numerous illnesses, including as cancer, neurological conditions, atherosclerosis, hypertension, ischaemia, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive lung disease, and asthma, have been linked to it (Birben *et al.,* 2012). Antioxidants are a class of a multitude of chemical substances clearly associated with large health benefits and lower risks of various age-related diseases.They also can stop the damaging actions of reactive oxygen species (ROS) Lü *et al.* (2009), which include "energised" or partially reduced forms of oxygen, some of which are "free radicals" with an unpaired electron in an orbital, and others that are "nonradical species," like singlet oxygen and hydrogen peroxide, whose reactivity is even higher than that of molecular oxygen's ground state (Halliwell, 2022). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx),play a crucial role in neutralizing ROS and maintaining redox homeostasis(Jomova *et al.,*

2024*)*.Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the primary enzymatic antioxidants present in cells that help to protect cells from ROS-induced damage(Pizzino *et al.,* 2017).A decrease in the activity of these enzymes is commonly associated with oxidative damage and has been linked to various diseases and toxicological effects*(*Nandi *et al.,* 2019).Studies have shown that MSG which is found in seasoning cubes has reduced the effects and increases the activity of antioxidants present in different research animals(Airaodion *et al.,* 2019). *Drosophila melanogaster* (fruit fly) is a well-established model organism in toxicology Chifiriuc *et al.* (2016) and genetic studies for over 70 years (Abdu-Allah *et al.,*2020) due to its short life cycle, genetic similarity to humans, and ease of maintenance (*News-Medical, 2025)*.According to comparative genomic analyses, about 75 % of the human genes linked to various disorders is conserved in *Drosophila* (Calap-Quintana *et al.,* 2017) which means more than half of *Drosophila* genes have orthologs in humans, which have made the fruit fly a leading model system for studying the mechanisms of human biology and disease(Bellen and Yamamoto, 2015). It is particularly suitable for evaluating the biological impact of dietary substances, including food additives.Therefore, as a well-established model organism with a short life cycle and advantages of genetic manipulation, the fruit fly has been increasingly employed to assess functions of antioxidants *in vivo* (Yi *et al.,* 2021).*Drosophila melanogaster* can be used as a convenient model system to experimentally test potential health effects of dietary components such as food enhancers and food additives(Chhabra *et al.,* 2013). There has been a lot of research on oxidative stress after MSG exposure in rodents, but there isn't much data from invertebrate models like *Drosophila melanogaster*, particularly when it comes to the combined effects of sodium and glutamate in commercial seasoning cubes. Moreover,

no evidence exists to connect long-term food intake to changes in *invivo* enzymatic antioxidant defences. This study fills this gap by assessing the survival rate and alterations in SOD, CAT, and GPx activity in *Drosophila melanogaster* exposed to different tasty cube chicken flavor doses.

1.2 Statement Of Problem

The widespread consumption of seasoning cubes (e.g.Tasty cubes chicken flavour) introduces compounds such as MSG, high sodium content, and preservatives into daily diets (Zanfirescu *et al.,* 2019).While pure MSG has been shown to induce oxidative stress and alter antioxidant enzyme activity in *Drosophila melanogaster* causing increased hydrogen peroxide (H_2O_2) and catalase activity,alongside reduced lifespan(Shivasharan *et al.,* 2012), no studies have evaluated the composite effects of chronic or excess use of seasoning cubes[commonly used in Africa],its MSG content and its overall effect on antioxidants levels.Thus, the specific impact on key antioxidant enzymes (SOD and GPx and CAT) remains unknown.

1.3 Justification of Study

Assessing the possible biochemical and physiological impacts of seasoning cube usage is crucial for gaining insights into public health, considering how common the use of seasoning cubes has become.Given the demonstrated oxidative effects of MSG and high-sodium diets in various organisms including *Drosophila* and cockroaches *(Airaodion et al., 2019*), Investigations assessing the potential effects of these flavor enhancers on survival rate and oxidative stress as well as on antioxidant levels can be conducted quickly, ethically, and informatively by using *Drosophila melanogaster*.It is important to assess commonly used flavoring agents in conditions closer to real-world consumer intake. Using *Drosophila melanogaster,*a validated model for

oxidative stress studies will provide cost-effective and biologically relevant insights(Abdulwahab *et al.,* 2025).*Drosophila melanogaster* offers a suitable model to examine both biochemical and survival outcomes in response to seasoning cube consumption(Alaraby *et al.,* 2016).Mechanistic insights into how seasoning cubes affect oxidative equilibrium in vivo will be produced by this study, which will concentrate on survival rate as well as changes in antioxidant enzyme activity, particularly superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The results may influence legislative decisions about the development and labelling of commercial flavour enhancers, influence dietary safety recommendations, and support public health awareness initiatives. By doing this, the study not only fills a knowledge gap but also promotes evidence-based strategies for enhancing consumer welfare and food safety.

1.4 Aim of Study

To investigate the modulatory effects of Tasty cube chicken flavour on survival rate and activity of selected antioxidant enzymes (SOD, CAT, GPx) in *Drosophila melanogaster.*

1.5 Research Questions

1. Does exposure to Tasty Cube seasoning affect the survival rate of *Drosophila melanogaster*?
2. How does Tasty Cube exposure influence the activity of antioxidant enzymes: SOD, CAT, and GPx?
3. Is there a dose-dependent relationship between Tasty Cube concentration and changes in survival and antioxidant enzyme activity?

1.6 Research Objectives

1. Determine the survival rate of *Drosophila melanogaster* following exposure to varying concentrations of Tasty Cube.
2. Quantify the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in treated flies.
3. Evaluate the presence of dose-dependent effects of Tasty Cube on survival and enzyme activity.

1.7 Research Hypothesis

1.7.1 Null Hypothesis (H_0): Tasty Cube exposure has no significant impact on survival rate or the activities of SOD, CAT, and GPx in *Drosophila melanogaster*.

1.7.2 Alternative Hypothesis (H_1): Tasty Cube exposure significantly affects survival rates and/or activities of SOD, CAT, and GPx in *Drosophila melanogaster*.

1.8 Scope of Study

This research investigates the impact of Tasty cube chicken flavour on survival rate and antioxidant enzyme activity (SOD, CAT, GPx) in *Drosophila melanogaster*. The study is limited to biochemical and lifespan analysis, excluding behavioral, histopathological, or genetic assays.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Seasoning Cubes and Food Additives

Seasoning cubes also known as bouillon cubes are small, concentrated blocks of herbs, spices, and flavor enhancers used to add taste and aroma to dishes. Widely used in households and restaurants, seasoning cubes dissolve easily in hot water or during cooking, enriching meals with savory flavors. They are particularly valued for their convenience, affordability, and versatility in preparing soups, stews, rice, and other ndishes.In Africa, seasoning cubes quickly gained popularity due to their adaptability to local cuisines and affordability compared to traditional spices(Archer *et al.,* 2022).Seasoning cube production in Nigeria began with the importation of popular brands like Maggi and Knorr, which quickly dominated the market. Recognizing the growing demand, multinational companies established local manufacturing facilities, allowing them to cater to Nigerian consumers more effectively.as time went on, local producers joined the market, bringing with them competitive pricing and Nigerian-style flavours. Nigeria is currently one of Africa's largest producers and consumers of seasoning cubes (*A Taste of Profit: Exploring the Business of Seasoning Cubes Manufacturing in Nigeria – Business Plans in Nigeria*, n.d.).Growing customer demand for quick and tasty cooking options is fuelling the market for seasoning Cubes. Demand for seasoning cubes, which provide quick and simple flavouring alternatives, has increased due to busy lifestyles and the rising popularity of homemade meals.These popular brands such as Knorr,Maggi,and Tasty cubes are commonly added to soup,stew,and sauce of different kinds especially in settings where access to fresh spice is limited.

These cubes are majorly composed of monosodiun glutamate[MSG],salt[sodium chloride],disodium inosinate,hydrogenated fats,starch,and variety of synthetic flavouring and preservatives as its major food additives (Utume *et al.,* 2020).While regulatory bodies like the FAO/WHO consider many of these components generally safe in moderation, evidence is emerging that chronic or excessive intake may pose significant health risks (Airaodion, 2019).Food additives are not solely responsible for taste improvement they may also influence physiological processes, either positively (as micronutrient-fortified products) or negatively (when rich in synthetic agents)(Paramasivam *et al.,*2024). Food additives are chemicals used in food processing to enhance food's flavour, sweetness, colour, stability, safety, expiration date, and other qualities(Mwale, 2023).Food additives can be derived from plants, animals or minerals, or they can be chemically synthesized. There are several thousand food additives used, all of which are designed to do a specific job*(World Health Organization: WHO, 2023)*.Typically, the amounts added are no more than what is necessary to have their technical effect.Varying nations have varying regulations on food additives, but they all need to be deemed safe regardless of where they are used in food(Dolan and Mozingo, 2022).Using food additives helps improve the quality of the final product and extends the shelf life of food on store shelves (Martins *et al.,* 2018).Monosodium glutamate has been classified as one of the common food additive that may cause problems especially with excessive use. Given the daily consumption of these seasonings in many households, it becomes crucial to understand how seasoning cubes, like Tasty cube chicken flavor, affect biological systems. This is especially relevant for long-term health, since food-based exposures often go unnoticed compared to pharmaceutical or environmental toxins.

Figure 2.1 Bullion cubes (*Wikipedia contributors, 2025)*

2.2 Composition of Tasty Cube Chicken Flavor (MSG, Sodium, Preservatives)

Tasty Cubes Chicken Flavor, like many bouillon or seasoning cubes (e.g. Terra, Maggi), is formulated primarily to enhance flavor and preserve food. According to product labels and ingredient listings, the typical composition includes:

1) Hydrolysed chicken protein
2) Monosodium Glutamate [MSG]
3) Salt
4) Starch
5) Spices [PepperandGinger]

2.2.1 Health Implications of Key Ingredients

2.2.1.1 Monosodium Glutamate (MSG):

Monosodium Glutamate (MSG) is one of the most widely used food-additives in commercial foods. Its application has increased over time and it is found in many different ingredients and processed foods obtainable in every market or grocery store. MSG gives a special aroma to processed foods which is known as umami in Japanese. This taste sensation is also called "savoury"(Xiong *et al.,* 2009).Despite being classified as "generally recognized as safe" (GRAS) by regulatory agencies such as the U.S. Food and Drug Administration (FDA), increasing evidence suggests that chronic or excessive MSG consumption may have detrimental effects on various biological systems(Geha *et al.,* 2000).

For around 2,000 years, the Chinese have been using specific seaweeds to improve the flavour of their meals. Glutamic acid was discovered to be the flavor-enhancing

ingredient in 1908.Techniques for removing glutamic acid from seaweeds were created shortly after.Protein hydrolysis, in which glutamic acid is liberated from protein by enzymatic fermentation or chemical means, is the process used to make MSG(Sano, 2009).MSG releases neurotransmitters that are essential for both healthy and diseased physiological processes by acting on glutamate receptors(Abdallah *et al.,* 2014).Glutamate is the most abundant excitatory neurotransmitter in the vertebrate brain.There are four kinds of ionotropic receptors [N-Methyl-D-Aspartate (NMDA),alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid (AMPA), delta, and kainite receptors) and three groups of metabotropic receptors (mGluR) found in glutamate receptors. The central nervous system has each of these receptor types. They are particularly prevalent in the hippocampus, amygdala, and hypothalamus, which regulate metabolic and autonomic processes(Zhu and Gouaux, 2016).According to estimates, the average person in the United Kingdom consumes approximately 4 grammes of MSG per week, which is similar to the 0.55 grammes that Americans ingest on a daily basis(Husarova and Ostatnikova, 2013).Since the liver is the primary organ involved in MSG processing, it is particularly susceptible to the harmful effects of this widely used food additive. MSG is harmful to liver cells because it produces too many ammonium ions and reactive oxygen species, according to earlier research(Tawfik and Al-Badr, 2012).Overloading ammonium ions is known to cause reactive oxygen species to develop, which then react with the polyunsaturated fatty acids in cell membranes to degrade mitochondrial and plasma membranes and cause the release of liver enzymes like ALT and AST(Umukoro *et al.,* 2015). MSG has be linked to cardiovascular diseases, renal and hepatic damage, neurotoxicity, and metabolic syndrome, according to recent preclinical and clinical research(Mondal *et al.,* 2024).Excessive MSG consumption has also been linked to

headaches, flushing, weakness, and numbness, as well as an aggravation of other symptoms like dermatitis, urticaria, ventricular arrhythmia, and stomach pain(Geha *et al.,* 2000).There is no discernible rise in glutamate levels in the brain that could result in neuronal damage because the usual blood plasma glutamate concentration is between 50 and 100 μmol/L. MSG administered systemically raises extracellular glutamate levels in parts of the brain other than the Blood Brain Barrier (BBB), which damages neurones and may increase the risk of neurogenerative disease(Onaolapo and Onaolapo, 2020). According to reports, MSG functions as an excitotoxin that contributes to the generation of free radicals, which in turn trigger signalling pathways that cause oxidative stress and inflammatory reactions that ultimately result in cell destruction and death(Rosa *et al.,* 2018).Furthermore, excessive consumption may unintentionally impact chromosome architecture and cell growth, which is connected to the development of malignancies.It is well recognised that long-term MSG use can have negative effects on development, metabolism, and the functioning of several organs, including the kidney, liver, heart, brain, and reproductive organs (Hazzaa *et al.,* 2020).

Figure 2.2 *Monosodium Glutamate (Clinic, 2022)*

2.2.2.2 Salt [Sodium Chloride]

The culinary idea behind bouillon is to use cooked meat and animal products (and/or vegetables) to produce a strong, delicious extract or essence. A large portion of bouillon's flavour improvement comes from its high sodium content, which is mostly from salt.However, bouillon has a high sodium content (and in addition with other foods) contributes to dietary sodium intake above recommended guidelines.In Sub-Saharan Africa, bouillon is widely used and frequently consumed(Archer *et al.,* 2022).For the body to operate normally, sodium is a vital nutrient. For an average adult, ~0.5 g of salt must be consumed daily.Despite reports that salt intake is generally lower in African regions than in other parts of the world, the average amount ingested in all African regions exceeds the WHO recommended intake levels(Powles *et al.,* 2013).World-wide dietary sodium intake, however, is more than the 5 g of salt (sodium chloride, NaCl) per day that the World Health Organisation (WHO) recommends (~2 g sodium per day)(Thout *et al.,* 2019).Chronically consuming too much salt might have detrimental effects on one's health. A major risk factor for elevated blood pressure (caused by increased water retention) is high sodium levels. This can lead to hypertension, cardiovascular disease (CVD), an increased risk of stroke, and other health issues(Strazzullo *et al.,* 2009).Additionally, lowering blood pressure and the rate of CVD are linked to a moderate reduction in sodium intake, indicating that reducing salt intake in the diet is an important strategy to fight CVD and the burden of related diseases (He *et al.,* 2013). In developed nations, processed foods account for 75–80% of sodium intake, with smaller amounts coming from naturally occurring sodium in food or sodium salt added during and after cooking (Farquhar *et al.,* 2015).While bouillon is consumed regularly across the population (e.g., on average 1.7–4.3 g per person a day)Chen and Oldewage-Theron

(2004), it is not known the relative contribution bouillon contributes to daily sodium intake in excess of recommended levels in African countries (in comparison with other foods). However, several publications directly link high daily sodium intake levels in African populations with bouillon cube consumption (Mezue, 2013). A high sodium consumption is significantly correlated with kidney disease, cardiovascular morbidity and mortality, and raises blood pressure (BP) while adversely affecting endothelial and cardiovascular function (Suckling *et al.,* 2007)

2.3 Oxidative Stress and Free Radical Biology

Oxidative stress is brought on by an imbalance between the body's capacity to detoxify reactive products and the generation and buildup of oxygen reactive species (ROS) in cells and tissues (Pizzino *et al.,* 2017).The term "reactive oxygen species" (ROS) refers to a broad category of molecular oxygen derivatives that are a typical feature of aerobic living (Sies and Jones, 2020).Living things generate reactive oxygen species (ROS) as a byproduct of regular cellular metabolism. They frequently leave the subcellular manufacturing site during a reduction process and are typically short-lived (Venditti *et al.,* 2015).They contribute to normal cell functions at low to moderate concentrations, but at high concentrations, they negatively alter cell constituents such proteins, lipids, and DNA(Birben *et al.,* 2012).Suitable amounts of ROS have beneficial effects such as wound healing, repairing processes, and killing invading pathogens (Bhattacharyya *et al.,* 2014). The shift in equilibrium between oxidant and antioxidant in favor of oxidants is termed “oxidative stress (Valko *et al.,* 2006).Unlike the majority of biological molecules, free radicals are tiny, diffusible molecules with an unpaired electron. Due to their tendency to be reactive, free radicals can take part in chain reactions, which can cause several molecules to be harmed by a single free radical initiation event (Jones, 2008).A free radical is

extremely reactive and indiscriminate in its reactions since it has an unpaired electron in its outer orbital (Averill-Bates, 2023).The superoxide anion (O2•-) and its protonated form, the perhydroxyl radical (HO2•), the hydroxyl radical (HO•), the peroxyl radical (ROO•), and the alkoxyl radical (RO•) are all examples of free radicals that are produced from oxygen (where R is a lipid or protein) (Halliwell and Gutteridge, 2015). Hydrogen peroxide (H2O2), singlet molecular oxygen (1O2), hypochlorous acid (HOCl), and organic hydroperoxides (ROOH) are examples of non-radical ROS (Redza-Dutordoir and Averill-Bates, 2016).

Cells are protected by the redox equilibrium between antioxidant levels and ROS levels.

2.3.1 Sources of Reactive Oxygen Species

Endogenous ROS formation occurs primarily via mitochondria, auto-oxidation of glucose, and enzymatic pathways;

2.3.1.1 Mitochondrial electron transport chain

Mitochondrial ROS, also known as mtROS or mROS, are reactive oxygen species (ROS) generated within mitochondria (Reichart *et al.,* 2018).In the course of oxidative phosphorylation, the electron transport chain on the inner mitochondrial membrane is where the majority of the generation of mitochondrial ROS occurs. Superoxide is created by partially reducing oxygen due to electrons from electron transport chains leaking at complex I and complex III. One of the two dismutases, superoxide dismutase 2 (SOD2) in the mitochondrial matrix and superoxide dismutase 1 (SOD1) in the mitochondrial intermembrane space, then rapidly converts superoxide to hydrogen peroxide. Superoxide and hydrogen peroxide produced during

this process are regarded as mitochondrial ROS when combined (Li *et al.,* 2013).Mitochondrial ROS are thought to be crucial for metabolic adaption in hypoxia at low concentrations.However,high mitochondrial ROS levels trigger autophagy and apoptosis processes that can cause cell death (Finkel, 2011). The main source of ROS during ageing is the mitochondria (Dai *et al.,* 2014).Mitochondria use oxygen oxidation to produce ATP ,when mitochondria are deficient, it results in high production of $O_2^{\bullet -}$, raising mitochondrial ROS (mtROS) levels, which lead to the accumulation of mitochondrial DNA (mtDNA) mutations, resulting in aging (De Almeida *et al.,* 2022). Furthermore, while the role of mtROS in aging remains unclear, it is clear that mtROS play a role in the development of age-related diseases (Chocron *et al.,* 2018).

2.3.1.2 Enzymatic systems

NADPH Oxidases

One important source of ROS, particularly in the cardiovascular system, is NADPH oxidases (NOX) (Montezano *et al.,* 2014).Cardiovascular pathology is facilitated by NOX-mediated excessive ROS generation (Kahles and Brandes, 2012).There are seven isoforms of NOX that have been found; they vary in where they are activated, what kind of ROS they make, and how they do it. These are NOX1, NOX2, NOX3, NOX4, NOX5, and Duox1 (Fukai and Ushio-Fukai, 2020). When NADPH first binds to the dehydrogenase domain, electrons are progressively moved from the NADPH substrate to the FAD cofactor and finally to the two heme groups in the transmembrane domain, resulting in the generation of ROS. Oxygen is the last electron acceptor on the other side of the membrane, where it is reduced to produce hydrogen peroxide or superoxide (Magnani and Mattevi, 2019).NOX regulation,

which affects ROS levels, plays a crucial role in developing aging and age-related diseases.

Endoplasmic Reticulum

The endoplasmic reticulum (ER) of eukaryotic cells is in charge of producing ROS, with ER oxirreductin-1 (ERO1) serving as the main source of these ROS (Zeeshan *et al.,* 2016).A healthy quantity of ROS is necessary for the formation of disulphide bonds, which is a crucial step in the machinery that folds proteins.Therefore, the proper organelle environment, including redox potential and metabolic requirements, is necessary for protein folding to occur successfully in the ER (Zhang *et al.,* 2018).ER also expresses one of the isoforms of NADPH oxidase, NOX4, which contributes to the vicious cycle of oxidative stress in the ER (Lee *et al.,* 2020).

Lipid Oxidases

With iron atoms at its core, lipoxygenases (LOX) are a class of enzymes that oxidise polyunsaturated fatty acids, primarily arachidonic acid, to produce a range of hydroperoxides as a byproduct of the ROS reaction (Zhong *et al.,* 2019).The ROS formation process occurs when Fe^{3+} present in activated LOX is reduced by the contact of this enzyme with its substrate (Watanabe *et al.,* 2017). LOX is primarily involved in the synthesis of prostaglandins, thromboxane, and leukotrienes.The ageing process is linked to increased LOX expression and activity, which produces a notable rise in ROS and adds to the impacts of aging-related molecular inflammation (De Almeida *et al.,* 2022).This impact was demonstrated in aged mice using certain LOX inhibitors, where an increase in LOX-dependent ROS generation was linked to an increase in LOX mRNA expression (Zou *et al.,* 2009).Consequently, LOX-induced oxidative stress can be regarded as a key factor in age-related inflammation.

2.3.1.3 Exogenous sources of ROS

A number of things can be exogenous sources of ROS, including food, alcohol, medications (halothane, doxorubicin, and metronidazole), industrial solvents, heavy metals (Fe, Cu, Co, and Cr), transition metals (Cd, Hg, Pb, and As), air pollution, physical stressors (UV, X-rays, and so on), and lifestyle choices (Jimenez *et al.,* 2022).When combined, these stresses cause inflammation and oxidative stress, which have a direct effect on human health, particularly in the aged. Numerous harmful substances, including metals and other compounds like ROS and RNS[Reactive Nitrogen Specie], are found in air pollution and contribute to the development of diseases. These dangerous particles can enter the body through the respiratory system or be consumed as pollutants, causing inflammation and oxidative stress on a local or systemic level (Mudway *et al.,* 2020).Additionally, radiation and chemotherapy can exacerbate oxidative damage in proteins, lipids, and DNA, increasing ROS and harming the vascular, TGI, and haematopoietic systems (De Almeida *et al.,* 2022).Thus, preventing its harmful consequences requires an understanding of the underlying processes that cause oxidative damage from exogenous sources and therapeutic targeting.

2.3.2 Types of ROS

The following ROS are frequently linked to oxidative stress:

2.3.2.1 Free radicals

A free radical is any chemical species with an unpaired electron in an atomic orbital that is able to exist on its own,rendering them highly reactive and unstable (Chandimali *et al.,* 2025).These reactive oxygen species (ROS) and reactive nitrogen

species (RNS) are produced both endogenously and exogenously (Mandal *et al.,* 2022).Endogenously, free radicals are a consequence of regular cellular processes (Phaniendra *et al.,* 2014).As an example, the electron transport chain occasionally leaks electrons during mitochondrial respiration, which combine with molecular oxygen to generate superoxide radicals (O2•−) (Turrens, 2003).The main types of radical ROS include the superoxide anion (•O2-), hydroxyl radical (•OH), nitric oxide (NO•), alkoxyl radicals (RO•), thiyl radicals (RS•), sulphonyl radicals (ROS•), and lipid peroxyl radical (•LOO-).

2.3.2.2 Non-radicals

Non-radical ROS include hydrogen peroxide (H2O2), singlet oxygen (1O2), dinitrogen dioxide (N2O2), ozone (O3), and hypochlorous acid (HOCl) (Sahoo *et al.,* 2021).

Table 2.1 (Sahoo *et al.,* 2021).

Sl. No.	Free radicals	Non-radicals
1	Superoxide anion ($\bullet O_2$-)	Hydrogen peroxide (H_2O_2)
2	Hydroxyl radical (•OH)	Hypochlorous acid (HOCl)
3	Lipid peroxyl ($\bullet LOO^-$)	Singlet oxygen ($^1O_2$)
4	Alkoxyl radicals (RO•)	Dinitrogen dioxide (N_2O_2)
5	Sulphonyl radicals (ROS•)	Ozone/trioxygen (O_3)

2.3.3 Biological Consequences Of Prolonged ROS Exposure

Human disease is closely linked to ROS signalling dysregulation. For instance, one of the basic features of neurodegeneration and a major factor in the progression of neuronal damage in Alzheimer's disease (AD) is oxidative stress (Hong *et al.,* 2024).ROS-mediated neuronal damage results from disruptions in redox processes, which are characterised by decreases in the activity of antioxidant enzymes like SOD and CAT and drops in antioxidant levels like ascorbic acid and tocopherol (Hajam *et al.,* 2022).The complicated terrain of carcinogenesis is influenced by ROS, which also have a variety of roles in the development and spread of cancer (Moloney and Cotter, 2017).Overproduction of ROS causes oxidative stress, which is linked to neoplastic transformation and results in protein alterations, lipid peroxidation, and DNA damage. The activation of redox-sensitive signalling pathways by ROS, including nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK), encourages the growth, survival, angiogenesis, and metastasis of tumour cells (Malla *et al.,* 2021).On the other hand, cancer cells frequently have stronger antioxidant defences to fend off damage from ROS, giving them a selective edge for tumour growth and survival (Kim *et al.,* 2019).Additionally, research has demonstrated that ATP depletion, whether by means of chemotherapy, radiation therapy, or the modification of glycolytic enzymes, can cause ROS-mediated death in cancer cells, underscoring the promise of combinatorial therapies that target ROS (Panieri and Santoro, 2016). Another biological effect of excessive exposure of the body to ROS is in DNA damage.Since DNA is one of the primary complex macromolecules that can be harmed by oxidative stress, detecting and fixing ROS-induced DNA damage is essential for cells to survive and operate normally (Yousefzadeh *et al.,* 2021).DNA damage,when not repaired can result in mutations and genomic instability, which are

linked to a number of illnesses, including cancer (Eyfjord and Bodvarsdottir, 2005). In cardiovascular development, ROS play a critical role in angiogenesis, cardiomyocyte proliferation and tissue regeneration.For instance, zebrafish research have demonstrated the crucial role that the HECT domain and the E3 ubiquitin-protein ligase 1 (hace1), which contains ankyrin repeats, play in the normal development and operation of the vertebrate heart in a manner that is dependent on ROS (Razaghi *et al.,* 2017).Hace1 expression inhibits NOX-dependent ROS production to preserve healthy cardiac development, while hace1 knockdown causes ROS buildup and cardiac abnormalities. ROS is a "double-edged" weapon that has a variety of functions in cell physiology. While ROS can do significant harm to macromolecules, an overabundance of them can lead to oxidative stress, which can cause a number of illnesses. However, ROS also serve as important signalling molecules that are involved in many physiological processes, including DNA repair, immunological response, gene expression regulation, circadian clock entrainment, and redox homeostasis.

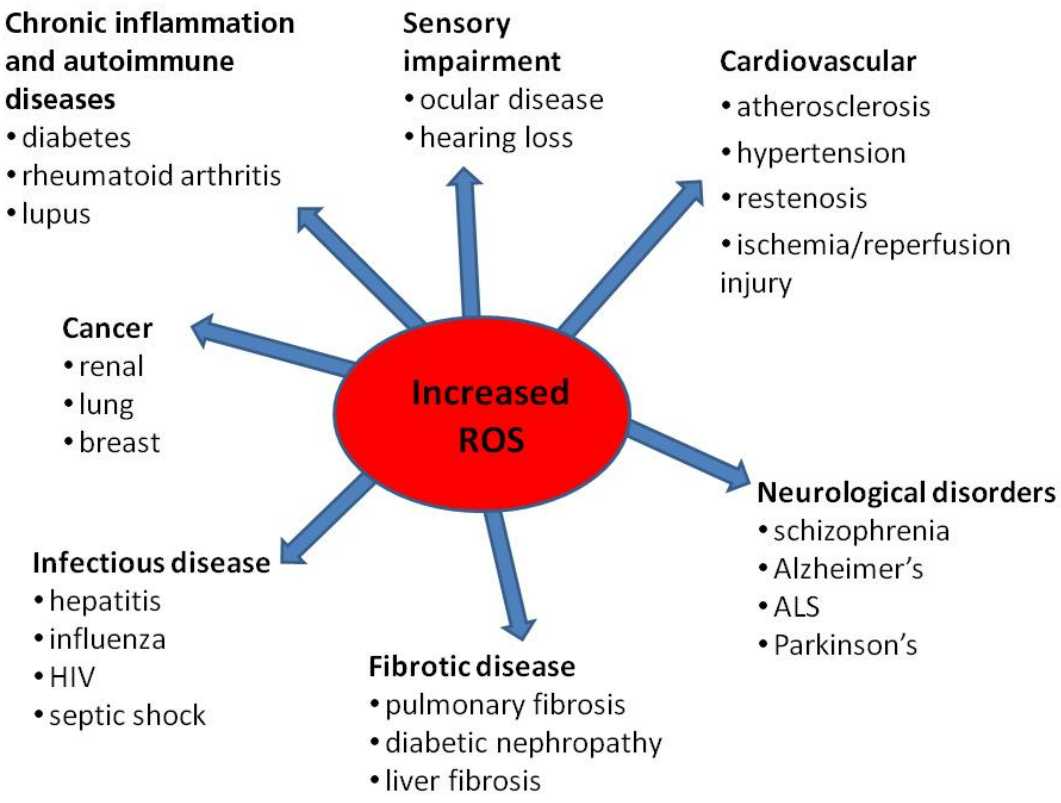

Figure 2.3 effects of increased ROS levels (Brieger *et al.,* 2012).

2.4 Role of Antioxidant Enzymes

Antioxidant enzymes are crucial for preventing and repairing molecular damage caused by free radicals in a number of contexts, as well as for the cellular defence system against their production.Prior to free radicals attacking biological components, antioxidant enzymes can stabilise or deactivate them. They work by lowering the free radicals' energy or by sacrificing some of their electrons for it, making it more stable (Krishnamurthy and Wadhwani, 2012).Antioxidants are enzymatic (endogenous) or non-enzymatic (exogenous) substances that act as a primary defence against reactive oxygen species (ROS) by preventing or preventing the production of free radicals. This reduces oxidative stress, boosts immunity, and lengthens healthy life (Ezema *et al.,* 2024).Exogenous enzymes must be obtained through diet because there is no synthetic pathway for them, whereas endogenous antioxidants are made within animal cells (Tan *et al.,* 2018). Free radicals and endogenous antioxidants are both byproducts of physiological processes and found in biological systems, but the former controls the latter. But when ROS production exceeds antioxidant production, pathogenic situations including oxidative stress and related illnesses arise (Husain *et al.,* 2023).Generally,antioxidants are compounds that can decrease the damage caused by ROS and RNS by converting them into less harmful molecules. Antioxidant enzymes are vital for the maintaining the homeostasis of oxidants,Such antioxidant enzymes include catalase (CAT),superoxide dismutase (SOD), glutathione peroxidase (GPx) and many others.

2.4.1 Catalase (CAT)

The tetrameric protein CAT, which contains heme, is a typical part of cellular peroxisomes. CAT prevents the buildup of H2O2 and shields cells from excessive ROS generation by converting hydrogen peroxide (H2O2) into water and molecular

oxygen (S. E. Lee and Park, 2021).Although CAT is expressed everywhere, it is mostly found in the peroxisomes of all mammalian cell type and human vascular cells (Rasheed, 2024). Structurally, it consists of four subunits, each containing a heme group essential for its catalytic activity(Ganguli *et al.,* 2019).The enzyme is responsible for breaking down H2O2 into H2O and molecular oxygen (O2).This reaction is essential because hydrogen peroxide, a consequence of aerobic respiration and other cellular functions, can become harmful if it builds up (Nandi *et al.,* 2019).Catalase effectively reduces this toxicity by quickly converting hydrogen peroxide into innocuous compounds.Because of its crucial function in cellular physiology, it effectively converts hydrogen peroxide—a precursor to extremely reactive hydroxyl radicals—into oxygen and water, as one of the main defence mechanisms against ROS, hence averting oxidative stress (Yuan *et al.,* 2021). By assisting in the maintenance of this equilibrium, catalase and other antioxidant enzymes shield cells from oxidative damage linked to ageing, inflammation, and a number of illnesses, such as cancer, neurological conditions, and cardiovascular diseases (Hewitt and Degnan, 2023).

2.4.2 Superoxide Dismutase (SOD)

All kingdoms of life have a class of metalloenzymes called superoxide dismutases (SODs). SODs are the first line of defence against damage caused by reactive oxygen species (ROS) (Younus, 2018).Widely distributed, SODs are the main regulating enzymes that microbes use to catalyse the transformation of superoxide into oxygen and H_2O_2 (Wang *et al.,* 2018).The superoxide anion free radical (O2-) is converted by these proteins into molecular oxygen and hydrogen peroxide (H2O2), lowering the O2-level that causes cell damage at high concentrations (Yasui and Baba, 2006).

SODs can be divided into four classes according to the metal cofactors found in their active sites: Iron SOD (Fe-SOD), manganese SOD (Mn-SOD), copper-zinc SOD (Cu, Zn-SOD), and nickel SOD (Fukai and Ushio-Fukai, 2011).SODs constitute a very important antioxidant defense against oxidative stress in the body.According to Zheng *et al.* (2023),SODs in eukaryotes are divided into three types according to their binding metal cofactors and cellular localization, namely, copper–zinc superoxide dismutase (Cu/ZnSOD or SOD1) present in the cytoplasm or secreted into the extracellular fluid, manganese-containing superoxide dismutase (Mn-SOD or SOD_2) present in the mitochondrial matrix, and extracellular superoxide dismutase (EC-SOD or SOD_3).The primary intracellular SOD enzyme, SOD1, is extensively found throughout the cell membrane, nucleus, and cytoplasm.Copper and zinc are necessary for SOD1's enzyme function, however zinc has no catalytic action and only affects the enzyme's molecular structure. SOD2 is an enzyme that contains a 96 kDa homotetramer of mitochondrial manganese and is found in the mitochondrial matrix (Dikalova *et al.,* 2017).The catalytic activity of enzymes is associated with manganese. Signal peptides, which are involved in oxygen disproportionation generated by respiratory enzyme chains, guide the cytoplasmic synthesis of SOD2 to the mitochondria. With the exception of erythrocytes, it is present in all mitochondria and cellular fluids, and its molecular weight varies according to source and distribution. SOD3, which is found in tissues, blood, lymph, and synovial fluid, is a member of Cu/Zn-SOD, which has Cu2+/Zn2+ as its auxiliary group.In most species, SOD3 is a 135 kDa homotetramer made up of two disulfide-linked dimers that release extracellular copper/zinc-containing SOD (ecSOD) (Zheng *et al.,* 2023).It prevents the body's metabolism from producing reactive oxygen species and prevents superoxide ions from being destroyed in both the internal and external environment.

Current research has shown the potential therapeutic applications of SOD in the prevention/control of various diseases(Younus, 2018).

2.4.3 Glutathione Peroxidase (GPx)

In mammals, the glutathione peroxidase family (GPX) is a crucial class of selenium-producing antioxidant enzymes (Zhao *et al.,* 2018).They reduce the toxicity of H2O2 or organic hydroperoxides by catalysing their reduction to water or corresponding alcohols. They are members of the same class of heme-free thiol peroxidases as peroxidases (Pei *et al.,* 2023).Three evolutionary groups make up the GPx family, which descended from an ancestor that contained Cys and catalysed the reduction of H2O2. Five of the eight known isoforms of GPx (GPx1-4 and GPx6) are selenoproteins. The three GPx enzymes that are not dependent on selenium use thiol chemistry instead of selenium chemistry (Brigelius-Flohé and Maiorino, 2012). While GPx6 is only present in humans as a selenoprotein, GPx1 is the most prevalent and abundant isoform of the GPx enzymes (Kryukov *et al.,* 2003).The anti-atherosclerotic properties of GPx-1 in endothelial cells Wagner *et al.* (2009) and an inhibitory potential against lipid peroxidation of GPx-4 in mice have been reported (Guo *et al.,* 2007). In mammals, GPX works with superoxide dismutase and catalase to form an enzymatic antioxidant system that reduces reactive oxygen species (ROS) and limits their toxicity.Formerly known as cytoplasmic GPX (CGPX), GPX1 is the first member of the GPx family to be identified and is found in practically all cell mitochondria and cytoplasm. It is also one of the most highly expressed and abundant selenium proteins in the human body Alehagen *et al.* (2021),Its primary function is to catalyse the reduction of harmful peroxides in the body, such as hydrogen peroxide, cholesterol peroxide, and long-chain fatty acid peroxide, by glutathione (GSH) (Handy and Loscalzo, 2022). Studies have indicated that GPX2 can be utilised as the

first line of defence against oxidative stress from food or intestinal flora. GPX2 is mostly expressed in the gastrointestinal tract, including intestinal epithelial cells and esophageal epithelium (Florian *et al.,* 2001). Hydrogen peroxide, tert-butanol peroxide, and other substances are among its catalytic substrates. GPX3 is predominantly present in plasma, GPX4 mainly protects cell membranes from oxidative attack, and studies have pointed out that there are three different subtypes, namely sperm karyotype, mitochondrial type and cytoplasmic type, which can catalyze complex lipid hydroperoxides such as phospholipids, cholesterol, and cholesterol esters in addition to H_2O_2 (Bersuker *et al.,* 2019). The first member of the Cys-based superfamily to be identified, GPX5 (also known as epididymal GPX, or EGPX), is a secreted protein found in the epididymis that has been shown to protect mouse sperm from hydrogen peroxide toxicity. Its active centre contains cysteine (Cys) rather than Sec (Luan *et al.,* 2019). In humans, GPX6 is a selenium protein with Sec as the active center. The final members to be identified in mammals based on Cys were GPX7 and GPX8, which were shown to have limited glutathione peroxidase activity. By controlling oxidative stress levels, GPX7 may alleviate non-alcoholic steatohepatitis (Wei *et al.,* 2012). Hepatocellular carcinoma cells' oxidative stress response can be inhibited by GPX8 (Lee *et al.,* 2021). GPx is particularly effective at handling lipid peroxides, thereby preventing lipid peroxidation and maintaining membrane integrity.In *Drosophila*, GPx activity has been reported to protect against dietary or environmental oxidative stressors, reinforcing its role as a key biomarker of antioxidant defense (Missirlis *et al.,* 2003). A malfunction in this Antioxidants cascade results in oxidative imbalance, a reduced lifespan, and heightened vulnerability to dietary or environmental contaminants.They are therefore extremely

pertinent to evaluating the toxicological effects of food additives, such Tasty Cube seasoning, in *Drosophila melanogaster*.

2.5 *Drosophila melanogaster* as a Model Organism

The family *Drosophilidae* includes the genus *Drosophila* of flies. Because they are frequently observed hovering around rotting or overripe fruit, members of this family are commonly referred to as "small fruit flies" and are also infrequently called pomace flies, vinegar flies, or wine flies. The *Tephritidae*, a related family of insects sometimes referred to as fruit flies or "real fruit flies," are very different from them. Tephritids mostly consume ripe or unripe fruit, and many of their species,especially the Mediterranean fruit fly which are regarded as dangerous agricultural pests (Atoki *et al.,* 2024). Among the many species of *Drosophila*, *D. melanogaster* has been used extensively in genetic studies since its introduction more than a century ago. It is a well-known model organism in the fields of developmental biology, particularly genetics and molecular biology, and biomedical research (Yamaguchi and Yoshida, 2018).The words "fruit fly" and "*Drosophila*" are frequently used interchangeably with *Drosophila melanogaster* in contemporary biological literature. However, the genus contains more than 1,500 species with a wide range in behaviour, appearance, and favoured breeding habitats (Rohner *et al.,* 2018).More precisely, *D. melanogaster* has been determined to contain over 65–70% of the genes that cause human disease (Pandey and Nichols, 2011), making it a useful model organism for studies in the fields of genetics, cell biology, molecular biology, and biochemistry.Due to its short life cycle, easy handling and maintenance in the lab, and quick generation turnover, *Drosophila* offers comparative benefits over other models for biological research, enabling large-scale studies (Rocha, 2013).

In toxicology research, *Drosophila* provides a unique opportunity to assess both survival outcomes (lifespan, mortality) and biochemical endpoints (enzyme activities such as SOD, CAT, and GPx), making it ideal for this study.

Figure 2.4 *:Drosophilia melanogaster (Wikipedia contributors, 2025)*

2.5.1 Taxonomy

Kingdom:Animalia

Class:Insecta

Order:Diptera

Family:Drosophilidae

Genus:*Drosophilia*

Species:*melanogaster*

2.5.2 Habitat

The tropics have the greatest diversity of *Drosophila species*, with multiple different variants within the genus.*Drosophila* expanded and created more than 800 distinct species on the Hawaiian islands (Atoki *et al.,* 2024). It was originally an African species, with all non-African lineages having a common origin (Baudry *et al.,* 2004).Its geographic distribution encompasses all continents and islands.They can be found in a variety of environments, including deserts, alpine regions, cities, marshes, and tropical rainforests. Many species of *Drosophila,* especially the melanogasters, simulans, and immigrans, are commonly referred to as domestic species because of their strong interaction with humans.*Drosophila* are found worldwide, and their extensive distribution has allowed studies of adaptations to different latitudes (Markow, 2015). *D. melanogaster* doesn't live alone. Many bacteria and other arthropods, including other Drosophila species, interact with their decomposing host materials.*D. melanogaster* is a common pest in homes, restaurants, and other places where food is served.

2.5.3 Physical Description

Wild fruit flies are yellow-brown in colour, with brick-red eyes and transverse black rings across the abdomen.The fly's body is separated into three major parts: the head, the thorax, and the abdomen. The head is somewhat round, with large, visible red compound eyes. These eyes are made up of hundreds of ommatidia and cover the majority of the head's surface.The wild type fly's brick-red eyes are owing to two pigments: xanthommatin, which is brown and generated from tryptophan, and drosopterins, which are red and derived from guanosine triphosphate (Ewart and Howells, 1998).Short antennae between the eyes look like tiny feathery or bristles projections and are used to detect odours, air currents, and vibrations (Kamikouchi *et al.,* 2009). The thorax is robust, with three pairs of legs and one pair of wings. The wings are transparent and membranous, with small veins showing, and span about 4 mm (Webb, 2010).When the fly is not moving, they are held flat over the back. Halteres, or modified hindwings, are tiny knob-like appendages located just behind the wings. These assist the fly maintain balance and orientation in flight (Dickerson, 2020). The *drosophila* leg consists of five segments: the coxa, trochanter, femur, tibia, and tarsus. They have five tarsal segments in their tarsus, which ends with the fly foot, which contains a variety of elements including as the claw and adhesive structures. *D. melanogaster's* major attachment devices are the pulvillus, a flexible elongated structure beneath the claw, and setae, spatula-shaped hair-like projections that are inset into the pulvilli. Although their claws can be used to stick to rough surfaces (Hüsken *et al.,* 2015).Males have sex combs on the first tarsal segment, which are tiny bristle-like projections on their front legs, utilised to connect to females during mating. The abdomen is divided into segments that taper towards the end. It often appears striped, with light and dark colouring alternating. Male abdomens are typically darker

and more rounder, whilst female abdomens are more pointed and striped. The black parts of the abdomen inspired the species' name (melanogaster which means "black-bellied"). They have sexual dimorphism; females are approximately 2.5 mm (0.10 in) length, while males are slightly smaller. Female bodies are up to 30% larger than an adult male's (Álvarez-Rendón *et al.,* 2022).Hormones do not impact the sex or physical appearance of fruit flies, as they do with humans (Kelley and Bayer, 2021). *Drosophila melanogaster* is separated from comparable species by the following combination of characteristics: The male protarsus has a single row of ~12 setae forming a sex comb. The male epandrial posterior lobe is small and nearly triangular. The female abdominal tergite 6 has a dark band running to its ventral margin. The female oviscapt is small, pale, without dorsodistal depression, and has 12-13 peg-like outer ovisensilla (Yuzuki and Tidon, 2020).

2.5.4 Lifecycle and reproduction

Under ideal growing circumstances of 25 °C (77 °F), the *D. melanogaster* lifespan is around 50 days from egg to death (Linford *et al.,* 2013).*D. melanogaster's* developmental time changes with temperature, as do many ectothermic species. The shortest development time (from egg to adult) is seven days at 28 °C (82 °F). Under crowded conditions, development time increases,Chiang and Hodson (1950)while the emerging flies are smaller.Females deposit approximately 400 eggs (embryos), five at a time, into rotting fruit or other appropriate material such as decaying mushrooms and sap fluxes. *Drosophila melanogaster* is a holometabolous bug that undergoes complete metamorphosis. Their life cycle is broken down into four stages: embryo, larva, pupa, adult (Fernández-Moreno *et al.,* 2007).The eggs are around 0.5 mm long and hatch after 12-15 hours.The resultant larvae grow for around four days (at 25 °C) before moulting twice (into second- and third-instar larvae) approximately 24 and 48

hours after hatching .To create the same microbial makeup in the larvae's guts that has benefited her, the mother deposits her excrement on the egg sacs (Blum *et al.,* 2013).The adults then eclose (emerge) after the larvae encapsulate in the puparium and go through a four-day metamorphosis (at 25 °C). Five distinct behavioural patterns are used by males to attract ladies. Initially, males extend and vibrate their wings horizontally to orient themselves while performing a courtship song. The man then lowers himself to the back of the female's belly to lick and tap her genitalia. The male finally tries copulation by curling his abdomen. Females can kick, move away, and extrude their ovipositor to reject males (Connolly and Cook, 1973).Copulation takes 15 to 20 minutes on average Houot *et al.* (2010),during which males transfer a few hundred, very long (1.76 mm) sperm cells in seminal fluid to the female.Sperm from several matings struggle for fertilisation in the two mushroom-shaped spermathecae and a tubular container that the females store the sperm in. It is thought that there is a last male precedent; over 80% of a female's children are sired by the last male to mate with her. In investigations on life extension, *D. melanogaster* is frequently employed to find genes that, when altered, are said to lengthen longevity,ageing,and other physiological mutations (Carnes *et al.*, 2015).

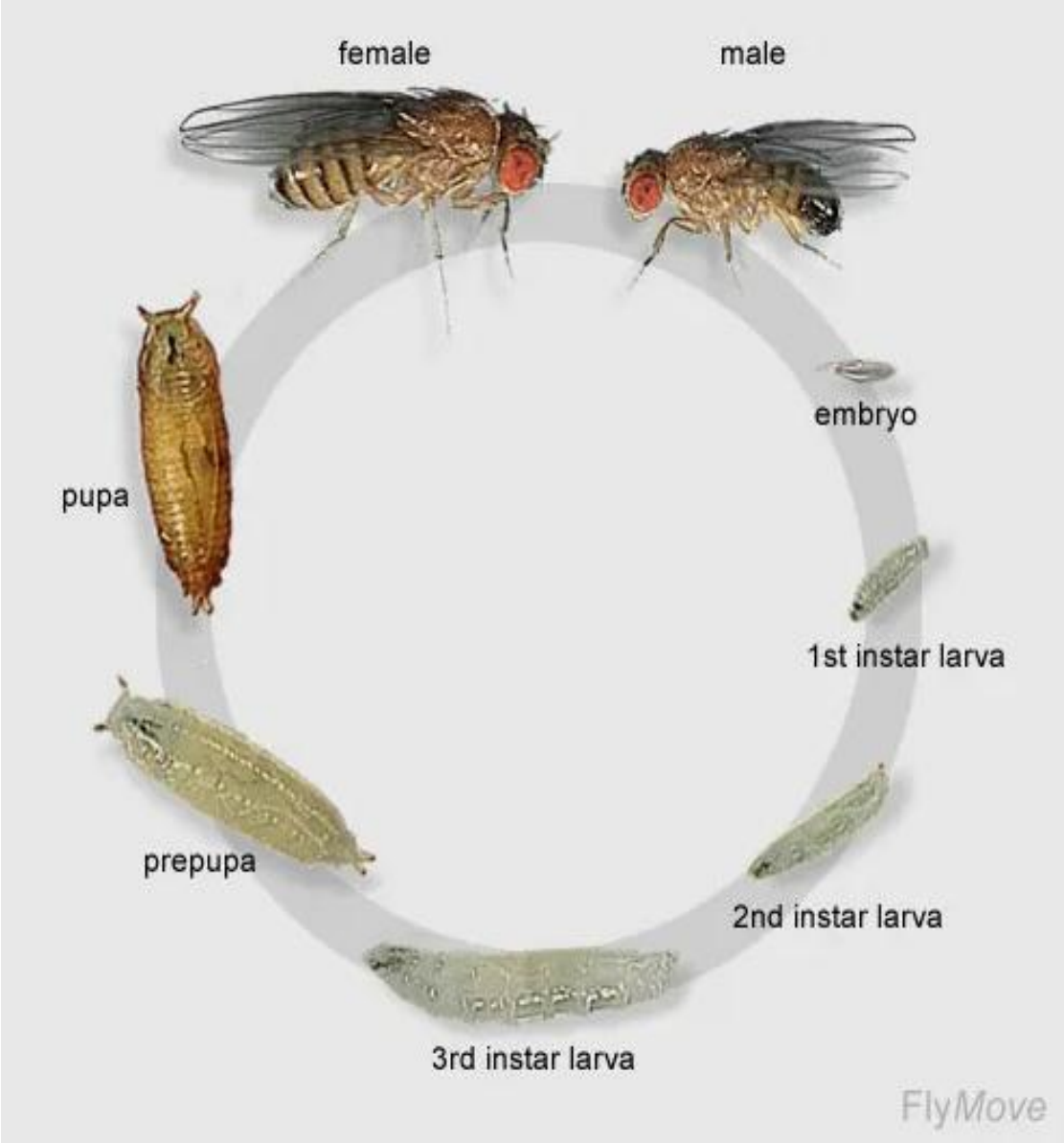

Figure 2.5: Lifecycle of *Drosophilia melanogaster* (*An Introduction to Fruit Flies*, 2017)

2.5.5 Drosophila culture

Drosophila are commonly used as teaching tools in high school biology classes to demonstrate the basic ideas of genetics and inheritance because they are typically inexpensive and easy to care for (Atoki *et al.,* 2024).Moreover, their use in labs is typically unhindered because they aren't any serious ethical or safety issues. Given that a female Drosophila can lay up to 100 eggs in a day for up to 20 days, an embryo at 25°C matures into a viable adult fly in roughly 5–10 days (Stocker and Gallant, 2008). Therefore, if necessary, it is quite simple to produce a huge number of flies for a scientific investigation. In the past, laboratory *Drosophila* were housed in containers with rotting banana pulp but nowadays, it is more customary to culture them in containers with a slurry-like food that is classically created from a combination of water, soy flour, yeast, corn syrup, malt extract, agar and cornmeal.The food must be both firm enough to prevent flies from being stuck in it and soft enough to allow the larvae to burrow through it and feed. Numerous modifications can be made to the basic recipe, and pre-mixed formulas are also available. To keep mites and other pests out of bottles and vials and to stop flies from escaping, foam or cotton wool plugs are used. It is possible to carefully anaesthetise Drosophila with carbon (IV) dioxide if handling individual flies is necessary. In most popular fly labs, flies are placed on absorbent pads connected to a carbon (IV) dioxide source, handled with a fine-tipped paintbrush, and examined under a stereomicroscope.A magnifying lens can be used in place of a stereomicroscope, and ether can be used in place of carbon (IV) oxide. Preserving Drosophila strains as living stocks is essential since gametes or embryos cannot currently be frozen efficiently. Given that this shortens the life cycle to

roughly 21-28 days, fly stocks are usually kept at 18 degrees Celsius. Under some circumstances, this suggests that fresh food should only be provided to each Drosophila stock few times to once in a month.

Figure 2.6*:*Culture of *Drosophilia melanogaster*(Atoki *et al.,* 2024)

2.5.6 Genetic And Toxicological Relevance Of *Drosophilia melanogaster*

The use of alternative small organism models in toxicology has grown tremendously in the last decade. While the fruit fly (*Drosophila melanogaster*) has been a premier model for developmental biologists and geneticists, its utility for toxicology studies has only recently seen a widespread emergence (Rand *et al.,* 2014).Drosophila are currently employed in mechanistic investigations of several important environmental pollutants and toxicants, including as mercury Rand et al. (2009), lead ,arsenic and solvents. The fruit fly has several benefits for research in the lab. Its short life cycle (from embryo to adult in about 10 days at 25°C), straightforward genetic makeup (about 15,000 genes on four chromosomes), and ease and affordability of lab care in comparison to other animal models are noteworthy.The value of *Drosophila* as a useful model organism for human diseases is growing (Chao *et al.,* 2017).They include metabolic illnesses, inflammatory diseases, infectious diseases, cardiovascular diseases, and neurodegenerative diseases. Comparative genomic investigations show that *Drosophila* conserves over 75% of the human genes associated with different diseases (Calap-Quintana *et al.,* 2017).This has facilitated the understanding of various aspects of an increasing number of human diseases.Since around a century ago, *Drosophila* has been employed as a biological model, which has aided in the development of genetics and other related fields.Thomas Hunt Morgan's discovery that genes are found on chromosomes is one of *Drosophila's* major contributions. This forms the basis of modern genetics (Obafemi *et al.,* 2024). Due to its compliance with current standards, D. melanogaster is now employed as a model in toxicology to conduct mechanistic investigations on a number of important environmental pollutants and toxicants (Alaraby *et al.,* 2016).Fruit flies' short biological life cycle makes them suitable for toxicological research spanning from infancy to maturity.

The ability to adapt and the knowledge of its genome have ultimately made it possible to conduct comprehensive genetic and toxicological researches (Ferrero *et al.,* 2020). Therefore, *Drosophila melanogaster* is a dependable and economical organism for toxicological study on food additives like seasoning cubes because to its genetic conservation, ease of experimental manipulation, and response to nutritional and chemical stimuli.

2.6 Previous Studies on MSG or Food Additives and Oxidative Stress

Monosodium glutamate (MSG) and related food additives have been the subject of numerous research that have examined its impact on antioxidant defence systems and oxidative stress in various biological models. These results offer crucial background information for comprehending the possible effects of Tasty Cube (Chicken Flavour) on *Drosophila melanogaster*. Studies on cells and animals have repeatedly demonstrated that MSG causes oxidative imbalance. For instance Abdou *et al.* (2025) reported that the organ damage in rodents caused by excessive MSG intake can be linked to dyslipidaemia, oxidative stress, and inflammation. Increased activity of α-ketoglutarate synthase and inducible nitric oxide synthase may lead to excess ROS generation and oxidative stress. Studies by Ogunmokunwa and Ibitoye (2025) on winstar rat also highlight the potential reproductive risks associated with MSG consumption and underscore the need for public health interventions to promote safe dietary practices.It also collectively illustrate the hormonal and oxidative stress changes that underpin the toxicological effects of MSG, offering new insights into its mechanisms of action and their implications for male reproductive health. Further research by Kesherwani *et al.* (2022) reported that that consumption of MSG at low doses may cause significant alterations that could be responsible for possible health issues including oxidative damage that may be neurotoxic and cause severe histologic

changes. Research on seasoning cubes, which contain MSG, sodium chloride, disodium inosinate, and preservatives, suggests much larger systemic effects. Airaodion et al. (2019) found that continuous use of seasoning cubes in rodents increased oxidative stress indicators and affected liver and kidney function indices. This demonstrates the possibility of additive or synergistic effects beyond MSG alone, due to the presence of excessive sodium and other preservatives. Further research on antioxidant enzymes adds additional evidence of vulnerability. Lushchak (2014) found that exposure to food additives and xenobiotics can decrease SOD, CAT, and GPx activities, resulting in disturbed redox homeostasis. These enzymes are well conserved in Drosophila and sensitive to oxidative stresses, making them useful biomarkers in food toxicity study. Collectively, these findings indicate that, while MSG and related compounds are generally considered safe at low doses, chronic or excessive use may cause oxidative stress and undermine antioxidant defences. However, there has been little investigation into the combined formulation of commercially available seasoning cubes, such as Tasty Cube, and their impact on both survival and antioxidant enzyme activity (SOD, CAT, GPx) in *Drosophila melanogaster*. This gap emphasises the uniqueness and significance of this study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area and Sampling

The experiment was conducted at the Biomedical Toxicology and Chemistry Research (Biotoxcs) Laboratory, located at University of Benin Central Research Laboratory(CRL), Benin City, Nigeria. The Tasty Cube Chicken Flavor seasoning sample was bought from New Benin market, Edo state, Nigeria. It was grinded to powder form with mortar and pestle.

3.2 Materials Used

Falcon tube,1000 microliter pipette,100 microliter micropipette,centrifuge,cotton wool, tissue paper, glass jar, foil paper, paint brush, homogenizing stick, Eppendorf tube, polyurethane foam, phosphate buffer, distilled water, Corn meal, agar, glucose, ethanol,UV-spectrophotometer.

3.2.1 Model Organism

The organism selected for this study is *Drosophilia Melanogaster.*It was bred at the Biomedical Toxicology and Chemistry Research (Biotoxcs) Laboratory.Located at University of Benin Central Research Laboratory (CRL), Benin City, Edo state, Nigeria.

3.3 Methods

3.3.1 Study design, grouping and meal preparation

Study subjects included male and female of *Drosophilia melanogaster* hatched within 48hrs or less. The fruit flies were fed with cornmeal-agar medium diet treated with Tasty Cube Chicken Flavor seasoning of different concentration (0.3g/ml, 0.15g/ml,

0.10g/ml, 0.05g/ml) in separate vials.The control subjects were 24-48 hrs old virgin male and female *Drosophilia melanogaster* flies fed with only a basic cornmeal diet.

The mixed gender pure drosophilia melanogaster was originally obtained from the University of Ibadan Laboratory stock.

Group A-control(200µl of distilled water + 9.8g of cornmeal)

Group B- 0.3g/ml (200µl of seasoning + 9.8g of cornmeal)

Group C- 0.15g/ml (200µl of seasoning + 9.8g of cornmeal)

Group D- 0.10g/ml (200µl of seasoning + 9.8g of cornmeal)

Group E- 0.05g/ml (200µl of seasoning + 9.8g of cornmeal)

For each group,there were three replicates containing 35 flies in each vials.

Table 3.1: Experimental Grouping

Control (A)	200µl of distilled water + 9.8g of cornmeal
Control(B)	200µl of distilled water + 9.8g of cornmeal
Control(C)	200µl of distilled water + 9.8g of cornmeal
Treatment 1A	200µl of 0.3g/ml seasoning + 9.8g of cornmeal
Treatment 1B	200µl of 0.3g/ml seasoning + 9.8g of cornmeal
Treatment 1C	200µl of 0.3g/ml seasoning + 9.8g of cornmeal
Treatment 2A	200µl of 0.15g/ml seasoning + 9.8g of cornmeal
Treatment 2B	200µl of 0.15g/ml seasoning + 9.8g of cornmeal
Treatment 2C	200µl of 0.15g/ml seasoning + 9.8g of cornmeal
Treatment 3A	200µl of 0.10g/ml seasoning + 9.8g of cornmeal
Treatment 3B	200µl of 0.10g/ml seasoning + 9.8g of cornmeal
Treatment 3C	200µl of 0.10g/ml seasoning + 9.8g of cornmeal
Treatment 4A	200µl of 0.05g/ml seasoning + 9.8g of cornmeal
Treatment 4B	200µl of 0.05g/ml seasoning + 9.8g of cornmeal
Treatment 4C	200µl of 0.05g/ml seasoning + 9.8g of cornmeal

Table 3.2 Meal Preparation

INGREDIENTS	STANDARD MEAL	HALF STANDARD MEAL	QUARTER STANDARD MEAL
cornmeal	52g	26g	13g
Agar agar	7.9g	3.95g	1.975g
Glucose	3.5g	1.75g	0.875g
Yeast	5g	2.5g	1.25g
Nipargin	1g	0.5g	0.25g
Ethanol	2ml	1ml	0.25ml
Water	850ml	425ml	212.5ml

3.3.2 Procedure for feed preparation

1. Measure 850ml of distilled water
2. Remove 150ml to mix cornmeal
3. Boil remaining 700ml 0f distilled water
4. Remove little quantity of the boiling water to mix the yeast
5. Add agar to the boiling water and stir till it has fully dissolved
6. Add the dissolved cornmeal and stir
7. Add the glucose and stir
8. Leave the mixture to boil for 2 minutes
9. Add the dissolved yeast
10. Dissolve the nipargin in ethanol
11. Remove the meal from the heat source,add the nipargin,and transfer into appropriate jars and allow to cool.

The same instructions were followed for half standard and quarter standard meal preparation,with the appropriate measurements of ingredients as provided in table 3.2

3.3.3 Experimental Design

The experimental design used for this study was a randomized controlled trial. *Drosophilia melanogaster* individuals were randomly assigned to different treatment groups, each exposed to a specific concentration of tasty cube chicken flavor. A controlled group was included, consisting of flies not exposed to the extract. This

design allowed for a comparison between the effects of different concentrations tasty cube chicken flavor on the antioxidant levels (Catalase, Sodium dismutase and Glutathione peroxidase) in *Drosophilia melanogaster*. To maintain consistency, The flies in each treatment group were raised and kept in identical settings, such as temperature, humidity, and illumination. To guarantee that the outcomes were comparable, the amount of time that each treatment group spent exposed to Tasty Cube chicken flavour seasoning was standardized. Appropriate replication was used throughout the experiment to improve the findings' statistical robustness. To find the bare minimum of flies needed in each group to attain sufficient statistical power, the sample size was calculated. In order to reduce confounding variables and assess the precise effects of different concentrations of Tasty Cube chicken flavour on antioxidant levels (Catalase, Sodium Dismutase, and Gluthathione Peroxidase) in *Drosophilia melanogaster*, this study used a randomised controlled trial design.

3.3.4 Breeding

The prepared meal was poured into glass jars to cool down and solidify.The flies from previous jars were transferred into the glass jar and covered with polyurethane foam that was pre-cut to fit the jar opening perfectly.The polyurethane foam is used in order For the experimental set up,35 flies were transferred into all the vials (15) containing the measured feed and seasoning treatment and covered with cotton wool.The flies were transferred into new meal after 5 days and the set up was left for 21 days before homogenization.

Figure 3.1**:** Experimental setup**.**

3.4 Experiment 1: Survival Assay

For the survival assay,flies of both gender of 1-2 days old were divided into four groups,with each group having three vials each.Each contained 35 flies each.The three groups had varied concentrations of Tasty cube chicken flavor seasoning as shown in Table 3.1. The survival assay was performed in three duplicates of each concentrations.throughout this experiment,the diet was changed every five days.The survival rate was calculated using all concentrations and both life and dead flies were counted daily for 21 days.By the end of the experiment,the data had been gathered and plotted as a proportion of live and dead flies .The result from this experiment was then compared to the control.

3.5 Homogenization and extraction of supernatant

Flies of both gender of 1-2 days old were divided into four groups,with each group having three vials each.Each contained 35 flies each.The three groups had varied concentrations of Tasty cube chicken flavor seasoning as shown in Table 3.1. Following a period of twenty one (21) days of exposure, the flies were placed in empty falcon tubes labelled appropriately for the experimental group and immobilised for four minutes in a freezer. Then, in order to determine the precise weight of flies in each group, empty Eppendorf tubes were labelled and weighed with a weighing balance. The immobilised flies from the freezer were then added to each tube with the proper labels. Phosphate buffer (PO4) was added in microlitres at a ratio of ten (10) times the calculated weight of flies in milligrammes in the eppendorf tube after the flies had been crushed inside the tubes after their precise weight had been determined. The eppendorf tubes were placed in a centrifuge and set to run at 4000rpm for seven (7) minutes. The supernatant was then collected from the sample using a micropipette and transferred

to a labelled eppendorf tube before being frozen for analysis.

Figure 3.2: Centrifuge with eppendorf tubes containing homogenized flies

3.6 Antioxidants Assays

3.6.1 Determination of Catalase (CAT)

Principle

This is based on the method of Cohen, *et al.,* (1970). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2), after the addition of the material containing the enzyme.

Catalase catalyses the reaction: $2H_2O_2 \longrightarrow 2H_2O + O_2$

The quantity of hydrogen peroxide decomposed is directly proportional to the concentration of the enzyme in the sample. The hydrogen peroxide produced in tissues is measured by reacting it with excess potassium permanganate ($KMNO_4$) and then measuring the residual $KMNO_4$ spectrophotometrically at 480 nm.

Catalase (CAT) activity was estimated by the method described by Cohen *et al.,* (1970).

3.6.1.1 Reagents Used

Hydrogen peroxidase (H_2O_2), Suphuric acid (6M) H_2SO_4

3.6.1.2 Preparation of Reagents

0.01M $KMnO_4$ was prepared by distilling 0.158g of $KMnO_4$ in 100ml of distilled water. Phosphate buffer (pH 7.4) 0.426g of $NaHPO_4$ and 0.240g of NaH_2PO_4 were weighed and mixed together in 100ml of distilled water. 6M H_2SO_4 and 32.3ml of concentrated H_2SO_4 were mixed to 66.7ml of distilled water.

3.6.1.3 Procedure

Sample homogenate (0.5 mL) was placed in ice – cold test tubes, the blank contained 0.5 mL distilled water. Cold phosphate-buffered H_2O_2 (30 mM, 5 mL) was added to both blank and sample tubes at fixed intervals, and were mixed by inversion. After 3 min, the reaction was stopped by rapid addition of 1 mL of 6 M H_2SO_4. The tubes were mixed thoroughly by inversion after which 7 mL of 0.01 M $KMNO_4$ was added. Absorbance was read at 480 nm within 3 min.

3.6.1.4 Calculations

1. Activity = $\frac{OD/min \times V}{M \times V \times L \times Y}$

Where OD = Absorbance

L= Light path

V= Total volume of reaction sample

M= Molar coefficient of H_2O_2 (40/m/cm)

V= Volume of sample

Y= mg protein in the sample

3.6.2 Estimation of superoxide dismutase activity (SOD)

This was determined according to the methods of Masra and Fridorich (1972)

3.6.2.1 Principle

Adrenaline undergoes auto oxidation rapidly to adrenochrome whose concentration can be determined at 420nm with the aid of a spectrophotometer. The auto oxidation of adrenaline depends on the presence of superanions. Superoxide dismutase inhibits the

auto-oxdiation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

3.6.2.2 Reagent and preparation

Carbonate buffer (0.05M) pH 10.2: This was prepared by dissolving 0.2014g of Na_2CO_3, 0.2604g $NaHCO_3$ and 0.0372g of EDTA in 100ml of distilled water. The pH was adjusted to 10.2 using Sodium hydroxide. Hydrochloric acid (0.005M): This was prepared by adding 0.044 concentration of HCl to 99.96mls of distilled water. Adrenaline solution (0.3mM): This was prepared by dissolving 0.01098g of adrenaline in 100mls of 0.005M HCL solution.

Assay Procedure

Sample homogenate (0.2 mL) was added to 2.5 mL of 0.05 M carbonate buffer (p^H 10.2) and allowed to equilibrate. The reaction was initiated by the addition of 0.3 mL of freshly prepared 0.03 mM adrenaline as substrate. The solution was mixed by inversion. The reference tube contained 2.7 mL of carbonate buffer and 0.3 mL of adrenaline, while the blank contained 2.5 mL of carbonate buffer, 0.2 mL of distilled water and 0.3 mL of 0.03 mM adrenaline. The increase in absorbance at 420 nm due to the formation of adrenochrome was monitored every 30 sec for 120 sec. One unit of SOD activity was taken as the amount of SOD necessary to cause 50 % inhibition of the oxidation of adrenaline to adrenochrome within 120 sec.

These were mixed and read at 420nm

1. $\%\text{inhibition} = \frac{(\text{O.D test} - \text{O.D ref}) \times 100}{\text{OD test}}$

Enzyme concentration can thus be calculated

$$\text{unit/mg protein} = \frac{\%\ \text{inhibition}}{50 \times Y}$$

Where Y = mg of protein in the volume of sample used.

3.6.3 Estimation of Gluthathione peroxidase (GPx)

This was determined according to Nyman (1959)

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown color disposition, read at 420nm.

3.6.3.1 Reagent and preparation

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100mls of distilled water.

3.6.3.2 Procedures

To an aliquot of sample (0.2 mL), 5 mL of phosphate-buffered H_2O_2, and 1.5 mL of pyrogallol were added. The reaction mixture was allowed to stand for 30 min at room temperature. A deep colour was formed, which was read at 430 nm.

3.6.3.3 Calculations

1. $$\text{Activity} = \frac{\text{OD/min} \times vt \times Df}{E \times Vs \times Y}$$

OD= Absorbance of test

Vt= Total volume of reaction mixture

Df = Dilution factor = 1

E= Molar extinction co-efficient (12/m/cm)

Vs= Volume of sample

Y= mg of protein used

3.7 Data Analysis

Analysis of variance (ANOVA), fishers LSD and sidak multiple comparison test to check for the p-value and the multiple comparison.

CHAPTER FOUR

The effect of Tasty Cube Chicken Flavour(TCS) on the survival of *Drosophila melanogaster* was assessed over a 21-day period (Table 4.1). The log-rank test for trend showed no significant difference in survival between the control and treated groups (p = 0.539). On day 5, the control group had a survival rate of 72.9%, while the groups administered 0.3 g /ml TCS, 0.15 g /ml TCS, 0.10 g /ml TCS, and 0.05 g /ml TCS recorded 76.2%, 77.3%, 66.7%, and 74.2% survival rates, respectively. At day 13, survival in the control group was 45.9%, whereas flies treated with 0.3 g /ml TCS, 0.15 g /ml TCS, 0.10 g /ml TCS, and 0.05 g /ml TCS concentrations showed 41.9%, 40.9%, 42.9%, and 40.4% survival, respectively. By the end of the experimental period (day 21), the control group maintained a 20.0% survival rate, while the 0.3 g/ml TCS, 0.15 g/ml TCS, 0.10 g/ml TCS, and 0.05 g/ml TCS treated groups showed 8.6%, 8.6%, 13.6%, and 11.2% survival, respectively. The survival curves presented in Figure 4.1 further illustrate the gradual decline in survival across all groups, with overlapping patterns and no concentration-dependent effect.

The effect of Tasty Cube Chicken Flavour on antioxidant enzyme activities was also evaluated (Table 4.2). For glutathione peroxidase (GPx), there was a significant difference among the groups (p = 0.009). The control group recorded the highest activity (0.178 ± 0.031), while flies treated with 0.3 g /ml TCS, 0.15 g /ml TCS, 0.10 g /ml TCS, and 0.05 g /ml TCS concentrations showed reduced activities of 0.074 ± 0.007, 0.116 ± 0.012, 0.083 ± 0.013, and 0.102 ± 0.004, respectively (Figure 4.2). For superoxide dismutase (SOD), the differences were also statistically significant (p = 0.029). The 0.15 g /ml TCS (0.223 ± 0.041) and 0.10 g /ml TCS (0.183 ± 0.071) groups showed elevated activities compared to the control (0.074 ± 0.014), while the lowest concentration (0.05 g /ml TCS) recorded markedly reduced activity (0.038 ±

0.003) (Figure 4.3). For catalase (CAT), no significant differences were observed among the groups ($p = 0.236$). The control group had a mean activity of 0.065 ± 0.016, while the treated groups showed 0.144 ± 0.030 (0.3 g /ml TCS), 0.122 ± 0.041 (0.15 g /ml TCS), 0.083 ± 0.012 (0.10 g /ml TCS), and 0.076 ± 0.020 (0.05 g /ml TCS) (Figure 4.4).

Table 4.1. Survival Analysis and Log-rank Test for Drosophila melanogaster Administered Different Concentrations Tasty Cube Chicken Flavour

Day	Control (%)	0.3g /ml TCS TCS (%)	0.15g /ml TCS (%)	0.10g /ml TCS TCS (%)	0.05g /ml TCS TCS (%)	Logrank test for trend	P value
0	90.6	89.5	96.6	94	89.9	3.113	0.539
1	90.6	89.5	95.5	92.9	89.9		
2	85.9	84.8	92	91.7	85.4		
3	80	83.8	81.8	81	80.9		
4	74.1	81.9	79.5	76.2	74.2		
5	72.9	76.2	77.3	66.7	74.2		
6	61.2	75.2	72.7	66.7	69.7		
7	61.2	73.3	71.6	61.9	66.3		
8	58.8	68.6	62.5	56	61.8		
9	55.3	61.9	59.1	52.4	51.7		
10	52.9	54.3	55.7	50	49.4		
11	49.4	50.5	53.4	47.6	46.1		
12	48.2	44.8	47.7	45.2	41.6		
13	45.9	41.9	40.9	42.9	40.4		
14	42.4	38.1	38.6	40.5	31.5		
15	41.2	38.1	37.5	35.7	30.3		
16	41.2	32.4	36.4	34.5	28.1		
17	37.6	25.7	33	31	27		
18	32.9	22.9	25	26.2	23.6		
19	30.6	14.3	22.7	23.8	20.2		
20	23.5	8.6	17	20.2	14.6		
21	20	8.6	13.6	17.9	11.2		

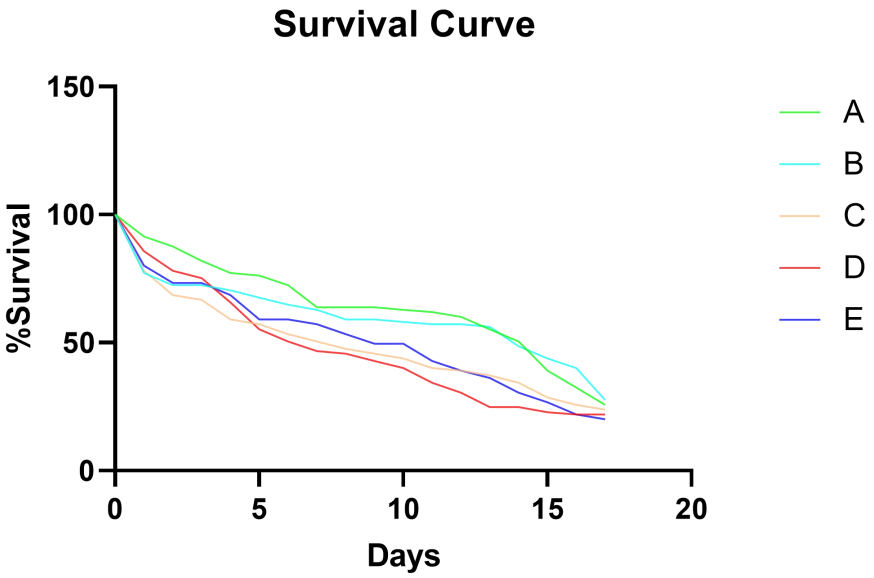

Figure 4.1. Survival curve showing survival rate of Drosophila melanogaster flies administered varied concentrations of Tasty Cube Chicken Flavour

Table 4.2: Effect of Tasty Cube Chicken Flavour on the Activities of Antioxidant Enzymes (Superoxide Dismutase, Catalase, and Glutathione Peroxidase) in Drosophila melanogaster

Parameters	Control	0.3 g /ml TCS	0.15 g /ml TCS	0.10 g /ml TCS	0.05 g /ml TCS	p-value
GPx (u/g Prot)	0.178 ± 0.031^{a}	0.074 ± 0.007^{c}	0.116 ± 0.012^{bc}	0.083 ± 0.013^{c}	0.102 ± 0.004^{bc}	0.009*
SOD (u/g Prot)	0.074 ± 0.014^{bc}	0.095 ± 0.010^{bc}	0.223 ± 0.041^{a}	0.183 ± 0.071^{ab}	0.038 ± 0.003^{c}	0.029*
CAT (u/g Prot)	0.065 ± 0.016	0.144 ± 0.030	0.122 ± 0.041	0.083 ± 0.012	0.076 ± 0.020	0.236

Values are Mean ± SEM (n = 3). $p < 0.05$ indicates significance by one-way ANOVA. Superscripts (^{a}, ^{b}, ^{c}) show Tukey's HSD comparison, where different letters indicate significant differences. GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase.

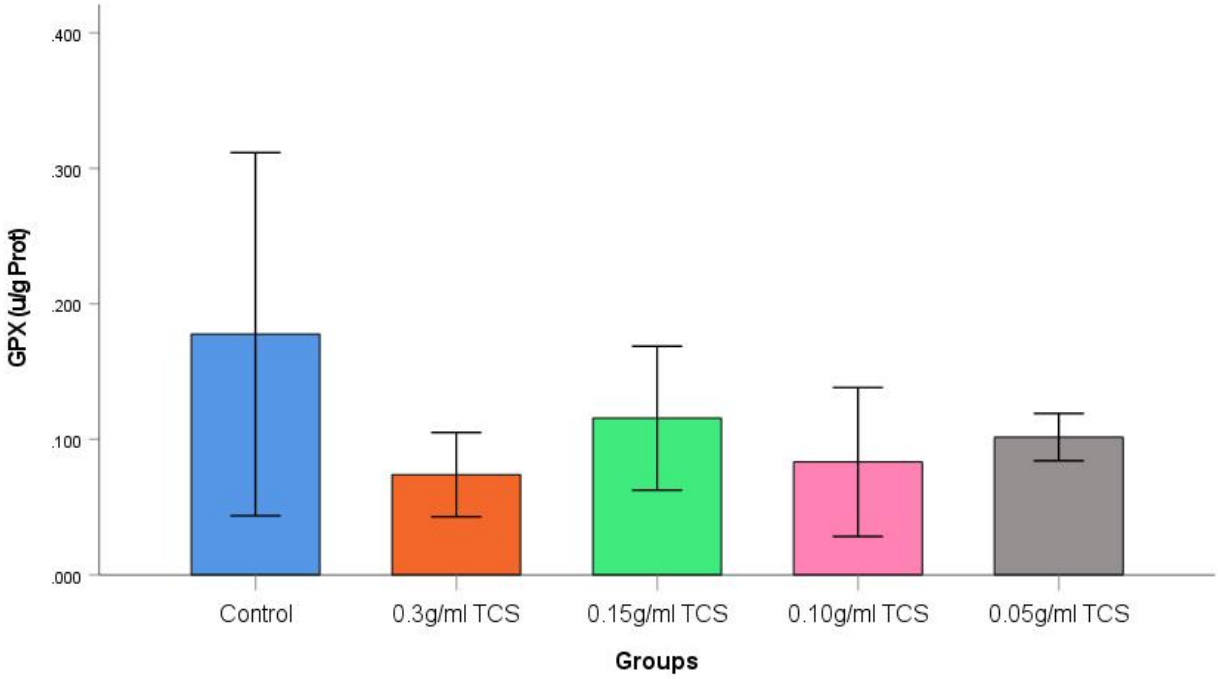

Figure 4.2: *Glutathione Peroxidase (GPx) Activity in Drosophila melanogaster Exposed to Tasty Cube Chicken Flavour.*

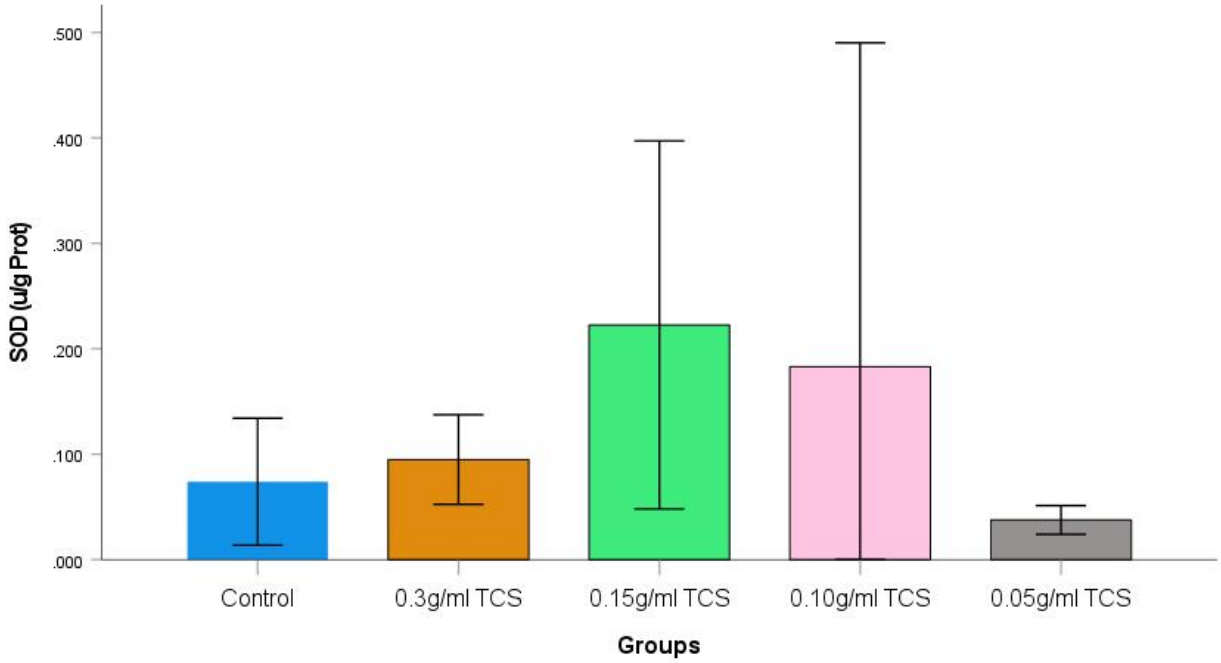

Figure 4.3: *Superoxide Dismutase (SOD) Activity in Drosophila melanogaster Exposed to Tasty Cube Chicken Flavour.*

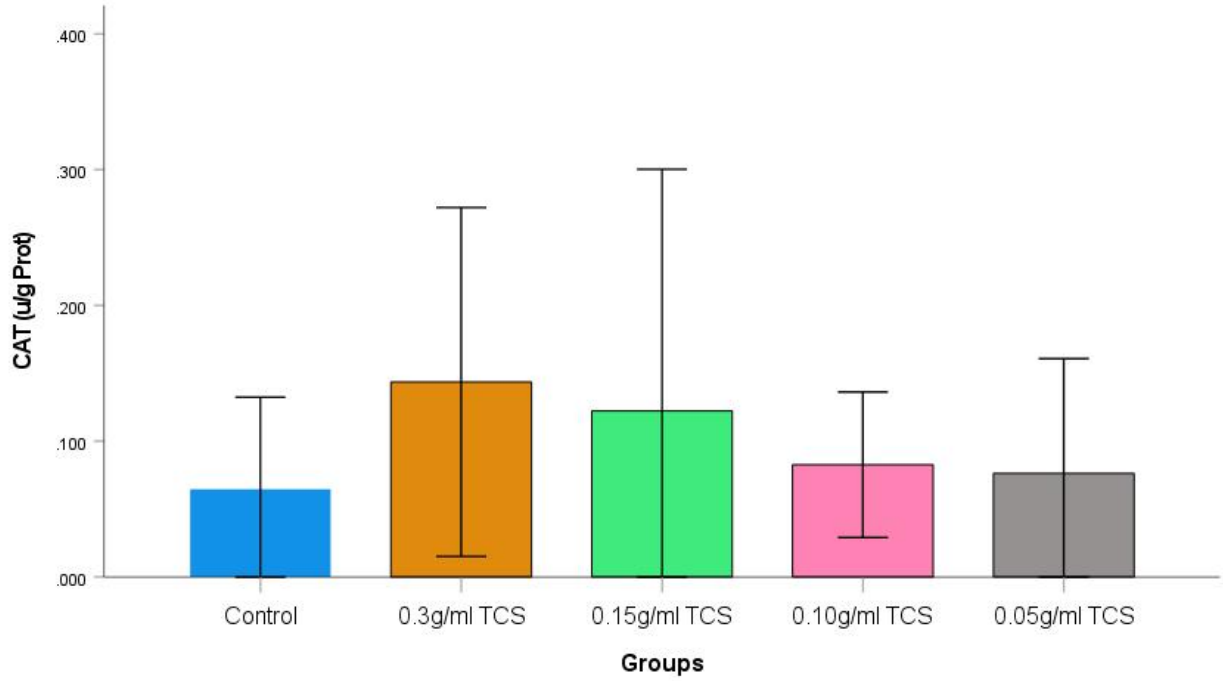

Figure 4.4: *Catalase (CAT) Activity in Drosophila melanogaster Exposed to Tasty Cube Chicken Flavour.*

CHAPTER FIVE

5.1 Discussion

The consumption of processed foods and artificial flavor enhancers has grown significantly in recent decades, particularly in developing nations. Seasoning cubes such as Tasty Cube Chicken Flavour are widely used across Africa due to their affordability and convenience. These cubes, however, contain high levels of monosodium glutamate (MSG), sodium chloride, and other additives, raising concerns regarding their long-term health effects (Airaodion *et al.,* 2019; Ibrahim *et al.,* 2021). Research has established that excessive intake of such additives is associated with oxidative stress, which occurs when the balance between reactive oxygen species (ROS) and the body's antioxidant defense systems is disrupted (Sies and Jones, 2020).

Antioxidants, particularly enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), play a central role in protecting the body against ROS-induced cellular damage (Pizzino *et al.,* 2017). Alterations in these enzymes' activity can serve as biomarkers of oxidative stress and may indicate potential risks to cellular homeostasis and survival.

Drosophila melanogaster, with its conserved antioxidant system, short lifespan, and genetic similarity to humans, is an ideal model organism for assessing such effects (Calap-Quintana *et al.,* 2017).

The survival analysis showed no significant differences between treated groups and controls ($p = 0.539$). All groups demonstrated a gradual decline in survival over the 21-day period, with overlapping survival curves. While there was a slight reduction in

survival in treated groups compared to the control (20% survival in control vs. 8.6–13.6% in treated groups at day 21), this difference was not concentration dependent.

These findings align with earlier reports that MSG and sodium in isolation can reduce survival in Drosophila through oxidative stress (Abolaji *et al.,* 2017). However, the absence of significant survival differences in this study suggests that the concentrations of Tasty Cube administered did not exert acute lethal toxicity. Similar outcomes were observed in rodent models where moderate seasoning cube exposure did not significantly reduce lifespan, though biochemical alterations were evident (Airaodion *et al.,* 2019).

GPx activity showed a significant decrease in treated groups compared to the control ($p = 0.009$), with the control group recording the highest activity (0.178 ± 0.031) and the 0.3 g /ml TCS TCS TCS treatment showing the lowest (0.074 ± 0.007). This reduction indicates impaired capacity to neutralize hydrogen peroxide and lipid peroxides.

These results agree with studies showing that MSG and sodium impair GPx activity, leading to oxidative imbalance (Lushchak, 2014). Reduced GPx activity has been linked to increased lipid peroxidation and membrane instability (Missirlis *et al.,* 2003). Thus, the findings suggest that Tasty Cube consumption compromises the antioxidant defense system in Drosophila, heightening vulnerability to ROS.

SOD activity differed significantly among the groups ($p = 0.029$). Interestingly, moderate concentrations (0.15 g and 0.10 g) produced elevated SOD activity relative to the control, while the lowest concentration (0.05 g) recorded markedly reduced activity (0.038 ± 0.003). This biphasic response suggests an adaptive upregulation of SOD at moderate exposure but enzyme suppression at low exposure.

This finding is consistent with earlier reports that oxidative stress can trigger compensatory increases in antioxidant enzymes as an adaptive mechanism (Halliwell, 2022). However, chronic exposure often overwhelms this defense, leading to eventual depletion of enzymatic activity (Nandi *et al.,* 2019). The reduced activity observed at the lowest dose may indicate insufficient stimulation of antioxidant defenses or enzyme inhibition by specific cube components.

CAT activity showed no significant differences among the groups ($p = 0.236$). While slight increases were recorded at higher concentrations, the changes were not statistically meaningful. This suggests that CAT, unlike SOD and GPx, was less sensitive to Tasty Cube exposure in this experimental setup.

Previous research has reported variable CAT responses to MSG, with some studies noting increased activity as an adaptive mechanism, while others found decreased activity due to enzyme inhibition (Abolaji *et al.,* 2017; Shivasharan *et al.,* 2012). The lack of significant change in this study may reflect a threshold effect, where the administered concentrations were insufficient to provoke marked CAT modulation.

The absence of significant survival differences contrasts with the significant alterations observed in antioxidant enzyme activity. This indicates that while Tasty Cube consumption did not acutely reduce lifespan, it disrupted antioxidant balance by suppressing GPx and altering SOD activity. These sub-lethal biochemical changes may predispose to long-term oxidative damage, even in the absence of immediate mortality.

This aligns with existing literature suggesting that biochemical alterations often precede visible physiological outcomes (Birben *et al.,* 2012). It underscores the

potential risk of chronic consumption of seasoning cubes, where oxidative stress accumulates over time without immediate lethality.

5.2 Conclusion

This study demonstrated that while Tasty Cube Chicken Flavour did not significantly affect survival rates of Drosophila melanogaster, it induced notable alterations in antioxidant enzyme activities. GPx activity was significantly suppressed, indicating reduced ability to counteract peroxides. SOD activity showed a dose-dependent adaptive response, while CAT remained largely unaffected.

The findings support the hypothesis that seasoning cube consumption disrupts antioxidant homeostasis without causing immediate lethality. These results contribute to the understanding of how food additives like MSG and sodium impact oxidative balance, reinforcing concerns raised in previous studies about their long-term health risks.

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5.3 Recommendation

1. Further Research: Long-term studies are needed to investigate chronic and multi-generational effects of seasoning cube exposure on survival, reproduction, and oxidative stress.

2. Dose-Response Studies: Future work should include a broader range of concentrations to better characterize the threshold for antioxidant enzyme modulation.

3. Public Health Awareness: Educational campaigns should highlight the potential risks of excessive seasoning cube consumption, particularly in communities where they are dietary staples.

4. Policy Consideration: Regulatory bodies should reassess the safety of commercial seasoning cubes, ensuring clear labelling and promoting moderate consumption.

5. Alternative Seasoning: Encouraging the use of natural spices and herbs could reduce dependence on synthetic flavor enhancers and lower associated health risks.

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