

TITLE PAGE

**EVALUATION OF METHANOLIC STEM EXTRACT OF *Diospyros mespiliformis*
(KAIWA) ON LIPID PEROXIDATION AND ANTIOXIDANT ACTIVITY OF
DIABETIC FRUIT FLIES**

BY

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A RESEARCH PROJECT

SUBMITTED TO THE

**DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, USMANU
DANFODIYO UNIVERSITY, SOKOTO,**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF
BACHELOR OF SCIENCES B.Sc. (HONS) DEGREE IN BIOCHEMISTRY.**

SUPERVISED

BY

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DECEMBER, 2025

Certification

Dedication

This work is lovingly dedicated to my father Late JP Auta Ibrahim, I miss you so much daddy, wished you were here to see your small girl achieve a degree which you always wanted for me.

Acknowledgements

My deepest gratitude goes to Dr. M. H. Yankuzo, my supervisor, who has been far more than a supervisor, but a father, mentor, role model, and source of wisdom and inspiration, throughout this academic journey. His guidance, patience, and fatherly support remain invaluable. I am equally grateful to my co-supervisor Abdulbariu Ogirima, whose mentorship, tireless guidance, day and night support, and sincere concern contributed immensely to the success of this work.

My special appreciation also goes to all the lecturers and staff of the Department, for the knowledge shared, encouragement and support throughout my studies.

My heartfelt and ceaseless appreciation goes to my mother, for making me who I am today. Your prayers, sacrifices, and unconditional love remain the backbone of every achievement in my life.

I express my unshakable appreciations to my friends from the start to the end who never left me and always stood by me to support and encourage me through every aspect; Jacob David, Susan Andy Garba, Hope, Favour, Daniel Sule, Christabel, Musa Chiroma, Mercy Owo, Mercy Peter, Clement Emmanuel, Nafisa, Alex, Abubakar Sadiq and most especially my bestie, Aisha Idris. I appreciate you all for never leaving or giving up on me.

To Lieutenant John Eteka, thank you so much for all you've done to help, support, and advice, and for never giving up on me. God bless you.

I also extend my special appreciations to my Daddy, Dr. Aliyu Fakandu, for all the mentorship, advises and guidance. Thank you sir.

Finally, to all family members, friends, and well-wishers who supported us in any form, may Allah the Almighty reward you abundantly.

Abstract

Diabetes mellitus is strongly associated with oxidative stress, lipid peroxidation, and impairment of antioxidant defense mechanisms. This study evaluated the effect of *Diospyros mespiliformis* stem bark extract on lipid peroxidation and antioxidant activity in *Drosophila melanogaster* subjected to a high-sugar diet (HSD) to induce diabetes-like metabolic dysfunction. Fruit flies were divided into control, HSD, metformin-treated, and extract-treated groups. Hyperglycemia, body weight, locomotor performance, superoxide dismutase (SOD) activity, total antioxidant capacity (TAC), and malondialdehyde (MDA) levels were assessed to determine metabolic and oxidative outcomes.

The HSD regimen produced significant increases in glucose concentration and body weight, along with impaired locomotor function, confirming successful induction of a diabetic phenotype. Antioxidant imbalance was evident through reduced SOD activity and elevated MDA levels, indicating enhanced lipid peroxidation. Treatment with *D. mespiliformis* extract significantly lowered glucose levels, reduced body weight, and improved climbing ability relative to untreated diabetic flies. The extract restored SOD activity, elevated TAC, and markedly decreased MDA levels to values comparable to the control and metformin groups, demonstrating potent antioxidant and anti-lipid peroxidative effects.

The findings show that *D. mespiliformis* effectively attenuates hyperglycemia, mitigates oxidative stress, and protects against lipid peroxidation in diabetic *Drosophila* models. The study supports its potential as a natural therapeutic candidate for managing oxidative complications associated with diabetes and highlights its relevance for future pharmacological and translational research.

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CHAPTER ONE

1.1 Introduction

Diabetes mellitus is one of the most prevalent metabolic disorders globally, characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Beyond glucose dysregulation, diabetes is strongly associated with enhanced oxidative stress, lipid peroxidation, and impaired antioxidant defense mechanisms, which contribute significantly to its complications (American Diabetes Association [ADA], 2022; Sun *et al.*, 2021). The increasing global burden of diabetes, projected to reach 643 million cases by 2030, highlights the urgent need for affordable and accessible therapeutic agents, particularly in low-resource settings (International Diabetes Federation [IDF], 2021).

Oxidative stress arises when the balance between the generation of reactive oxygen species (ROS) and the antioxidant defense system tilts towards the former, leading to cellular and tissue damage (Sies and Jones, 2020). ROS attack polyunsaturated fatty acids within cell membranes, initiating lipid peroxidation, which compromises membrane integrity and disrupts normal cellular functions. Malondialdehyde (MDA), a by-product of lipid peroxidation, is commonly used as a biomarker of oxidative stress and has been found to be elevated in diabetic conditions (Ayepola *et al.*, 2019).

Experimental models using fruit flies (*Drosophila melanogaster*) have gained significant recognition in biomedical research due to their genetic similarities to humans, short life cycle, and well-characterized metabolic pathways (Pandey and Nichols, 2011). *Drosophila* has been widely employed to model diabetes mellitus, lipid metabolism disorders, and oxidative stress, providing insights into disease mechanisms and therapeutic interventions (Troutwine *et al.*, 2020).

Medicinal plants have been utilized for centuries to treat various ailments, and current pharmacological research continues to highlight their bioactive potential against chronic

diseases. *D. mespiliformis* Hochst. ex A. DC., commonly known as African ebony or jackalberry, is a widely distributed plant in sub-Saharan Africa. It is traditionally employed for the management of fever, infections, and metabolic disorders, with its leaves, bark, and fruits reported to possess significant antioxidant and anti-inflammatory activities (Adzu *et al.*, 2019; Musa *et al.*, 2021). The phytochemical constituents of *D. mespiliformis* include flavonoids, tannins, saponins, and phenolic compounds, many of which are linked to free radical scavenging and modulation of oxidative pathways (Okoli *et al.*, 2020).

Given the rising interest in natural antioxidants and the therapeutic potential of *D. mespiliformis*, investigating its effect on lipid peroxidation and antioxidant activity in a diabetic model is both timely and necessary.

1.2 Statement of the Problem

Diabetes mellitus remains a major cause of morbidity and mortality worldwide, largely due to its complications linked to oxidative stress and lipid peroxidation. Current therapeutic approaches, while effective in glycemic control, often fail to adequately address oxidative damage, leaving patients vulnerable to long-term complications such as cardiovascular disease, neuropathy, and nephropathy (Brownlee, 2021). Moreover, the high cost and side effects of synthetic drugs limit their accessibility and compliance, particularly in resource-constrained settings.

Despite the well-documented traditional use of *D. mespiliformis*, there is limited experimental data on its role in modulating oxidative stress in diabetic conditions, especially in *in vivo* models like *Drosophila melanogaster*. This gap underscores the need for scientific validation of its antioxidant and protective effects in the context of diabetes.

1.4 Justification/Significance of the Study

This study is justified on several grounds. Firstly, it addresses the global health challenge of diabetes, particularly the oxidative stress component often neglected in conventional therapy.

By evaluating *D. mespiliformis*, a plant widely available in Africa, this research provides scientific validation for its traditional use and highlights its potential as a source of affordable therapeutic agents.

Secondly, the study contributes to the growing body of knowledge on plant-based antioxidants and their mechanisms of action in diabetic models. Findings from this work may inform the development of phytotherapeutics targeting oxidative stress pathways, thereby improving diabetes management and reducing complications.

Finally, employing *Drosophila melanogaster* as a model enhances translational relevance due to its conserved metabolic pathways with humans, and at the same time provides a cost-effective and ethically viable experimental approach.

1.3 Aim and Objectives of the Study

1.3.1 Aim:

To evaluate the effect of *D. mespiliformis* on lipid peroxidation and antioxidant activity in diabetic-induced fruit flies (*Drosophila melanogaster*).

1.3.2 Objectives:

- a. To determine the levels of lipid peroxidation (MDA) in diabetic fruit flies treated with *D. mespiliformis*.
- b. To assess the activities of key antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) in the treated groups.
- c. To evaluate non-enzymatic antioxidant levels (glutathione and related compounds) in diabetic fruit flies administered *D. mespiliformis*.
- d. To compare the biochemical outcomes between treated, untreated, and control groups.

CHAPTER TWO

Literature Review

2.1 Oxidative Stress

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the ability of biological systems to detoxify these reactive intermediates or repair the resulting damage (Pizzino *et al.*, 2017). ROS include free radicals such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($\bullet OH$), and non-radical derivatives such as hydrogen peroxide (H_2O_2), which are generated as natural by-products of aerobic metabolism (Sies and Jones, 2020). While low-to-moderate levels of ROS are essential for physiological processes such as cell signaling and immune defense, excessive ROS accumulation can damage biomolecules including lipids, proteins, and nucleic acids, ultimately disrupting cellular homeostasis (Phaniendra *et al.*, 2015).

The mitochondria are the primary source of endogenous ROS, especially through electron leakage at complexes I and III of the electron transport chain. Other intracellular sources include peroxisomes, cytochrome P450 metabolism, and NADPH oxidase activity (Forrester *et al.*, 2018). In addition, exogenous factors such as xenobiotics, pollutants, radiation, and poor dietary habits can significantly elevate ROS production (Liguori *et al.*, 2018).

Persistent oxidative stress has been implicated in the onset and progression of a wide range of diseases, including neurodegenerative disorders, cancer, cardiovascular disease, and metabolic syndromes such as diabetes mellitus (Hayes *et al.*, 2020). In diabetes, hyperglycemia exacerbates ROS generation via pathways such as glucose autooxidation, protein glycation, and polyol pathway activation, thereby promoting tissue injury and diabetic complications (Brownlee, 2021).

The body possesses a complex antioxidant defense system, comprising enzymatic and non-enzymatic mechanisms, to neutralize ROS and prevent cellular damage (Sun *et al.*, 2021).

However, when the rate of ROS production overwhelms these defense mechanisms, oxidative stress occurs, leading to lipid peroxidation, protein oxidation, and DNA fragmentation (Birben *et al.*, 2012). This makes oxidative stress both a marker and mediator of cellular dysfunction in chronic disease conditions.

Recent studies emphasize that oxidative stress is not only a pathological by-product but also an active contributor to disease pathogenesis. For example, Vona *et al.* (2021) demonstrated that chronic oxidative imbalance drives persistent inflammation and vascular damage, two hallmarks of diabetic complications. Similarly, Rani *et al.* (2022) highlighted that oxidative stress interacts with metabolic dysregulation to exacerbate β -cell dysfunction in diabetes, underscoring the critical role of ROS in disease progression.

2.2 Lipid Peroxidation

Lipid peroxidation is a free radical-mediated process in which reactive oxygen species (ROS) attack polyunsaturated fatty acids (PUFAs) within cell membranes, leading to the formation of lipid radicals and reactive aldehydes (Ayala *et al.*, 2014). This process is initiated when a hydroxyl radical ($\bullet\text{OH}$) abstracts a hydrogen atom from a PUFA, producing a lipid radical that subsequently reacts with molecular oxygen to form lipid peroxyl radicals ($\text{LOO}\bullet$). These radicals propagate chain reactions that destabilize cell membranes, alter membrane fluidity, and impair membrane-bound enzymes and receptors (Phaniendra *et al.*, 2015).

One of the most commonly measured by-products of lipid peroxidation is malondialdehyde (MDA), which serves as a reliable biomarker of oxidative damage (Ayepola *et al.*, 2019). Other secondary products, such as 4-hydroxynonenal (4-HNE), also contribute to cytotoxicity by forming adducts with proteins and nucleic acids, disrupting cellular signaling, and promoting apoptosis (Gaschler and Stockwell, 2017).

Lipid peroxidation plays a dual role in biology. On one hand, it contributes to normal physiological signaling; for example, lipid hydroperoxides participate in immune modulation

and eicosanoid synthesis (Yin *et al.*, 2011). On the other hand, excessive lipid peroxidation is pathogenic, leading to cellular dysfunction and tissue injury (Sultana *et al.*, 2013). In chronic diseases such as diabetes mellitus, cardiovascular disease, and neurodegenerative disorders, elevated lipid peroxidation is a consistent hallmark of oxidative stress-mediated damage (Vona *et al.*, 2021).

Recent studies underscore the clinical relevance of lipid peroxidation as both a biomarker and a therapeutic target. Oyenih *et al.* (2022) demonstrated significantly elevated MDA levels in diabetic patients compared to non-diabetic controls, linking lipid peroxidation to poor glycemic control and cardiovascular risk. Similarly, Sharma *et al.* (2021) observed that elevated lipid peroxidation in diabetic animal models was accompanied by reduced antioxidant enzyme activity, suggesting a vicious cycle of oxidative imbalance.

Experimental evidence further shows that lipid peroxidation disrupts mitochondrial membranes, leading to impaired ATP production and amplification of ROS generation, a self-perpetuating mechanism of cellular injury (Gaschler and Stockwell, 2017). Moreover, lipid peroxidation products such as 4-HNE have been shown to activate pro-inflammatory signaling pathways, thereby linking oxidative damage to chronic inflammation (Ismail *et al.*, 2020).

Because of its central role in disease pathology, mitigating lipid peroxidation is a key therapeutic strategy. Antioxidants, both enzymatic (e.g., superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (e.g., vitamin C, vitamin E, polyphenols), function to intercept free radicals, terminate peroxidation chain reactions, and repair oxidized molecules (Birben *et al.*, 2012). Plant-derived phytochemicals, including flavonoids and phenolic compounds, have attracted attention for their ability to inhibit lipid peroxidation and protect cellular structures (Musa *et al.*, 2021).

2.3 Antioxidant Defense Systems in Living Organisms

To counterbalance the continuous production of reactive oxygen species (ROS), living organisms have evolved complex antioxidant defense systems. These defenses consist of enzymatic antioxidants, non-enzymatic antioxidants, and repair/removal mechanisms, which together maintain redox homeostasis and protect cellular integrity (Sies and Jones, 2020).

Enzymatic Antioxidants

The enzymatic defense system provides the first line of protection against ROS. Key enzymes include:

- **Superoxide dismutase (SOD):** Converts superoxide anions ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2), thereby reducing the burden of highly reactive superoxide radicals (Lobo *et al.*, 2010).
- **Catalase (CAT):** Located primarily in peroxisomes, catalase rapidly decomposes hydrogen peroxide into water and oxygen, preventing the formation of hydroxyl radicals ($\bullet OH$) through the Fenton reaction (Ighodaro and Akinloye, 2018).
- **Glutathione peroxidase (GPx):** Utilizes reduced glutathione (GSH) as a substrate to reduce H_2O_2 and lipid hydroperoxides, thereby preventing lipid peroxidation (Brigelius-Flohé and Maiorino, 2013).

These enzymes work synergistically; for example, SOD generates H_2O_2 from $O_2^{\bullet-}$, which is then removed by catalase or GPx, ensuring minimal accumulation of harmful intermediates.

Non-Enzymatic Antioxidants

Non-enzymatic antioxidants are low molecular weight compounds, many of which are derived from diet. Major examples include:

- **Glutathione (GSH):** A tripeptide that directly scavenges ROS and serves as a cofactor for GPx. Depletion of GSH is a hallmark of oxidative stress (Forman *et al.*, 2009).
- **Vitamins:** Vitamin E (α -tocopherol) protects cell membranes by terminating lipid peroxidation chain reactions, while vitamin C (ascorbic acid) scavenges ROS in aqueous environments and regenerates oxidized vitamin E (Traber and Stevens, 2011).

- **Plant-derived phytochemicals:** Flavonoids, phenolic acids, and carotenoids provide additional antioxidant defense, either by scavenging free radicals or chelating redox-active metals (Pisoschi and Pop, 2015).

Repair and Removal Systems

Beyond scavenging ROS, organisms possess repair mechanisms that restore oxidatively damaged molecules. For instance, DNA repair enzymes correct oxidative lesions such as 8-oxoguanine, while proteasomal degradation removes oxidized proteins that cannot be repaired (Sharma *et al.*, 2021). Damaged lipids are similarly metabolized and replaced by normal biosynthetic processes.

Physiological Significance

Antioxidant defense systems are essential not only for protection but also for redox signaling. Moderate ROS levels act as secondary messengers in processes such as cell proliferation, immune response, and apoptosis (Sies and Jones, 2020). However, overwhelming ROS production leads to oxidative stress when antioxidant defenses are insufficient.

Recent studies emphasize that impaired antioxidant defenses contribute to the pathology of chronic diseases. For example, Rani *et al.* (2022) observed reduced activities of SOD, CAT, and GPx in diabetic models, correlating with elevated lipid peroxidation and tissue injury. Similarly, Oyenih *et al.* (2022) reported that restoration of antioxidant enzyme activity through plant extracts significantly reduced oxidative stress markers in diabetic rats.

Thus, antioxidant defense systems represent a turning point between ROS production and detoxification. Strategies aimed at enhancing endogenous antioxidant capacity, including the use of medicinal plants such as *Diospyros mespiliformis* remain promising in the prevention and management of oxidative stress-related disorders.

2.4 Diabetes Mellitus and Oxidative Stress

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association [ADA], 2022). Beyond glucose imbalance, oxidative stress has emerged as a pivotal factor in the onset and progression of diabetes and its associated complications (Rani *et al.*, 2022). Excessive generation of reactive oxygen species (ROS) in diabetic states overwhelms the antioxidant defense system, resulting in lipid peroxidation, protein oxidation, DNA damage, and impaired cellular signaling (Brownlee, 2021).

Several biochemical pathways link hyperglycemia to oxidative stress. One key mechanism is glucose autooxidation, where glucose undergoes non-enzymatic reactions with molecular oxygen to produce free radicals. Another mechanism is the polyol pathway, in which excess glucose is reduced to sorbitol by aldose reductase, consuming NADPH and reducing cellular antioxidant capacity (Obrosova, 2019). Furthermore, advanced glycation end-products (AGEs) formed under hyperglycemic conditions interact with their receptors (RAGE), stimulating NADPH oxidase activity and further increasing ROS production (Singh *et al.*, 2014). Collectively, these processes form a vicious cycle of oxidative imbalance.

Mitochondria also play a crucial role in diabetes-associated oxidative stress. Hyperglycemia enhances electron flow through the mitochondrial respiratory chain, leading to electron leakage and excessive generation of superoxide anions ($O_2^{\bullet-}$) (Sies and Jones, 2020). This contributes to mitochondrial dysfunction, reduced ATP production, and initiation of apoptotic pathways (Pinti *et al.*, 2019).

Oxidative stress is not only implicated in the pathogenesis of diabetes itself but also in its complications. Diabetic nephropathy is associated with oxidative injury to renal glomeruli and tubules, while diabetic neuropathy results from ROS-mediated damage to peripheral nerves and Schwann cells (Forbes and Cooper, 2019). Similarly, diabetic retinopathy has

been linked to ROS-induced microvascular damage and lipid peroxidation in retinal cells (Stitt *et al.*, 2016). Cardiovascular complications, which account for the majority of diabetes-related deaths, are strongly correlated with oxidative stress-driven endothelial dysfunction and vascular inflammation (Vona *et al.*, 2021).

Recent studies continue to reinforce the role of oxidative stress in diabetes. For example, Sharma *et al.* (2021) reported significantly elevated levels of malondialdehyde (MDA) and reduced antioxidant enzyme activities in diabetic rats, suggesting enhanced lipid peroxidation and compromised defense mechanisms. In another study, Oyenihini *et al.* (2022) observed that oxidative stress markers were directly correlated with poor glycemic control and cardiovascular risk among diabetic patients, emphasizing the clinical importance of redox imbalance in disease progression.

2.5 Lipid Peroxidation in Diabetic Model

Lipid peroxidation is a prominent biochemical feature of diabetes mellitus, arising from chronic hyperglycemia and excessive generation of reactive oxygen species (ROS). In diabetic states, elevated ROS attack polyunsaturated fatty acids in cell membranes, leading to the accumulation of toxic lipid peroxidation by-products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (Ayepola *et al.*, 2019). These products are widely employed as biomarkers of oxidative stress in experimental and clinical diabetes studies (Sharma *et al.*, 2021).

Experimental models have consistently shown a strong association between hyperglycemia, lipid peroxidation, and tissue injury. For instance, Sharma *et al.* (2021) demonstrated that diabetic rats exhibited significantly higher MDA levels compared to controls, alongside decreased antioxidant enzyme activity. Similarly, Oyenihini *et al.* (2022) reported that lipid peroxidation correlated positively with glycated hemoglobin (HbA1c) levels in diabetic patients, underscoring its role in glycemic dysregulation and vascular complications.

In addition to serving as a marker of oxidative imbalance, lipid peroxidation directly contributes to diabetic pathology. Peroxidized lipids compromise membrane integrity, impair insulin signaling, and activate pro-inflammatory pathways (Ismail *et al.*, 2020). Lipid peroxidation products such as 4-HNE can form adducts with proteins, altering their function and further exacerbating insulin resistance (Gaschler and Stockwell, 2017). Moreover, these aldehydes are capable of diffusing across membranes, spreading oxidative damage beyond their site of origin.

Fruit fly (*Drosophila melanogaster*) models have been particularly useful in elucidating lipid peroxidation under diabetic conditions. Due to the conservation of metabolic and oxidative pathways between *Drosophila* and mammals, diabetic fruit fly models induced by high-sugar diets or genetic manipulation exhibit increased ROS production and lipid peroxidation, mimicking human diabetic complications (Troutwine *et al.*, 2020). For example, Na *et al.* (2019) observed elevated MDA levels and reduced catalase activity in high-sugar-fed *Drosophila*, suggesting that lipid peroxidation contributes significantly to metabolic stress in this model.

Furthermore, lipid peroxidation has been linked to specific diabetic complications in model organisms. In *Drosophila*, increased ROS and lipid peroxidation were associated with reduced lifespan, locomotor deficits, and neuronal degeneration, paralleling diabetic neuropathy in humans (Hughes and Tadjuidje, 2019). These findings support the relevance of lipid peroxidation not only as a biomarker but also as a mechanistic driver of diabetic pathology.

Given this evidence, therapeutic interventions targeting lipid peroxidation are considered crucial in diabetes research. Antioxidants from natural sources, such as polyphenols, flavonoids, and other phytochemicals, have demonstrated efficacy in reducing MDA levels and improving antioxidant enzyme activity in diabetic models (Musa *et al.*, 2021).

2.6 *Diospyros mespiliformis* (Botanical, Phytochemical, and Pharmacological Perspectives)

Diospyros mespiliformis Hochst. ex A. DC., commonly known as African ebony or jackalberry, is a perennial tree belonging to the family Ebenaceae. It is widely distributed across tropical and sub-Saharan Africa, including Nigeria, Ghana, Cameroon, and South Africa, where it grows in savannah woodlands, riverine areas, and well-drained soils (Adzu *et al.*, 2019). The tree can reach heights of up to 25 meters, with dense, dark-green foliage and small edible fruits that serve as a food source for both humans and animals (Musa *et al.*, 2021). Traditionally, various parts of the plant, including the leaves, bark, roots, and fruits have been employed in African ethnomedicine for the treatment of fever, dysentery, infections, wounds, and metabolic disorders.

Phytochemical Composition

Phytochemical studies reveal that *D. mespiliformis* is rich in bioactive secondary metabolites, including flavonoids, tannins, saponins, alkaloids, terpenoids, and phenolic compounds (Okoli *et al.*, 2020; Musa *et al.*, 2021). These phytochemicals are known to possess antioxidant, anti-inflammatory, and antimicrobial activities. Flavonoids and phenolics, in particular, contribute to the plant's ability to scavenge free radicals, inhibit lipid peroxidation, and chelate pro-oxidant metals (Adzu *et al.*, 2019). The plant also contains sterols and triterpenoids, which have been associated with anti-diabetic and hepatoprotective effects (Oladunmoye *et al.*, 2020).

Pharmacological Properties

The pharmacological potential of *D. mespiliformis* has been validated in several experimental studies. Extracts of the plant have demonstrated:

- **Antioxidant activity:** Several studies have reported significant radical scavenging activity of leaf and bark extracts in vitro, often attributed to their flavonoid and phenolic content (Okoli *et al.*, 2020). In animal models, administration of the extracts reduced MDA levels and improved antioxidant enzyme activities (Musa *et al.*, 2021).
- **Antidiabetic effects:** Research indicates that *D. mespiliformis* extracts lower blood glucose levels, possibly by enhancing insulin secretion and improving glucose utilization (Adzu *et al.*, 2019).
- **Antimicrobial activity:** Both aqueous and ethanol extracts of the plant exhibit inhibitory effects against bacterial and fungal pathogens, supporting its traditional use in treating infections (Eze *et al.*, 2020).
- **Anti-inflammatory properties:** The extracts inhibit inflammatory mediators such as nitric oxide and cytokines, providing further therapeutic relevance in oxidative stress-related conditions (Oladunmoye *et al.*, 2020).

Toxicological Considerations

Toxicity studies suggest that *D. mespiliformis* is relatively safe at therapeutic doses, though high doses may produce hepatotoxic effects in experimental animals (Adzu *et al.*, 2019). This underscores the importance of dose optimization in therapeutic applications.

Relevance to Diabetes and Oxidative Stress

The bioactive constituents of *D. mespiliformis*, particularly its phenolics and flavonoids, are strongly linked to antioxidant and anti-lipid peroxidative activities. By scavenging ROS and enhancing endogenous antioxidant defenses, the plant may mitigate oxidative damage associated with diabetes mellitus. For instance, Musa *et al.* (2021) demonstrated that ethanol leaf extract of *D. mespiliformis* significantly reduced MDA levels and increased catalase activity in diabetic rats, suggesting its potential as a natural adjunct in diabetes management.

Collectively, the botanical richness, phytochemical diversity, and pharmacological properties of *D. mespiliformis* justify further exploration of its role in modulating oxidative stress and lipid peroxidation in diabetic models such as *Drosophila melanogaster*.

2.7 Antioxidant Composition and Bioactivity of *D. mespiliformis*

The antioxidant potential of *D. mespiliformis* is largely attributed to its rich composition of polyphenols, flavonoids, tannins, and other phytoconstituents that actively neutralize reactive oxygen species (ROS) and inhibit oxidative damage (Okoli *et al.*, 2020). Phytochemical analyses consistently report high concentrations of phenolic compounds and flavonoids in the leaves, bark, and fruits of the plant, which are known to act as hydrogen donors, metal chelators, and chain-breaking antioxidants (Musa *et al.*, 2021).

Antioxidant Composition

- **Flavonoids:** These compounds scavenge free radicals such as hydroxyl and superoxide radicals, and modulate signaling pathways that regulate oxidative stress and inflammation (Adzu *et al.*, 2019).
- **Phenolic acids:** Phenolic constituents, including gallic acid and related derivatives, inhibit lipid peroxidation by stabilizing free radicals and preventing chain propagation (Oladunmoye *et al.*, 2020).
- **Tannins:** Reported to exert antioxidant activity through protein-binding and ROS scavenging mechanisms (Musa *et al.*, 2021).
- **Vitamins and micronutrients:** The fruits contain appreciable levels of vitamin C and other micronutrients, which further enhance their antioxidant potential (Eze *et al.*, 2020).

Bioactivity Evidence

In vitro assays provide strong evidence of the antioxidant capacity of *D. mespiliformis*. For example, Okoli *et al.* (2020) demonstrated that ethanol extracts of the plant exhibited significant scavenging activity in DPPH and ABTS radical assays, comparable to standard

antioxidants such as ascorbic acid. Similar findings were reported in ferric-reducing antioxidant power (FRAP) assays, indicating electron-donating properties of the extracts.

In vivo studies further support its antioxidant bioactivity. Musa *et al.* (2021) showed that administration of *D. mespiliformis* leaf extract to diabetic rats significantly reduced malondialdehyde (MDA) levels and restored activities of key antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT). These findings highlight the extract's ability to both reduce lipid peroxidation and enhance endogenous antioxidant defenses. In another study, Adzu *et al.* (2019) observed that aqueous extracts improved glutathione levels in oxidative stress-induced models, suggesting modulation of non-enzymatic antioxidant pathways.

Mechanistic Insights

The antioxidant activity of *D. mespiliformis* is proposed to involve multiple mechanisms:

1. Direct scavenging of ROS and reactive nitrogen species (RNS).
2. Inhibition of lipid peroxidation by stabilizing PUFA radicals and preventing chain propagation.
3. Upregulation of endogenous antioxidant enzymes via modulation of redox-sensitive transcription factors such as Nrf2 (Oladunmoye *et al.*, 2020).
4. Anti-inflammatory effects that indirectly reduce ROS production by inhibiting pro-oxidant enzymes like inducible nitric oxide synthase (iNOS) (Musa *et al.*, 2021).

Relevance to Diabetes Models

Given the established role of oxidative stress in diabetic pathology, the antioxidant composition of *D. mespiliformis* makes it a promising therapeutic candidate. Its ability to reduce MDA levels and improve antioxidant enzyme activity aligns with the biochemical goals of mitigating oxidative damage in diabetes (Sharma *et al.*, 2021; Oyenihini *et al.*, 2022). Moreover, the use of *Drosophila melanogaster* as a model offers a platform to further

validate these bioactivities in vivo, particularly with regard to lipid peroxidation and antioxidant defense modulation.

Chapter Three

3.0 Materials and Methods

The materials used in this study are listed in Table 3.1

Table 3.1.: List of materials used in the study

S/N	Materials/Equipment /Reagents	Types/Size	Manufacturer/ Sources	Cities/States/Provinces	Countries
1	ELISA plate reader	S/N:IFTDL2310 07015	Infitek	Lixia	China
2	Centrifuge	S/NBEPDL2308 09001	Bioevopeak	Lixia	China
3	Eppendorf tube	1.5ml (500 pieces) LOT NO: 202301			Germany
4	Water Distiller machine	LWDB-400M	Laboid International, Solan	Pradesh	India
5	Absolute Ethanol	283041 5 litres	Loba-chemie pvt Ltd	Mumbai	India
6	Pipette	1000µl, 200 µl, 100 µl, and 10 µl	Eppendorf AG	Hamburg	Germany
7	Pipette tips	Pt1000-1-BS, PT0200-9-45 F1670801 1000µl, 200 µl, 100 µl, and 10 µl	Shanghai Gene Era Bio-Science	Zhejiang	China

8	Latex hand gloves	4 packs	Unigloves SDN	Senawang	Malaysia
9	Measuring Cylinder	50ml	New England Biolabs Inc	Hitchin	UK
10	Scott bottle	100ml bottle	CAMRET	Sokoto	Nigeria
11	Refrigerator-Freezer	Model: RC- 67WSI	Hisense	Hisense	China
12	Distilled water	2 Litres	CAMRET	Sokoto	Nigeria
13	Icepacks	4 packs	CAMRET	Sokoto	Nigeria
14	Phosphate buffered saline (PBS) tablet	1 Tablet	Oxoid	Basingstoke Hemisphere	England
15	Baker's Yeast		STK Royal Ltd	Lagos	Nigeria
16	Agar-Agar		Titan Biotech	Rajasthan	India
17	Methyl Paraben		Molychem	Mumbai	India
18	Corn Flour		Faso Farine Mais Blanc	Ouagadougou	Burkina Faso
19	Sucrose		(FFMB) LOBA Chemie	Mumbai	India
20	Electronic weighing scale		Pvt Ltd Kern and Sohn	Balingen	Germany
21	Handheld homogenizer		Ltd Hangzhou Miou Instrument Co., Ltd	Hangzhou	China

3.1 Preparation of *D. mespiliformis* (Kaiwa) Bark Extract

The stem extract of *D. mespiliformis* (Kaiwa) was obtained from Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University Sokoto.

3.2 Fly Stock and Husbandry

Wild-type *D. melanogaster* were obtained from the *Drosophila* Laboratory at the Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University, Sokoto. The stock was maintained under standard laboratory conditions to ensure genetic stability and optimal physiological health prior to experimental use. Flies were reared in sterile *Drosophila* culture vials containing a freshly prepared cornmeal–yeast–agar diet, and maintained at a controlled temperature range of 22–25 °C, relative humidity of 55 ± 5%, and a 12:12 h light–dark cycle.

To maintain a healthy colony and prevent overcrowding, flies were transferred to fresh diet every 48–72 hours. Only healthy, active adult flies were selected for the study. Prior to experimentation, newly emerged flies were allowed to acclimate for 3–5 days, ensuring full physiological maturation and reducing age-related variability.

Throughout the pre-experimental and experimental phases, all cultures were handled under aseptic conditions to prevent microbial contamination. Vials, cotton plugs, and feeding materials were routinely sterilized, and all procedures were conducted in a clean environment to preserve the integrity of the experimental units.

3.3 Experimental Design

Drosophila melanogaster flies, aged 3 to 5 days, were induced with diabetes by feeding them a 30% high sucrose diet for 14 days, with a diet change on day 7. On day 14, body weight and glucose levels were measured to confirm the induction of diabetes. The flies were then divided into three groups (n = 30/group) in triplicate: sucrose diet (SD), extract diet (ED), and

metformin diet (MD), along with a control group fed a normal diet, also in triplicate. All groups continued on their respective diets for 7 more days. The nutritional composition of the experimental diets is provided in Table 3.2. Following the intervention, changes in body weight, locomotor performance, superoxide dismutase (SOD), malondialdehyde (MDA), and Total Antioxidant Capacity (TAC) were assessed across the groups.

Table 3.2: Nutritional composition of experimental groups

	Control	High Sucrose Diet	Extract Diet	Metformin Diet
Corn-flour	100g	100g	100g	100g
Agar-Agar	10g	10g	10g	10g
Yeast	10g	10g	10g	10g
Methylparaben	10g	10g	10g	10g
Distilled Water	1.5 L	1.5 L	1.5 L	1.5 L
Sucrose	-	30%	-	-
Extract	-	-	600µg/ml	-
Metformin	-	-	-	16mg/10ml

HSD- High Sucrose Diet; ED- Extract Diet; MD- Metformin Diet

3. 4 Measurement of Body Weight

The body weight of the flies was assessed across the experimental groups using a precise electronic weighing scale. Ten flies per group were anesthetized on ice for 3–5 minutes and transferred into a pre-weighed 1.5 ml microcentrifuge tube (Eppendorf tube). The tubes were re-weighed to determine the weight in milligrams (mg). This process was repeated for three biological replicates, and the average weight per group was calculated.

3.5 Climbing assay

Also referred to as the locomotor performance assay, this test was conducted following the method described by Adeshina et al 2024. Briefly, thirty flies were introduced into an empty 50 mL vial marked with a line 6 cm from the bottom and allowed to acclimate. The flies were

gently tapped to the bottom of the vial, and the number of flies crossing the 6 cm mark within 10 seconds was recorded. The assay was repeated three times, and the average percentage of flies crossing the mark was calculated for each group.

3.6 Haemolymph Extraction

Following cold anaesthesia, a pooled group of thirty flies per experimental group was transferred into 1.5 mL Eppendorf tubes containing 200 µL of phosphate-buffered saline (PBS). The flies were homogenized using a handheld homogenizer and centrifuged at $8000 \times g$ for 10 minutes at 4 °C. The resulting supernatant was carefully collected, transferred into a sterile 1.5 mL tube, and stored at -20 °C for subsequent biochemical analyses.

3.7 Biochemical Assays

3.7.1 Quantification of Glucose

Glucose levels in whole fly extracts were measured using a Spinreact™ kit (Girona, Spain) following the manufacturer's instructions. The absorbance of samples and standards was read at 505 nm using microplate reader with a blank serving as the reference. Glucose concentration was calculated using the formula:

$$\text{Glucose concentration (mmol/mL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

3.7.2 Malondialdehyde (MDA) Assay

MDA levels were measured using a lipid peroxidation assay kit (Solarbio Life Science, Beijing, China) according to the manufacturer's protocol. Absorbance was measured at 593nm using microplate reader. The final MDA concentration was calculated using the formula:

$$\text{MDA (nmol/mL)} = 5 \times [6.45 \times (\Delta A_{532} - \Delta A_{600}) - 1.29 \times \Delta A_{450}]$$

3.7.3 Superoxide Dismutase Quantification

The concentration of SOD was quantified using a commercially available ELISA kit, absorbance of 450nm was taken in accordance with the protocol provided by the manufacturer.

3.8 Statistical Analysis

The normality of the data was assessed using the Shapiro-Wilk test, and all data were found to be parametric. Data are presented as means $\pm$ standard deviation (SD). Unpaired t-test was conducted to compare two groups. One-way analysis of variance (ANOVA) was applied to compare variables across the groups. Bonferroni post hoc test was then conducted to compare the means. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were carried out using Jamovi (Version2.3.28) and GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, California).

CHAPTER FOUR

4.0 RESULTS

4.1 Evaluation of Glucose Levels Following Diabetes Induction

Table 4.1 shows that diabetes induction was confirmed by measuring glucose levels, which were significantly elevated in the 30% sucrose HSD group.

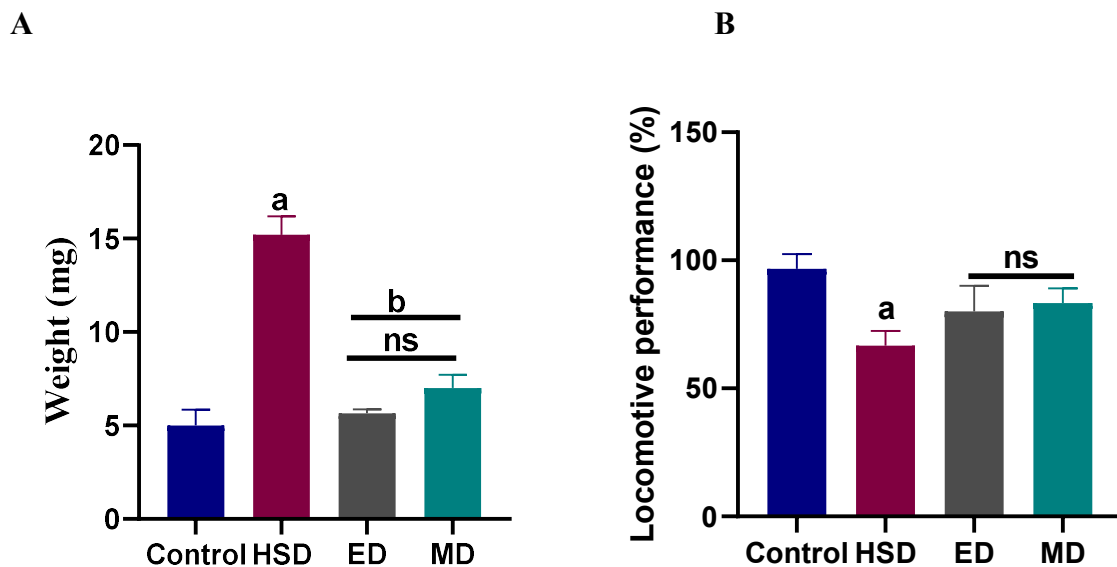
Table 4.1: Confirmation of Glucose Induction

Experimental groups	Parameters			
	Control	HSD 1	HSD 2	HSD 3
Glucose (mg/dL)	52.41 ± 7.07	188.9 ± 15.91*	201.9 ± 6.01*	182.2 ± 6.93*

HSD- High sugar diet. Statistical significance difference compared to control group $p < 0.05$ was represented by *. No statistical difference compared to control group $p < 0.05$ was represented by ns. Data were reported as mean ± SD (n = 200).

4.2 Evaluation of Weight and Locomotor Parameters

Figure 4.2A-B shows that HSD caused a significant increase in body weight in *Drosophila melanogaster*, confirming diet-induced obesity ($p < 0.05$). Administration of MSEDMD or metformin significantly reduced this weight gain relative to the HSD group, indicating protection against sugar-related metabolic stress. The HSD regimen also led to reduced locomotor performance; however, MSEDMD markedly improved climbing activity, restoring it to levels close to those of the control group ($p < 0.05$). Metformin also enhanced locomotor ability, though not significantly compared with controls, suggesting that MSEDMD may offer equal or superior support for motor coordination under hyperglycemic conditions.

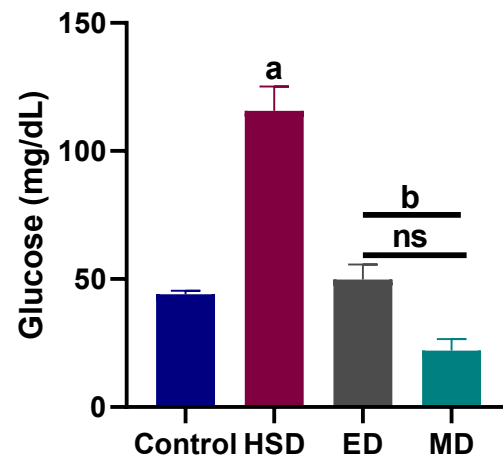


HSD- High sugar diet; ED- Extract diet containing MSEDMD; MD- Metformin diet. Statistical significance difference compared to control group $p < 0.05$ was represented by *, while statistical significance compared to HSD group $p < 0.05$ was represented by #. No statistical difference compared to control group $p < 0.05$ was represented by ns. Data were reported as mean $\pm$ SD ($n = 30$).

4.3 Effect of MSEDm on HSD-Induced Hyperglycemia and Carbohydrate Metabolic

Dysregulation

HSD led to pronounced disturbances in carbohydrate metabolism in *Drosophila melanogaster*, with glucose levels significantly elevated compared to controls ($p < 0.05$). Treatment with MSEDm produced a significant reduction in glucose levels relative to the HSD group ($p < 0.05$), demonstrating antihyperglycemic effects comparable to metformin and an overall improvement in carbohydrate regulation (**Figure 4.3**).

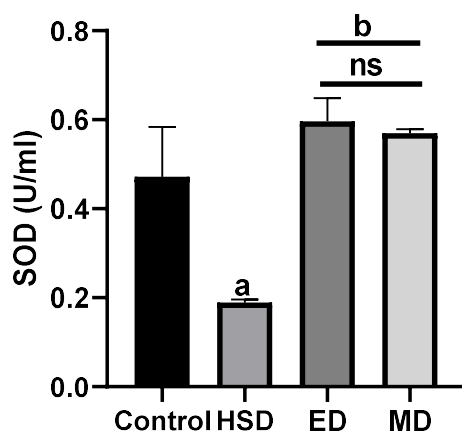


HSD- High sugar diet; ED- Extract diet containing MSEDm; MD- Metformin diet. Statistical significance difference compared to control group $p < 0.05$ was represented by *, while statistical significance compared to HSD group $p < 0.05$ was represented by #. No statistical difference compared to control group $p < 0.05$ was represented by ns. Data were reported as mean $\pm$ SD ($n = 30$).

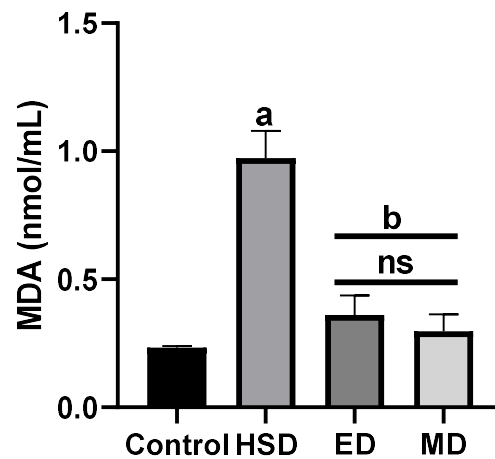
4.4 Assessment of Antioxidant Response and Oxidative Stress Markers in HSD-Fed *D. melanogaster* Treated With MSES

HSD exposure in *Drosophila melanogaster* resulted in a reduction in SOD significant to control ($p < 0.05$). MDA levels were elevated, indicating heightened lipid peroxidation. Administration of MSES improved TAC beyond HSD levels and significantly lowered MDA concentrations to near-control values ($p < 0.05$), highlighting its potent antioxidant and lipid-protective properties. Comparable effects were also observed in metformin-treated flies (Figure 4.4A and 4.4B).

A



B



HSD- High sugar diet; ED- Extract diet containing MSED; MD- Metformin diet. Statistical significance difference compared to control group $p < 0.05$ was represented by *, while statistical significance compared to HSD group $p < 0.05$ was represented by #. No statistical difference compared to control group $p < 0.05$ was represented by ns. Data were reported as mean $\pm$ SD ($n = 30$).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The significant elevation of glucose levels in HSD-fed flies confirms effective hyperglycemia induction. The control group maintained glucose levels within the normal physiological range, whereas all HSD groups showed marked increases in glucose concentration, aligning with established *Drosophila* diabetic models. High-sugar feeding is known to elevate circulating glucose, activate insulin resistance pathways, and trigger metabolic disturbances similar to type 2 diabetes in mammals (Na *et al.*, 2019; Musselman *et al.*, 2011).

The degree of hyperglycemia observed further validates the model as one capable of inducing downstream oxidative stress mechanisms. Hyperglycemia-generated reactive oxygen species (ROS) arise through glucose autooxidation, advanced glycation end-product formation, and mitochondrial overload—processes that have been documented extensively in both mammalian and *Drosophila* systems (Rani *et al.*, 2022; Brownlee, 2021). The elevated glucose values seen in this study therefore provide a biochemical foundation for examining oxidative stress and antioxidant modulation by the extract.

HSD exposure caused a significant increase in body weight, indicating diet-induced obesity. This is consistent with previous reports showing excessive sugar intake in *Drosophila* promotes triglyceride accumulation, insulin signaling disruption, and metabolic burden (Troutwine *et al.*, 2020). Weight gain is also a key feature associated with insulin resistance and poor glycemic control, and thus strengthens the diabetic phenotype.

Locomotor performance was markedly impaired in HSD-fed flies, which aligns with studies demonstrating that hyperglycemia-induced ROS accumulation leads to neuromuscular degeneration and reduced motor coordination (Hughes and Tadjidje, 2019). Climbing

defects are often used as functional readouts of metabolic and neurological damage in diabetic *Drosophila* models.

Treatment with MSEDm significantly improved locomotor ability and reduced weight relative to the untreated HSD group. This suggests that the extract supports metabolic homeostasis and may counteract ROS-mediated neuromuscular damage. Interestingly, the locomotor recovery produced by MSEDm was comparable to or better than metformin, indicating the extract's strong therapeutic potential.

MSEDm treatment significantly reduced glucose levels in diabetic flies, demonstrating antihyperglycemic activity. This parallels findings in rodent models where *D. mespiliformis* extracts improved glucose tolerance, enhanced insulin secretion, and modulated carbohydrate metabolism (Musa *et al.*, 2021; Adzu *et al.*, 2019).

The extract's glucose-lowering effect may be attributed to its phytochemicals—particularly flavonoids, tannins, and phenolic acids—which are known to enhance glucose uptake, stimulate insulin pathway components, and inhibit carbohydrate-digesting enzymes (Oladunmoye *et al.*, 2020). These bioactivities likely contribute to the improved glycemic profile observed.

The similar antihyperglycemic effect seen with metformin further supports the therapeutic potential of MSEDm. Both interventions appear to counteract metabolic dysregulation triggered by the high-sugar diet.

HSD-fed flies showed a significant reduction in SOD activity, indicating compromised antioxidant defense. Reduced SOD activity is a hallmark of hyperglycemia-induced oxidative stress as excessive ROS overwhelm enzymatic defenses (Sharma *et al.*, 2021). Low SOD levels promote the accumulation of superoxide radicals, exacerbating cellular damage.

Administration of MSEDm restored SOD activity toward control levels. This indicates that the extract enhances endogenous antioxidant defense, likely through phytochemicals that

support enzyme activity or activate antioxidant regulatory pathways such as Nrf2. Similar improvements in antioxidant enzymes have been documented in earlier studies using *D. mespiliformis* extracts (Musa *et al.*, 2021).

HSD feeding significantly increased MDA levels, confirming elevated lipid peroxidation. MDA is a widely accepted biomarker of oxidative membrane damage and is typically elevated in diabetic conditions due to ROS attack on polyunsaturated fatty acids (Ayepola *et al.*, 2019).

Treatment with MSEDm markedly reduced MDA levels to near-control values, demonstrating strong anti-lipid peroxidation activity. This aligns with *in vitro* and *in vivo* studies reporting that *D. mespiliformis* flavonoids and polyphenols are effective scavengers of hydroxyl and superoxide radicals, preventing peroxidative chain reactions (Okoli *et al.*, 2020). The reduction in MDA achieved by MSEDm was comparable to metformin, highlighting the extract's ability to stabilize cell membranes and counteract oxidative damage.

5.2 Conclusion

The present investigation demonstrated that exposure to a high-sugar diet successfully induced a diabetic and oxidative stress state in *Drosophila melanogaster*, characterized by elevated glucose levels, increased body weight, reduced locomotor activity, suppressed antioxidant defenses, and heightened lipid peroxidation. Treatment with *D. mespiliformis* extract significantly ameliorated these metabolic and oxidative disturbances, producing effects comparable to those of metformin. The extract lowered glucose concentrations, reduced malondialdehyde levels, restored superoxide dismutase activity, improved total antioxidant capacity, and enhanced both physiological and behavioral outcomes in the diabetic flies. These findings strongly suggest that the antioxidant-rich phytochemicals present in *D. mespiliformis* contribute to its ability to modulate redox balance and protect against lipid peroxidation under hyperglycemic stress. The study therefore supports the therapeutic potential of *D. mespiliformis* as a natural agent capable of mitigating oxidative complications associated with diabetes.

5.3 Recommendations

Based on these results, it is recommended that:

- a. Further research explore the molecular pathways through which the extract exerts its antioxidant and antihyperglycemic effects, particularly with regard to gene expression, enzyme regulation, and redox-sensitive signaling mechanisms.
- b. Expanding the study to include other oxidative stress markers, inflammatory mediators, and mitochondrial function would provide deeper mechanistic insight.
- c. Comparative studies involving different extraction solvents, plant parts, and dosage gradients would help establish optimal conditions for therapeutic application.
- d. Investigations in higher animal models are also essential to determine the translational relevance of these findings for human diabetes management.

- e. Finally, standardization of phytochemical composition and safety profiling will be important steps toward developing *D. mespiliformis* into a viable, evidence-based phytotherapeutic option.

5.4 Limitations

Limitations include:

- a. The findings may not fully replicate the complex metabolic interactions in higher vertebrates, limiting direct extrapolation to humans.
- b. Variability in phytochemical composition of the plant extract depending on geographical location and extraction method may affect reproducibility.
- c. The scope is confined to biochemical parameters; molecular mechanisms (e.g., gene expression studies) are beyond the current design.

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