

**HYPOGLYCEMIC EFFECT OF METHANOL STEM EXTRACT OF *DIOSPYRUS*
MESPILIFORMIS, (KAIWA) ON DIABETES INDUCED *DROSOPHILA*
*MELANOGASTER***

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF
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CERTIFICATION

This is to certify that the research project have been conducted by SANI, Maryam Muhammad (2010304041) and has been approved as meeting part of the requirements for the award of Bachelor of Science (B.Sc) degree in Biochemistry of Usmanu Danfodiyo University, Sokoto.

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DEDICATION

This work is dedicated to my family for their prayers ,sacrifices encouragement and Support.

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TABLE OF CONTENT

Title page.....	i
Certification	ii
Dedication	iii
Acknowledgement	iv
Abstract	v
Table of contents.....	vi
List of tables.....	vii
CHAPTER ONE.....	1
1.0 Introduction	1
1.1 Background of study	1
1.2 Statement of problem.....	4
1.3 Justification of study	4
1.4 Aim and objective	5
CHAPTER TWO.....	6
2.0 Literature review	6
2.1 Overview of diabetes Mellitus	6
2.2 Hypoglycemic agent and mechanism	7
2.2.1 Sulfonylureas	7
2.2.2 Meglitinides	8
2.2.3 Biguanides	8
2.2.4 Thiazolidinediones	8
2.2.5 α -Glucosidases inhibitors	9
2.2.6 Dipeptides peptidase -4 (DPP-4) inhibitors	9

2.2.7 Sodium – Glucose cotransporter – 2 inhibitors (SGLT-2).....	9
2.2.8 Cycloset	10
2.2.9 Limitations of current therapy	10
2.3 Ethnopharmacology of <i>Diospyros mespiliformis</i>	11
2.4 Bioactive phytochemical in <i>Diospyros mespiliformis</i>	12
2.5 <i>Drosophila melanogaster</i> as a model organism in metabolic research	13
CHAPTER THREE.....	16
3.0 Materials and method	16
3.1 Extracts	18
3.2 Fly stock and husbandry	18
3.3 Experimental design	18
3.4 Measurement of body weight	20
3.5 Climbing assay	20
3.6 Haemolymph Extraction	20
3.7 Biochemical Assay	20
3.7.1 Quantification of Glucose	20
3.7.2 Trehalose Assay	21
3.7.3 Glycogen Assay	21
3.8 Statistical Analysis	20
CHAPTER FOUR	23
4.0 Result	23
4.1 Assessment of Glucose levels after induction of diabetes	23
4.2 Evaluation of weight and locomotor parameters	25
4.3 Effect of MSEDm on HSD induced hyperglycemia and carbohydrate metabolic deregulation.....	27

CHAPTER FIVE.....	29
5.1 Discussion	29
5.2 Conclusion	31
5.3 Recommendation	31
5.4 Limitations	32
References	33

LIST OF TABLES

Table 3.1.: List of materials used in the study.....	16
Table 3.2: Nutritional composition of experimental groups.....	19
Table. 4.1: Effect of MSEDm on Weight and Locomotive Performance.....	26

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder associated with impaired glucose regulation. This study evaluated the hypoglycemic effect of *Diospyros mespiliformis* stem bark extract on diabetes-induced *Drosophila melanogaster*. Adult flies were exposed to a high-sugar diet (30% sucrose) for 14 days to induce hyperglycemia. Experimental groups received extract-supplemented diet (MSEDM) or metformin, while controls were maintained on a standard diet. Glucose, trehalose and glycogen levels, as well as weight and locomotor activity, were assessed. The high-sugar diet caused a significant rise in glucose concentration ($p < 0.05$), increased body weight ($p < 0.05$), and reduced locomotor performance ($p < 0.05$). Treatment with *Diospyros mespiliformis* significantly reduced glucose levels ($p < 0.05$) and trehalose levels ($p < 0.05$) compared with the diabetic group, showing antihyperglycemic activity comparable to metformin. Both MSEDM and metformin also produced a significant reduction in glycogen content relative to the high-sugar group ($p < 0.05$). Locomotor activity improved markedly in extract-treated flies ($p < 0.05$), and weight gain was reduced ($p < 0.05$). These results demonstrate that *Diospyros mespiliformis* possesses hypoglycemic properties and offers protection against diet-induced metabolic disturbances, supporting its potential as a natural antidiabetic candidate.

CHAPTER ONE

1.0 Introduction

1.1 Background of the Study

Diabetes mellitus is a major public health problem worldwide. Current global estimates indicate that this condition affects about 415 million people and is projected to escalate to 642 million by the year 2040 (Al-Lawati, 2017). Diabetes Mellitus is growing worldwide, but it remains an underestimated health threat, comprising about 366 million people globally (Schwandt, 2012). Diabetes is one of the top 10 causes of death globally, and together with cardiovascular disease, cancer, and respiratory disease, these conditions account for over 80% of all premature non communicable disease (NCD) deaths (Lin *et al.*, 2020). Individuals with diabetes have a 2–3-fold higher risk of all-cause mortality, and its presence is associated with increased mortality from infections, cardiovascular disease, stroke, chronic kidney disease, chronic liver disease, and cancer (Yang *et al.*, 2019; Bragg *et al.*, 2017). Although significant progress has been made in promoting population health and extending life expectancy, diabetes remains the second biggest negative contributor to global health-adjusted life expectancy (Chen *et al.*, 2019). Furthermore, diabetes Mellitus imposes a significant economic burden encompassing both direct and indirect costs, as patients often spend large portions of their income on drugs, diagnostic tests, and hospital visits (Koronkwo *et al.*, 2015).

In Nigeria, the burden of diabetes is also alarming. According to the International Diabetes Federation (2021), approximately 3.62 million Nigerians aged 20–79 years are living with diabetes, and about 53% of these cases remain undiagnosed. Similarly, recent reports estimate that more than 11 million Nigerians may be living with diabetes, with many cases yet to be detected (Independent Newspaper Nigeria, 2024; Nigerian Tracker, 2025).

Diabetes mellitus can be classified into two major categories: Type 1 and Type 2 diabetes, where Type 2 diabetes accounts for about 90% of all cases. Type 1 diabetes, previously known as insulin-dependent diabetes, is an autoimmune disorder that occurs due to the destruction of the pancreatic beta cells, leading to significantly reduced secretion of insulin (Katsaru *et al.*, 2017). Type 2 diabetes, also known as non-insulin-dependent diabetes, is the most common form of diabetes, with its prevalence rapidly rising worldwide. It is a hereditary condition resulting from insulin resistance, insufficient insulin secretion, or a combination of both, largely affecting an older population than Type 1 diabetes (DeFronzo *et al.*, 2015). Diabetes confers a huge economic burden due to its management costs, and its complications continue to rise. The conventional medications used in diabetes treatment, which focus on insulin secretion and insulin sensitization, often cause unwanted side effects that lead to poor compliance and treatment failure (Tan *et al.*, 2019).

Available therapies currently in use for the treatment and management of diabetes include insulin and several oral hypoglycemic agents such as metformin, sulfonylureas, α -glucosidase inhibitors, and meglitinides (Ansari *et al.*, 2019). However, these drug intended to boost insulin sensitivity and increase insulin secretion along with reducing plasma glucose levels by increasing glucose excretion or uptake in adipose tissue are usually associated with many side effects (Patel *et al.*, 2012). These include weight gain, hypoglycemia, gastrointestinal disturbances, liver injury, renal failure, hypersensitivity reactions, flatulence, diarrhea, and abdominal bloating. In addition, these drugs have other disadvantages, including drug resistance and a lack of therapies to prevent long-term complications of the disease (Ansari *et al.*, 2022).

The complications associated with insulin and oral antidiabetic agents, together with limited drug tolerability, adverse effects, and high costs, have accelerated the search for alternative medicines with better efficacy, potency, and fewer side effects (Gaokar and Hullati, 2020).

There has been an increase in interest in drug discovery research focused on natural antidiabetic agents, particularly those derived from medicinal plants, which could enhance β -cell function and treat diabetes-associated complications with fewer adverse effects (Oh, 2015). Medicinal plants have been used traditionally throughout the ages to treat various ailments due to their availability and safety (Gaokar and Hullati, 2020). Ethnomedicine is a traditional health-care practice followed by indigenous people and is considered the origin of systems such as Ayurveda, Siddha, Unani, Nature Cure, and even modern medicine (Vedavathy, 2003).

Traditional natural medicines are extensively prescribed in many Asian countries (Dar et al., 2017). Nearly 75% of globally used herbal medicines have been developed based on traditional medical knowledge (Ansari, 2021). Biologically active components derived from medicinal plants have yielded several clinically used drugs and continue to play a crucial role in drug discovery (Gu *et al.*, 2013). The commonly prescribed synthetic drug metformin, which possesses glucose-lowering properties in Type 2 diabetes, was derived from plant sources, and the search for similar plant-based drugs continues (Osadebe *et al.*, 2014). The management of diabetes using herbal medicines has proven to be more advantageous than synthetic drugs due to their accessibility, affordability, reduced complications, and fewer side effects (Gaokar and Hullati, 2020). The convenience of availability, lower cost, and relatively low risks of adverse reactions have made plant-based medicines an important alternative treatment option, especially in rural or developing regions (Salehi, 2019). Unlike synthetic drugs, plant-based medicines do not interrupt the body's natural healing process; rather, they support and enhance it, leading to steady and holistic recovery (Kaur & Valecha, 2014).

Diospyros mespiliformis, commonly known as African ebony, Jackal-berry, or Monkey guava, is a large deciduous tree mainly found in the African savannah. It can grow up to 25 meters in height and has a dense evergreen canopy (Ahmad and Mahmud, 2017). Different

parts of the plant have been widely documented in ethnobotanical use for treating bacterial, fungal, viral, parasitic, and inflammatory-related ailments (Ramadwa and Meddows, 2023). The bark, roots, and stem are traditionally used for the management of diabetes mellitus (Mudau *et al.*, 2022). *D.mespiliformis* has reported antidiabetic properties, and isolated compounds such as plumbagin, lupeol, and betulin have shown inhibitory activity in α -glucosidase enzyme inhibition assays (Mohammed *et al.*, 2009).

However, existing reviews conclude that the pharmacological effects of several compounds identified in *D.mespiliformis* have not been fully investigated, and further research is still needed to understand the molecular mechanisms underlying the reported pharmacological activities of its extracts and isolated compounds (Ramadwa and Meddows, 2023).

1.2 Statement of the Problem

Diabetes mellitus remains a major global health concern, yet the effectiveness of conventional antidiabetic drugs is often limited by side effects, high cost, and poor long-term tolerability (Patel *et al.*, 2012; Tan *et al.*, 2019; Ansari *et al.*, 2022). Although *D. mespiliformis* has been traditionally used for managing diabetes and contains bioactive compounds with reported antidiabetic properties (Mohammed *et al.*, 2009; Mudau *et al.*, 2022), there is still insufficient scientific evidence confirming the hypoglycemic activity of its fruit, Kaiwa. Furthermore, its mechanism of action has not been evaluated in a controlled experimental model (Ramadwa and Meddows-Taylor, 2023). Therefore, this study aims to investigate the antihyperglycemic potential and safety of Kaiwa fruit extract using a high-sugar diet-induced diabetic *Drosophila melanogaster* model.

1.3 Justification/Significance of the Study

Diabetes mellitus remains a major global health burden, and the limitations of conventional antidiabetic drugs highlight the need for safer, affordable alternatives (IDF, 2023; Atanu *et al.*, 2020). *D. mespiliformis* (Kaiwa) is traditionally used for managing diabetes and contains

phytochemicals with potential glucose-lowering properties, but scientific evidence supporting its effectiveness is still limited (Mukhtar *et al.*, 2022; Adewoye *et al.*, 2021). The use of *D. melanogaster* as a diabetic model is appropriate because its metabolic pathways closely resemble those of mammals and it is efficient for evaluating hypoglycemic activity (Musselman *et al.*, 2018; Pasco and Leopold, 2012). Therefore, this study is justified by the need to experimentally validate the hypoglycemic potential and mechanisms of *D. mespiliformis* using a diabetic-induced *Drosophila* model

1.4 Aim and Objectives of the Study

1.4.1: Aim

To investigate the hypoglycemic effect of methanol stem extract of kaiwa (*D. mespiliformis*) on Diabetes Induced fruits fly

1.4.2: Specific Objectives:

- a) To induce diabetes mellitus (Type II) model in *Drosophila*
- b) To assess the physical parameters including weight and locomotory performance after treatment with extract.
- c) To assess the biochemical parameters including Glucose, Trehalose and Glycogen after treatment with extract.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Diabetes Mellitus and metabolic implications

Diabetes mellitus is a serious, chronic metabolic disorder that's characterized by high sugar level either when the pancreas does not produce enough insulin, or when the body cannot effectively use insulin (Bereda,2021). Type 2 diabetes Mellitus (T2DM) which results from the body's inability to respond properly to the action of insulin produced by the pancreas (Amin, 2018). T2DM constitutes about 85–95% of all diabetes in high-income countries with a higher percentage in low- and middle-income countries due to rapid sociocultural changes, ageing populations, increasing urbanization, reduced physical activity and unhealthy lifestyle and behavioral patterns (WHO,2019). The International Diabetes Federation report of 2017 suggested that 451 million adults globally had diabetes in 2017, and 693 million individuals are expected to suffer from Diabetes mellitus by 2045 (IDF,2017). The World Health Organization (WHO) also estimates that more than 19% of the world's total adult population will suffer from Diabetes mellitus by the year 2030(Danaei *et al.*,2011). The pathophysiology of T2DM involves insulin resistance and β -cell dysfunction. Insulin-sensitive tissues fail to respond effectively to insulin, while pancreatic β -cells produce insufficient insulin to maintain normal glucose levels, together leading to persistent hyperglycemia (Galicia-Garcia *et al.*, 2020; Roden and Shulman, 2019). This hyperglycemic state is exacerbated by dysregulated glucagon secretion from pancreatic alpha-cells, which inappropriately stimulates hepatic glucose output even in the fed state (Dunning and Gerich, 2007). Furthermore, a characteristic dyslipidemia is common, typified by elevated triglycerides, low levels of high-density lipoprotein (HDL) cholesterol, and a preponderance of small, dense low-density lipoprotein (LDL) particles (Feingold and Grunfeld, 2023).

The long-term implications of these metabolic disturbances are severe and underscore the necessity for effective interventions. Chronic hyperglycemia drives the development of microvascular complications, including retinopathy, nephropathy, and neuropathy, which are leading causes of blindness, kidney failure, and lower-limb amputations, respectively (Cole and Florez, 2020). Additionally, T2DM is a major risk factor for macrovascular complications, such as atherosclerotic cardiovascular disease, stroke, and heart failure, which account for the majority of mortality in this patient population (Arnett *et al.*, 2019).

2.2 Hypoglycemic agent and mechanism of action

The effective management of diabetes mellitus requires an interprofessional approach that integrates lifestyle modification including diet and physical activity with pharmacologic therapy to achieve individualized glycemic goals (Liu and Ang, 2019). As type 2 diabetes mellitus progresses and pancreatic β -cell function continues to decline, healthcare providers often emphasize the need for patients to combine lifestyle changes with oral hypoglycemic medications for optimal glycemic control (Gatwood *et al.*, 2018). A wide range of oral antidiabetic agents is available, each with distinct mechanisms that target various metabolic defects in T2DM (Ganesan *et al.*, 2023). These include sulfonylureas, meglitinides, biguanides, thiazolidinediones, α -glucosidase inhibitors, DPP-4 inhibitors, SGLT2 inhibitors, and bromocriptine (Cycloset).

2.2.1 Sulfonylureas

Sulfonylureas (e.g., glipizide, glyburide, gliclazide, glimepiride) are among the earliest and most widely used oral antidiabetic drugs (Thulé and Umpierrez, 2014). They function as insulin secretagogues by stimulating glucose-independent insulin release from pancreatic β -cells (Timmons and Boyle, 2022). Their mechanism involves binding to ATP-sensitive

potassium (K-ATP) channels on β -cells, leading to channel inhibition, membrane depolarization, calcium influx, and subsequent insulin secretion (de Wet and Proks, 2015).

2.2.2. Meglitinides

Meglitinides (repaglinide and nateglinide) are also insulin secretagogues but have a faster onset and shorter duration of action than sulfonylureas, making them particularly effective for controlling post-prandial hyperglycemia (Guardado-Mendoza *et al.*, 2013). They bind to a distinct site on the SUR1 subunit of the K-ATP channel separate from the sulfonylurea binding site yet ultimately produce the same cascade of cell depolarization, calcium influx, and insulin release (Tahrani *et al.*, 2016; Tran *et al.*, 2015).

2.2.3 Biguanides

Metformin, the only biguanide currently in use, is the most commonly prescribed first-line medication for T2DM due to its strong efficacy and safety profile (Tehrani *et al.*, 2016). It primarily reduces hepatic glucose production and enhances hepatic insulin sensitivity, while having modest effects on peripheral glucose uptake (Rena *et al.*, 2017). Metformin also reduces hyperinsulinemia, lowers fasting glucose levels, increases intestinal glucose utilization, and stimulates GLP-1 secretion, although its complete cellular mechanisms are still not fully understood (Foretz *et al.*, 2014).

2.2.4 Thiazolidinediones (TZDs)

Thiazolidinediones (pioglitazone and rosiglitazone) are insulin sensitizers that act on intracellular pathways to improve insulin action in adipose tissue, liver, and muscle (Yamanouchi, 2010). Their primary mechanism is the activation of peroxisome proliferator-activated receptor-gamma (PPAR- γ), a nuclear receptor that enhances glucose uptake and increases adiponectin levels thereby improving insulin sensitivity and promoting fatty acid oxidation (Gor *et al.*, 2020; Viera *et al.*, 2019).

2.2.5 α -Glucosidase Inhibitors (AGIs)

Agents such as acarbose, miglitol, and voglibose delay carbohydrate digestion and absorption by inhibiting key enzymes (glucoamylase, sucrase, maltase, and isomaltase) in the small intestine (Derosa *et al.*, 2012). This slows the breakdown of complex carbohydrates to simple sugars and reduces post-prandial blood glucose spikes by approximately 3 mmol/L (Kumar *et al.*, 2011). AGIs are especially useful for patients with elevated post-meal glucose excursions (Akmal *et al.*, 2024).

2.2.6 Dipeptidyl Peptidase-4 (DPP-4) Inhibitors

DPP-4 inhibitors (e.g., sitagliptin, vildagliptin, saxagliptin, linagliptin) enhance incretin activity by preventing the degradation of GLP-1 and GIP two hormones that stimulate insulin secretion and suppress glucagon release (Capuano *et al.*, 2013). GLP-1, secreted by intestinal L-cells, has a short natural half-life of less than 2 minutes (Pathak *et al.*, 2010), while GIP, produced by K-cells, has a half-life of 5–7 minutes (Gupta, 2011). By prolonging incretin activity, DPP-4 inhibitors improve glucose homeostasis without causing hypoglycemia and are valuable in managing patients at cardiovascular risk (Katsina and Baharadhi, 2023).

2.2.7 Sodium-glucose cotransporter-2 inhibitors (SGLT2)

SGLT2 inhibitors such as dapagliflozin, canagliflozin, empagliflozin, and ertugliflozin—lower blood glucose by reducing renal glucose reabsorption in the proximal convoluted tubule, thereby increasing urinary glucose excretion (Fonseca-Correa and Correa, 2021). These drugs inhibit SGLT2 transporters, reducing glucose reabsorption by 30–60% and lowering HbA1c by 0.5–1.0% (Plosker, 2014). Beyond glycemic control, they also provide cardiovascular and renal protective benefits (Padda *et al.*, 2025).

2.2.8 Cycloset (Bromocriptine)

Cycloset is a quick-release dopamine D2 receptor agonist that exerts central effects on the hypothalamus, particularly the suprachiasmatic nucleus, to restore normal circadian dopaminergic activity (FDA, 2020). This improves insulin sensitivity, reduces hepatic glucose output, and attenuates excessive sympathetic tone without stimulating insulin secretion or causing hypoglycemia (Veroscience, 2023; AAFP, 2013). Cycloset provides a unique, centrally mediated mechanism of glucose control compared to other antidiabetic agents.

2.2.9 Limitations of current therapy

Despite their widespread use, current oral antihyperglycaemic agents are hampered by significant limitations. A major concern is their side effect profile; for instance, sulphonylureas are associated with hypoglycaemia and weight gain (Silbert *et al.*,2018) while the weight-neutral metformin (Sanchez-Rangel and Inzucchi 2017) frequently causes gastrointestinal upset. TZDs can lead to weight gain, oedema, and heart failure (yau *et al.*,2013). and the AGIs acarbose causes Gastrointestinal effects such as abdominal pain, bloating, flatulence, and diarrhoea (He *et al.*,2014). Newer classes also present challenges: DPP-4 inhibitors may produce allergic reactions as serious as angioedema and anaphylaxis (Karagiannis *et al.*,2014), while SGL2 inhibitors can cause polyuria resulting in dehydration with postural hypotension (Shi *et al.*,2020) .Furthermore, the cardiovascular safety of several agents is a major concern for physicians and regulatory bodies, as highlighted by the unexpected outcomes with rosiglitazone(Alhamdramy, 2016).Cost is another critical factor, with many newer agents being expensive, although generic metformin and SU provide a cheap and effective alternative (Vaughan *et al.*,2020)). Perhaps most critically, many patients still fail to achieve optimal glycemic control, as monotherapies like metformin have a cumulative incidence of secondary failure of 21% at 5 years, a figure that is even higher for sulphonylureas (jeon *et al.*,2018). These collective limitations encompassing a wide range of

side effects, cost, cardiovascular risks, and inadequate long-term efficacy create a compelling rationale for investigating alternative treatments, such as natural products, for the management of T2DM.

2.3 Ethnopharmacology of *Diospyros mespiliformis*

D.mespiliformis Hochsr. ex. A. DC, commonly known as Jackal Berry or African Ebony In Nigeria, the plant is popularly known in Hausa as Kanya and in Yoruba as Igidudu., is a member of the Ebenaceae plant family. *Diospyros* is a general name that means “divine”, and *mespiliformis* comes from the Greek words “mesos”, which means “half”, and “pilos”, which means “bullets”. The plant is widely distributed throughout Africa, Asia, and parts of Europe (Ramadwa and Meddows-Taylor,2023). The leaves are simple and alternate in arrangement and dark green in coloration. The plant is dioecious and flowers in the months of April and May and matured fruits are large yellow berries (Dangoggo *et al.*,2012).The fruits of the species are edible, while the bark, leaves, stem and roots are employed for various purposes (Gnonlonfin *et al.*, 2018).Traditionally, different parts of this plant including its leaves, stem bark, roots, and fruits have been extensively used(Ebbo *et al.*,2020).The ethnopharmacological relevance of *D. mespiliformis* stems from its rich phytochemical diversity, which includes alkaloids, flavonoids, tannins, saponins, cardiac glycosides, phenolic acids, terpenoids, and steroids (Aliyu *et al.*, 2009; Lawal *et al.*, 2017).These compounds are known to contribute to its broad spectrum of biological activities, including antioxidant, antimicrobial, anti-inflammatory, antimalarial, hepatoprotective, and antidiabetic effects (Bello *et al.*, 2019). Flavonoids and phenolics in particular are recognized for their ability to modulate oxidative stress, a central factor in diabetic pathophysiology, by scavenging reactive oxygen species and enhancing endogenous antioxidant defenses (Egharevba and Kunle, 2010). In African traditional medicine, decoctions or infusions of *D. mespiliformis* bark and roots are commonly used to lower blood sugar levels and restore

vitality in individuals exhibiting symptoms of diabetes. The use of this plant in ethnomedicine aligns with scientific reports demonstrating its antihyperglycemic activity in diabetic animal models (Ebbo *et al.*, 2020). Methanol extracts of the stem bark have been shown to significantly reduce fasting blood glucose, improve insulin sensitivity, and enhance glycogen storage, indicating possible modulation of carbohydrate metabolism and pancreatic function (Adebayo *et al.*, 2020). Furthermore, the antioxidant constituents of *D. mespiliformis* such as quercetin, betulinic acid, lupeol, and tannins are believed to contribute to its hypoglycemic mechanism by protecting pancreatic β -cells from oxidative damage and preserving insulin secretion (Aliyu *et al.*, 2009; Bello *et al.*, 2019). These bioactive compounds may also exert peripheral effects by enhancing glucose uptake in muscle and adipose tissue or inhibiting intestinal α -glucosidase enzymes, thereby reducing postprandial hyperglycemia (Adebayo *et al.*, 2020).

2.4 Bioactive Phytochemicals in *Diospyros mespiliformis*

The hypoglycemic potential of *D. mespiliformis* is strongly associated with its rich profile of bioactive phytochemicals (Ebbo *et al.*, 2019). The plant contains several classes of secondary metabolites, including flavonoids, phenolic compounds, tannins, saponins, alkaloids, terpenoids, and anthraquinones, which collectively contribute to its glucose-lowering effects (Shamsuddeen, 2023). These phytochemicals play important biochemical roles that can influence carbohydrate metabolism in diabetes-induced *D. melanogaster* (Birse *et al.*, 2020). Flavonoids in *D. mespiliformis* are particularly important for its hypoglycemic activity (Agbadoronye *et al.*, 2021). Compounds such as quercetin, catechin, and kaempferol derivatives have been identified in various parts of the plant, as Shamsuddeen (2023) reported. These molecules improve glucose handling by enhancing insulin-like signaling, increasing peripheral glucose uptake, and modulating pathways that regulate trehalose and glycogen

metabolism in *Drosophila* according to Birse *et al.* (2020). Flavonoids also inhibit digestive enzymes like α -amylase and α -glucosidase, reducing glucose spikes after feeding a mechanism relevant to high-sucrose-diet (HSD) induced diabetic flies (Jibril, 2023).

The phenols in *D. mespiliformis* scavenge reactive oxygen species and restoring redox balance, which supports more efficient glucose utilization (Bababe, 2024). Tannins possess anti-inflammatory and enzyme-modulating activities, which may improve insulin sensitivity and reduce metabolic stress associated with diabetes, and also by reducing systemic oxidative stress and inflammation, tannins can support restoration of normal carbohydrate metabolism in *Drosophila* (Adamu *et al.*, 2023)

Saponins in *D. mespiliformis* play an additional role in hypoglycemic action (Agbadoronye *et al.*, 2021). They are known to reduce intestinal glucose absorption, protect insulin-producing cells, and modulate lipid and carbohydrate pathways, according to Ebbo *et al.* (2019). In *Drosophila*, saponins may help suppress excessive trehalose production and prevent abnormal glycogen accumulation associated with insulin pathway disruption, as Birse *et al.* (2020) described. Other bioactives, including alkaloids, terpenoids, and anthraquinones, contribute antimicrobial, anti-inflammatory, and cytoprotective properties that support overall metabolic stability (Shamsuddeen, 2023). These compounds can enhance survival, improve physiological resilience, and indirectly support glucose regulation in diabetic flies (Kabr , 2024).

2.5 *Drosophila melanogaster* as a Model Organism in Metabolic Research

D. melanogaster, commonly known as the fruit fly or vinegar fly, is a premier model organism with a research history spanning over a century (Giansanti *et al.*, 2025). Its adoption in laboratories was driven by practical advantages: it is cost-effective due to its high reproductive rate (30–50 eggs per day), short generation time (approximately ten days at 25 °C), and minimal space requirements (Kaufman, 2017). Furthermore, its short lifespan (an

average of three months) facilitates efficient longevity and lifespan assays (Pandey and Nichols, 2011).

The utility of *D. melanogaster* extends far beyond genetics and developmental biology (Tolwinski, 2017) to become a powerful system for studying human physiology and disease. A key strength is the high degree of evolutionary conservation; approximately 60% of fly genes are conserved with humans, and about 75% of these have matches to known human disease genes (Reiter *et al.*, 2001).

Critically for metabolic research, *Drosophila* possesses tissues and systemic pathways that are analogous to those in mammals. For instance, while it lacks a discrete liver, its fat body performs equivalent functions in energy storage, metabolism, and immune response, making it a valid model for studying hepatic diseases (Victor *et al.*, 2025). The fly's insulin signaling pathway is also remarkably similar to humans. Despite having seven insulin-like peptides with partially redundant functions, the core pathway components are conserved and have been instrumental in studying various aspects of diabetes and the diabetic state (Álvarez-Rendón *et al.*, 2018). These similarities extend to fundamental metabolic regulation, including nutrient sensing and energy homeostasis, allowing *Drosophila* to effectively model disorders like diabetes and obesity (Garofalo, 2002; Owusu-Ansah and Perrimon, 2014; Chatterjee and Perrimon, 2021).

As a result, *D. melanogaster* has emerged as a very versatile and potent model in recent years for investigating metabolism and metabolic disorders, including both type 1 and type 2 diabetes (Álvarez-Rendón *et al.*, 2018). It provides an invaluable system for modeling a wide range of human pathologies, from neurodegenerative disorders and cancer to cardiac conditions and muscular diseases (Victor *et al.*, 2025). Its relatively simple nervous system further makes it an ideal model for studying neurodevelopment, neurodegeneration, and associated locomotor activity using automated systems (Tello *et al.*, 2022). In summary, due

to its genetic power, physiological parallels, and experimental practicality, *D. melanogaster* stands as a robust and effective animal model for studying metabolic homeostasis and related human diseases (Chatterjee *et al.*, 2021; Reiter *et al.*, 2001).

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 Molychem	Rajasthan	India

17	Methyl Paraben	Faso Farine	Mumbai	India
18	Corn Flour	Mais Blanc (FFMB) LOBA Chemie Pvt Ltd	Ouagadougou	Burkina Faso
19	Sucrose	Kern & Sohn Ltd	Mumbai	India
20	Electronic weighing scale	Hangzhou Miou Instrument Co., Ltd	Balingen	Germany
21	Handheld homogenizer		Hangzhou	China

3.1 Extract

The methanol stem extract of *D.mespiliformis* was provided at the Centre for Advanced Medical Research and Training (CAMRET).

3.2 Fly Stock and Husbandry

The Harwich strain of *Drosophila melanogaster* was obtained from the Fly Laboratory at the CAMRET, Usmanu Danfodiyo University, Sokoto. The flies were cultured in vials and maintained under optimal conditions: a temperature range of 22–25 °C, controlled humidity ($55 \pm 5\%$), and a natural light-dark cycle. They were fed a standard cornmeal diet, and the fly media was replaced every three days to prevent contamination.

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, glucose, trehalose, glycogen 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 μ L 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 Trehalose Assay

Trehalose levels were quantified using a colorimetric assay kit (Solarbio Life Science, Beijing, China) in accordance with the manufacturer's protocol. Absorbance was measured at 620 nm using microplate reader. The trehalose concentration was determined using the formula:

$$\text{Trehalose (mg/g sample)} = 10 \times \text{Concentration from y-axis sample}$$

3.7.3 Glycogen Assay

Glycogen levels in whole fly extracts were analyzed using a colorimetric assay kit (Elabscience, Houston, USA) as per the manufacturer's guidelines. Absorbance readings were taken at 620 nm with the Microplate Reader. The glycogen concentration was calculated using the formula:

$$\text{Glycogen (mg/g fresh weight)} = \frac{\Delta A1}{\Delta A2} \times m \times f \times 10 \times 1.11$$

$\Delta A1 = OD_{\text{sample}} - OD_{\text{blank}}$; $\Delta A2 = OD_{\text{sample}} - OD_{\text{blank}}$; m =content of standard (0.01mg); f = dilution factor of sample in sample preparation; 10= dilution factor of reaction system; 1.11 = conversion factor (glucose to glycogen)

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 Assessment of Glucose Levels After Induction of Diabetes

As illustrated in Figure 4.1, exposure to a 30% sucrose high-sugar diet resulted in a marked rise in glucose levels, confirming successful induction of a diabetic state in the flies.

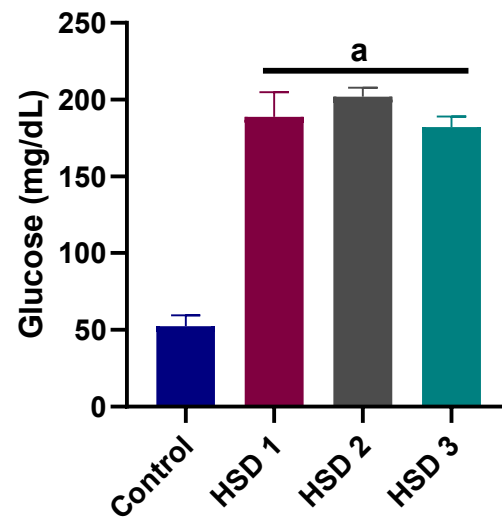


Figure 4.1: HSD- High sugar diet. Statistical significance difference compared to control group $p < 0.05$ was represented by **a**. No statistical difference compared to control group $p < 0.05$ was represented by **ns**. Data were reported as mean $\pm$ SD ($n = 200$).

4.2 Evaluation of Weight and Locomotor Parameters

Table 4.1 shows that a high-sugar diet (HSD) caused a significant increase in body weight in *Drosophila melanogaster* confirming diet-induced obesity ($p < 0.05$). Administration of MSEDm 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, MSEDm 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 MSEDm may offer equal or superior support for motor coordination under hyperglycemic conditions.

Table. 4.1: Effect of MSEDm on Weight and Locomotive Performance.

Experimental groups	Parameters	
	Weight (mg) (%)	Locomotive performance
Control	5.00 ± 0.85	95.00 ± 7.07
HSD	15.20 ± 0.99 ^a	65.00 ± 7.00 ^a
ED	5.65 ± 0.21 ^{ns b}	75.00 ± 7.00 ^{ns}
MD	7.00 ± 0.71 ^{ns b}	85.00 ± 7.07 ^{ns}

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 **a**, while statistical significance compared to HSD group $p < 0.05$ was represented by **b**. No statistical difference compared to control group $p < 0.05$ was represented by **ns**. Data were reported as mean ± SD (n = 30).

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

A 14-day high-sugar diet (HSD) led to pronounced disturbances in carbohydrate metabolism in *Drosophila melanogaster*, with glucose levels significantly elevated compared to controls ($p < 0.05$). Although trehalose levels increased under HSD, the change was not statistically significant. Treatment with MSEDm produced a significant reduction in glucose and trehalose levels relative to the HSD group ($p < 0.05$), demonstrating antihyperglycemic effects comparable to metformin and an overall improvement in carbohydrate regulation. Additionally, both MSEDm and metformin markedly decreased glycogen levels compared with the HSD group (Figure 4.3A–C).

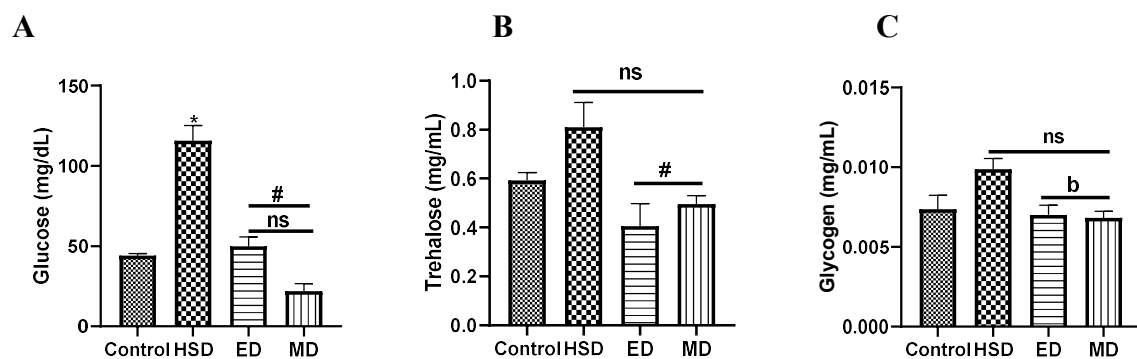


Figure 4.3: HSD- High sugar diet; ED- Extract diet containing MSEDm; MD- Metformin diet. Statistical significance difference compared to control group $p < 0.05$ was represented by *, while statistical significance compared to HSD group $p < 0.05$ was represented by #. No statistical difference compared to control group $p < 0.05$ was represented by ns. Data were reported as mean $\pm$ SD ($n = 30$).

CHAPTER 5

5.1 Discussion

In this study, a high-sugar diet successfully induced a diabetic condition in *Drosophila melanogaster* as shown by significant increases in glucose, body weight, and altered carbohydrate storage. Treatment with *Diospyros mespiliformis* extract (ED) markedly improved these parameters and produced effects that were comparable to those of metformin (MD).

Phytochemical investigations have consistently shown that *D. mespiliformis* contains multiple bioactive compounds such as flavonoids, phenolics, saponins, tannins, alkaloids and steroids (Shamsuddeen, 2023). Previous mammalian studies offer strong support for these findings. In alloxan-induced diabetic rats, methanolic stem bark extract of *D. mespiliformis* significantly lowered blood glucose and improved biochemical markers, with effects comparable to metformin (Muhammad *et al.*, 2020). Therefore, the hypoglycemic effect observed in *Drosophila* is consistent with established animal studies, suggesting that the extract may act through mechanisms similar to or complementary with metformin.

The extract also prevented the excessive weight gain induced by a high-sugar diet. Prevention of diet-induced obesity has been reported for *D. mespiliformis*, where extracts reduced weight and improved metabolic parameters, likely through modulation of lipid and carbohydrate metabolism (Adamawa State Polytechnic, 2022). In the current study, ED restored body weight close to the control group, whereas MD was slightly less effective, suggesting that the extract may offer similar or superior protection against diet-related metabolic stress.

Locomotor performance, which is commonly impaired in diabetic flies due to neuronal and mitochondrial dysfunction, was decreased by high-sugar feeding but improved significantly after treatment with ED. The improvement was slightly greater than that produced by MD, suggesting that antioxidant effects may play a role. This is consistent with reports of strong

antioxidant activity and metabolite protection by *D. mespiliformis* in chemically induced oxidative stress models (Sani *et al.*, 2025). Since oxidative stress is a major contributor to diabetic complications, the antioxidant activity of the extract may contribute to improved neuromuscular performance.

Carbohydrate markers further demonstrated metabolic disruption. Glycogen and trehalose levels increased under a high-sugar diet, reflecting impaired insulin signaling and excessive carbohydrate storage. Treatment with both ED and MD significantly reduced these parameters, indicating restored carbohydrate regulation. These effects may be mediated by inhibition of carbohydrate-metabolizing enzymes such as α -amylase and α -glucosidase, which has been reported for *D. mespiliformis* extracts (Kabre *et al.*, 2024). Inhibition of these enzymes reduces post-prandial hyperglycemia and promotes improved glucose homeostasis, which aligns with the trends observed in this study.

Overall, the comparable performance of ED and MD across glucose levels, body weight, locomotion, trehalose, and glycogen suggests that *D.mespiliformis* possesses meaningful antidiabetic potential. While metformin is a well-established standard drug, the plant extract produced similar effects in many parameters and even slightly better responses in locomotion and weight restoration.

Although the present findings are promising, further mechanistic studies are needed to clarify how the extract acts. Previous research has proposed antioxidant, enzyme-inhibitory, and organ-protective mechanisms (Kabre *et al.*, 2024; Muhammad *et al.*, 2020; Shamsuddeen, 2023). Future work should also assess bioactive compounds using advanced profiling methods, as recent studies have identified diverse metabolite signatures with pharmacological relevance (Sani *et al.*, 2025).

5.2 Conclusion

This study evaluated the hypoglycemic effect of *D.mespiliformis* extract in a *Drosophila* model of diet-induced diabetes. Exposure to a high-sugar diet successfully induced hyperglycemia, weight gain, impaired locomotion, and carbohydrate dysregulation, confirming a diabetic-like condition in the flies. Administration of the extract significantly reversed these metabolic disturbances. The extract reduced glucose, trehalose, and glycogen levels, prevented excessive weight gain, and improved locomotor performance. Importantly, the effects of the extract were comparable to those of metformin, the standard antidiabetic drug used as a positive control. In several parameters, including body weight and locomotion, the extract performed slightly better than metformin, suggesting additional metabolic or antioxidant benefits.

Overall, the findings demonstrate that *D.mespiliformis* possesses meaningful antihyperglycemic and metabolic regulatory properties. The extract was able to restore glucose homeostasis and improve physiological function in diabetes-induced flies. These results support the traditional use of the plant for diabetes management and indicate its potential as a natural therapeutic alternative or complementary treatment to existing antidiabetic drugs. Further studies are recommended to explore the underlying mechanisms, identify the active compounds, and evaluate long-term safety in higher animal models.

5.3 RECOMMENDATION

Based on the findings of this study, *D. mespiliformis* extract showed hypoglycemic activity in diabetes-induced *Drosophila melanogaster*. Therefore, it is recommended that:

- a. Further studies should be carried out on dose optimization, to determine the most effective and safest concentration for glucose reduction.
- b. Bioactive compounds responsible for the hypoglycemic effect should be isolated and identified, using chromatographic and spectroscopic methods.
- c. Extended studies using mammalian models are encouraged, to validate the potential for clinical application in diabetes management.
- d. Combination studies with standard antidiabetic drugs should be considered, to assess possible synergistic or additive effects.

5.4 LIMITATIONS

The study was conducted only on *D.melanogaster*, which may not fully reflect physiological responses in higher animals or humans.

Only biochemical glucose levels were assessed, while other metabolic markers such as insulin signaling, lipid profile, or oxidative stress parameters were not measured.

The research did not investigate individual bioactive components present in the plant extract, therefore the exact compounds responsible for the effect remain unknown.

Only short-term exposure was evaluated, and long-term safety or toxicity of the extract was not explored.

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