Arab American University
Faculty of Graduate Studies

Antidiabetic and antioxidant activity of Ocimum basilicum and Gundelia tournefortii extracts in a mouse model

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This thesis was submitted in partial fulfillment of the requirements for the Master`s degree in Cellular and Molecular Biosciences

February /2024

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Thesis Approval

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Declaration

I certify that the work provided in this thesis, unless otherwise referenced, is the researchers work and has not been submitted for a higher degree to any other university or institution.

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Dedication

I dedicate this thesis to my husband Tareq and my children Alma and Elham, my parents, sisters, brothers, all the family members and friends.

Acknowledgment

First of all, my immeasurable thanks to Allah, who has enabled me to accomplish this work.

I wish to express my deepest gratitude to my thesis supervisors Prof. Dr. Hilal Zaid and co-supervisor Dr. Feras Al Battah for their supervision, constant encouragement, indispensable guidance throughout this work, constructive comments and for their valuable criticism.

Many thanks for my biggest supporter and my friend Najlaa Bassalat for her help all the time, and to all my colleagues in the Department of Biology and Biotechnology at Arab American University for their help and cooperation.

I would thank my husband, my mother, father, sisters, brothers and friends for their continuous love and support.

Thank you all …

Abstract

This thesis discusses chronic diabetes, especially type II diabetes, where the disease and its types were introduced in the introduction of the letter, as well as the introduction of *Ocimum basilicum* and *Gundelia tournifortii* plants, which were used to examine the effectiveness of their extracts at the blood sugar level through the use of experimental mice, and dividing mice into different groups (a healthy group, a healthy group received extract, a group with diabetes, a group with diabetes and received extract). With consideration of the ethics of dealing with animals, provide appropriate conditions for their breeding, and make the experiment. The disease was triggered by the use of streptozotocin with a dose of 50 mg/kg body weight, a diabetogenic agent for Type II diabetes, through injections into the abdomen and the destruction of Type B pancreatic cells. The mice received the extract via the gavage. During the experiment, the presence of any behavioral changes was monitored, in addition to monitoring weight, food, and water consumption, noting the difference in urination between groups, as it increases significantly in groups with diabetes compared to the other. The two plants were also evaluated as antioxidant plants by examining melanoaldehyde in several organs. Lastly, an assessment was conducted to see how well the plant affected the blood sugar levels of both diabetic and healthy mice. There was no significant effect ($p \leq 0.05$) of both plant extracts in biochemical parameter such as lowering blood sugar, Weight loss, consumption of food and water, our results were not approved with previous studies on the two plants at the cell level, as the extract of both plants succeeded to enhance the translocation of GLUT4 to the plasma membrane of L6myc muscle cells.

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List of abbreviation

GLUT4 ……. Glucose Transporter 4

WHO ……. World Health Organization

NCDs …… Non-Communicable Diseases

DFUs …… Diabetic Foot Ulcers

T1DM ……... Type1 Diabetes Mellitus

T2DM ……Type2 Diabetes Mellitus

BMI ……... Body Mass Index

GRS ……. Genetic Risk Score

STZ ……. Streptozotocin

GLUT2 ……... Glucose Transporter 2

FFAs ……... Free Fatty Acids

PKA …… Protein Kinase A

HSL .....Hormone- Sensitive Lipase

ROS ……..... Reactive Oxygen Species

SOD …….....Superoxide Dismutase

CAT …….....Catalase

MDA ……. …. Malondialdehyde

TBA …….2-Thiobarbituric acid

LDL-c ……. Lipoprotein-Cholesterol

PM ……...Plasma Membrane

OGTT ……. Oral Glucose Tolerance Test

GTT ……. Glucose Tolerance Test

IV ……. Intravenously

IP ……... Intraperitoneally

IVGTT …….. Intravenously glucose tolerance test

O. basilicum…. Ocimum basilicum

G. tournifortii…Gundelia tournifortii

1. Chapter One: Introduction

1.1 Diabetes

Diabetes is categorized as a long-term, extremely high blood glucose level endocrine disorder that is non-communicable disease. In their Action Plan to address the non-communicable diseases (NCDs) problem, the United Nations and the World Health Organization (WHO) have designated diabetes as one of the five priority non-communicable illnesses (NCDs) (Banatvala and Bovet, 2023).

Managing and preventing the long-term consequences that diabetes patients face requires intricate, expensive, and time-consuming work. (Cole and Florez, 2020). By 2045, 693 million individuals are predicted to be affected globally due to of its rapid global spread (Stanciu et al., 2020). The Diabetic Association expects that the prevalence of diabetes in 30.3 million people in the USA in 2015, is anticipated to further rise to 642 million by 2040. Its rise is higher in low to middle-income countries compared to high-income countries in other words, the pervasiveness of diabetes is the most elevated in the Middle East (Kadan et al., 2013). International Diabetes Federation has identified diabetes as a significant, special health and economic costly burden issue affecting 58 million people in Europe in 2017 (Pandey et al., 2020).

Diabetes mellitus causes a host of negative effects and complications, including cardiovascular disease (Kadan et al., 2013), diabetic nephropathy, which damages the kidney's blood vessel wall over time (Süntar, 2020), impairing the kidney's ability to filter waste and fluid from the body,

and affects the brain, particularly cognition and thinking. Lately, there has been a lot of discussion about diabetes type 3, or "brain diabetes," and how it increases the risk of Alzheimer's disease (Stanciu et al., 2020).

Since saliva contains glucose, excessive salivary glucose levels in uncontrolled diabetes can have negative effects on the mouth, teeth, and breath by promoting the growth of pathogenic bacteria that lead to periodontitis and gum disease (Nazir et al., 2018).

Vision problems and blindness are in sometimes, the first symptoms of diabetes leading to diabetic retinopathy (Cheng et al., 2019), and diabetic macular edema (DME) may appear (Fu-Shin et al., 2022; Kaštelan et al., 2020).

Leg or foot ulcers are the most frequent wounds in diabetes patients. High blood sugar also damages the skin and makes it difficult for the body to heal wounds. About 20% of diabetic individuals worldwide have diabetic wounds (Patel et al., 2019). In addition to being expensive, the growing prevalence of diabetic foot ulcers (DFUs) has a significant mortality impact (Bellomo et al., 2022).

Diabetes mellitus damages the circulatory system, which reduces blood flow, and damages nerves, impairing the health of the sexual organs in both sexes and causing sexual dysfunction, such as erectile dysfunction in diabetic men (Shindel and Lue, 2015).

1.1.1 Type1 Diabetes Mellitus (T1DM) or Insulin dependent

Chronic Type 1 Diabetes Mellitus is an autoimmune condition characterized by the T-cell-mediated death of pancreatic beta cells, which causes insufficient insulin synthesis from

pancreatic beta cells (increasing reduction in insulin production) and secretion, which results in hyperglycemia (Burrack et al., 2017).

T1DM is a common one in children and is referred to as insulin- dependent diabetes (Oyibo, 2022). Clinically, T1DM often manifests as many weeks of weight loss, intense thirst, and/or excessive urine (polydipsia and/or polyuria) brought on by hyperglycemia. This was formerly thought to be the initial sign of the sickness (Dayan et al., 2021).

It affects millions of people globally, and its effects must be avoided with specific, cautious management. Nowadays, the majority of T1DM patients are being treated with exogenous insulin replacement in addition to the conventional approach of routine blood glucose monitoring (Akil et al., 2021).

1.1.2 Type 2 Diabetes Mellitus (T2DM) Type 2 diabetes mellitus is the cause of about 90% of all diabetes cases, and this condition is becoming more common worldwide. According to estimates from the International Diabetes Federation, there will be 629 million individuals with diabetes worldwide by the year 2045, up from the current 425 million cases. T2DM is primarily characterized by insulin resistance, particularly in skeletal muscle and the liver, and compromised pancreatic insulin production (Laakso, 2019).

Currently, the two biggest issues are type 2 diabetes (T2D) and insulin resistance. The hormone insulin, which lowers blood sugar, is crucial for the liver and skeletal muscles. After attaching to its receptors in the cell membrane, insulin activates metabolic processes. For instance, it stores glucose in the liver and skeletal muscles, starts glucose use in the muscles, and controls the

expression of genes involved in lipid synthesis and glucose transport (Rafiq and Jeppesen, 2021).

Plasma glucose levels rise after meal consumption to a threshold level that causes pancreatic beta-cells to produce insulin. Under normal circumstances, this insulin promotes the absorption of carbohydrates at critical sites for storage and consumption, including skeletal muscle and adipose tissue, where proteins and carbohydrates are stored as lipids. However, the overeating and inactivity that characterize modern lifestyles upset this system and can result in serious conditions such as metabolic syndrome, obesity, T2D, and cardiovascular disease (Lee et al., 2022).

Physiologically, insulin resistance is defined as a condition of decreased responsiveness in insulin-targeting tissues to high physiological insulin levels. Insulin resistance is the primary clinical symptom of T2DM. Before non-physiologic increased plasma glucose levels, insulin resistance develops. In the prediabetic condition, insulin levels increase to meet normal insulin requirements. This results in chronic hyperinsulinemia, hyperglycemia-induced -cell failure, and ultimately T2DM (Kahn, 2003; Lee et al., 2022).

Moreover, insulin prevents the liver's lipolysis, which decreases acetyl-CoA levels and pyruvate carboxylase activity. For insulin to lessen gluconeogenesis, pyruvate carboxylase and glycerol synthesis must decrease. Insulin resistance is characterized by a greater need for insulin to keep blood glucose levels within normal range (Rafiq and Jeppesen, 2021).

Several previous studies show many non-genetic risk factors for T2DM. Non-genetic risk factors include unhealthy lifestyles such as low physical activity (less exercise), a diet rich in saturated

fat and low in fiber as salad, unhealthy habits like smoking, body mass index (BMI), age, waist circumference, history of gestational diabetes mellitus, elevated blood pressure, dyslipidemia, and different drug treatments (diuretics, unselected β-blockers, statins and glucocorticoids).

In addition, there are a genetic risk factors scores as studies mentioned, which play role as a predictive measure of disease susceptibility by aggregating the effects of individual loci into a single genetic risk score (GRS) (Laakso, 2019).

1.2 Animals model to study biological phenomena(diseases)

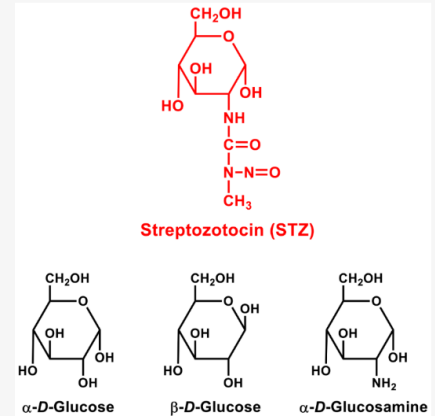
Scientists have known for more than 2400 years that studying illnesses and complicated biological events in laboratory animals may inform us a great deal about people. Infectious disease, immunology, oncology, neurology, endocrinology, and behavior science as well as diabetes, cancer, and other disorders are best studied using animal models. As a result, the largest and most prestigious research institutes have complete animal departments where they can generate and care for animal models to support their studies, and other institutes are in the process of doing the same (Pandey et al., 2020).

There are many animal models to understand biological function in humans, each with a setting based on its specific uses. For example, there are "exploratory models" aimed at understanding the fundamental mechanism of action in biological systems and "explanatory models" aimed at understanding complex biological problems. These models may be supported by a physical, bio-informatics, or mathematical model system, but the last one we focus on and use more is the "predictive model"(Pandey et al., 2020).

1.2.1 Diabetic animal model

There are several methods have been used for developing animal models of diabetes, such as chemically induced diabetes. The most effective diabetogenic medications include alloxan and streptozotocin (STZ). These chemical substances are most often used to kill beta cells in diabetic animal models (Zhu, 2022).

The glucose transporter 2 (GLUT2) carries these lethal glucose analogs, which tend to accumulate in pancreatic beta-cells because STZ is structurally identical to glucose. Alloxan and STZ toxicity to -cells exhibit interspecies variation, which is thought to be related to the variation in GLUT2 expression between species. However, in other species, a dosage of 150 mg/kg is required to create diabetes. In rats, a greater dose of 50 mg/kg and beyond can cause permanent diabetes (Dufrane et al., 2006; Eizirik et al., 1994; Pandey et al., 2020; Zhu, 2022).



(Zhu, 2022)

STZ has a half-life because of its rapid hepatic metabolism and rapid renal elimination. After STZ has left the body, the effects of diabetic hyperglycemia may be to blame for the kidney or

liver's prolonged functional impairment. This is the basis for a study into the mechanisms underlying STZ diabetes-related issues in the heart, muscles, and other pertinent organs (Akinlade et al., 2021).

1.3 Hypothesis of STZ

The environment is home to several strains of the gram-positive bacteria Streptomyces achromogenes, which are in charge of producing STZ. In the late 1950s, it was initially identified as an antibiotic. Later, research revealed that STZ specifically destroys insulin-producing islet cells. It was revealed that STZ, a glucosamine-nitrosourea compound, may cause considerable cytotoxicity largely by harming cellular DNA and proteins by covalent modifications, even though alternative routes were also thought to be implicated (Zhu, 2022).

1.4 Insulin's Anti-Lipolysis Effects

In controlling the metabolisms of lipids and glucose, insulin is essential. Insulin slows lipid catabolism and free fatty acids (FFA) oxidation, increases lipid synthesis and storage, lowers plasma FFA levels, and reduces plasma FFA levels. The main hormone that prevents lipolysis is insulin. The production of FFAs from adipocytes was suppressed by the addition of glucose and insulin to a growth medium as early as 1960, according to in vitro investigations (Al-Sulaiti et al., 2018; Zhao et al., 2020).

1.4.1 Insulin Mechanism of Action Against Lipolysis

Insulin-induced anti-lipolysis is a pretty well-understood mechanism. Insulin controls adipocytes' absorption of glucose and initiates the movement of fatty acid transporters and adipocytes'

uptake of FFA. Insulin activates tyrosine phosphorylation by binding to particular membrane insulin receptors, which subsequently interact with IRS-1 and IRS-2 to activate the phosphatidylinositol 3-kinase (PI3K) complex. After that, the PKB/Akt pathway activates phosphodiesterase 3B (PDE3B) to block both basal and catecholamine-induced lipolysis. Phosphodiesterase catalyzes the breakdown of cAMP into inactive 5′-AMP, which lowers the degree of Protein Kinase A (PKA) activation and inhibits lipolysis by decreasing the activity of hormone-sensitive lipase (HSL) (Al-Sulaiti et al., 2018; Carpentier and Metabolism, 2021; Zhao et al., 2020).

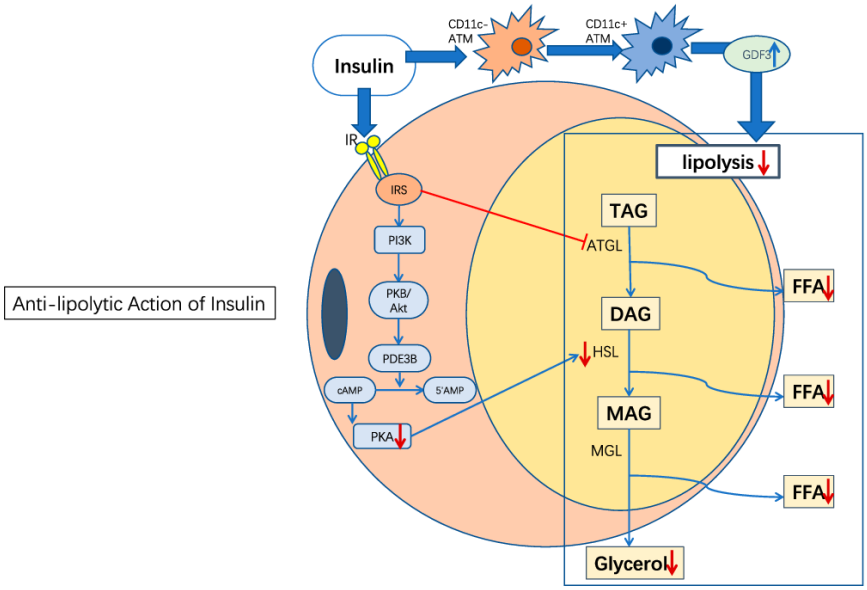


Figure A: Insulin Mechanism of Action Against Lipolysis

(Zhao et al., 2020).

1.4.2 Correlation between triglyceride storage and lipolysis in insulin-sensitive tissues:

Aberrant triglyceride buildup and lipolysis can be noticed in insulin-sensitive tissues as an early indicator of insulin resistance conditions, before postprandial or fasting hyperglycemia manifests. Many of the fundamental metabolic abnormalities that are characteristic of insulin resistance syndrome and type 2 diabetes, are attributable to and amplified by increased free fatty acid (FFA) flow from adipose to (liver and muscle) tissue as a result of anomalies in fat metabolism (Czech et al., 2013; Lewis et al., 2002).

People with systemic insulin resistance have impaired insulin's capacity to both increase glucose absorptions in adipocytes and skeletal muscle and to reduce hepatic gluconeogenesis. When lipid emulsion infusions are used to produce hypertriacylglycerolaemia or high circulating Non-esterified fatty acids NEFA levels in human volunteers, hyperinsulinaemic clamps can be utilized to identify systemic insulin resistance levels in human volunteers (Czech et al., 2013).

1.5 Antioxidant effects

An increase in blood sugar triggers a series of actions that eventually lead to an increase in free radical generation and oxidative stress in several organs, including the pancreas (Davì et al., 2005). Reactive oxygen species (ROS) have been established in several studies to cause harm to cells and tissues (Fatehi-Hassanabad et al., 2010).

Active biomolecules known as free radicals are produced physiologically during metabolic processes and/or by immune cells (Staveness et al., 2016). Free radicals have physiological functions in a wide range of molecular processes, such as cell-cell communication, synaptic plasticity, memory formation, defense against pathogen invasion, cell-cell interactions, cell

proliferation, autophagy, apoptosis, and aging (Bokkon, 2012). Oxidative stress develops when free radical formation rises over the physiological range and overwhelms cellular antioxidant defenses (Angelova and Abramov, 2018; Halliwell and Gutteridge, 2015; Maritim et al., 2003; Yaribeygi et al., 2020).

Superoxide dismutase (SOD), glutathione, and catalase (CAT), among other endogenous antioxidant substances, shield cells from free radicals, particularly ROS. Studies have revealed that chronic disorders like diabetes significantly lower the levels of enzymatic and non-enzymatic antioxidants in the blood and cells (Oroojan et al., 2020; Shrilatha and andrology, 2007).

An imbalance between the body's ability to protect itself against free radicals produced by oxygen is known as oxidative stress. Additionally, free radicals weaken the body's antioxidant defenses, disrupting enzyme function and causing an increase in lipid peroxidation (Bouabid et al., 2020; Oroojan et al., 2020; Rochette et al., 2014).

Chronic diabetic problems are believed to be primarily caused by increased oxidative stress and alterations in antioxidant capacity, which are shown in both clinical and experimental diabetes (Motawi at el, 2013).

When exposed to free radicals, unsaturated fatty acids can undergo lipid peroxidation and the synthesis of electron-friendly lipids. One of the most harmful forms of aldehydes is malondialdehyde (MDA), (one of the final products of polyunsaturated fatty acids peroxidation in the cells) (Stalikas and Konidari, 2001), which damages tissues and lipids by triggering lipid peroxidation (Oroojan et al., 2020; SABOURI et al., 2010). Malondialdehyde level is commonly

known as a marker of oxidative stress and antioxidant status (Stalikas and Konidari, 2001). The most used technique for detecting MDA involves measuring the condensation product with 2-thiobarbituric acid (TBA). However, TBA assay for MDA has drawbacks including interference from other oxidation lipid products, poor reproducibility, and harsh conditions, which are needed in sample preparation (Stalikas and Konidari, 2001).

Utilizing antioxidant substances will be crucial in minimizing the effects of chronic illnesses like diabetes. In this way, administering chemicals of plant origin is linked to fewer negative effects (Harasym and Oledzki, 2014; Oroojan et al., 2020).

According to reports, medicinal plants and their extracted active components can protect against oxidative stress and tissue damage by boosting the CAT and SOD enzymes' antioxidant activity. By bolstering the antioxidant defense system in diabetic instances, it is possible to stop the evolution of this illness as the weakening of the antioxidant system in the pancreas or islets of Langerhans causes the difficulties of diabetes (Demidchik and botany, 2015; Oroojan et al., 2020; Rahbarian et al., 2016).

1.6 Herbal Medicinal Plants

The Middle East and North Africa have the second highest rates of diabetes growth globally, according to earlier studies, and by 2035, 96.2% more people are expected to have the disease, adding to the social and economic burden in many nations (Abuyassin and Laher, 2016; Saravanan V Sathasivampillai et al., 2017).

A metabolic condition with a broad spectrum of complications is referred to as the "diabetes syndrome," which emphasizes the urgent need for novel therapeutic approaches. Since they are often thought to be accessible and secure, traditional medicine made from plants has been used

for generations to treat a wide range of illnesses, including diabetes (Saravanan V. Sathasivampillai et al., 2017; Tahrani et al., 2011).

Traditional medicines "[include] diverse health practices, approaches, knowledge, and beliefs incorporating plant, animal, and/or mineral-based medicines, spiritual therapies, manual techniques, and exercises applied alone or in combination to maintain well-being, as well as to treat, diagnose, or prevent illness" (Karunamoorthi et al., 2013)

The term "medicinal plants" refers to any plant or plant components, including flowers, fruits, seeds, leaves, berries, bark, and roots, that are utilized for therapeutic purposes. Plants are selected based on several factors, including cost, availability, comorbidities, progression stage, and safety (Verma et al., 2018). A plant is considered medicinal if it includes substances that can be employed therapeutically or as building blocks for the semi-synthesis of chemo-pharmaceuticals in one or more of its organs (Karunamoorthi et al., 2013).

Medicinal plants most likely make up a single, more significant functional category of plants globally. Due to the existence of phytochemical ingredients such as secondary metabolites, the use of medicinal plants as traditional medicine is well recognized in rural parts of many developing nations (Rabizadeh et al., 2022).

Naturally occurring phytochemicals that contain defensive mechanisms and protect against numerous diseases may be found in medicinal plants, leaves, vegetables, and roots (Hussein, 2010). The study of medicinal plants, particularly those used as folk medicines, has to be strengthened to encourage the use of herbal remedies and the identification of their potential. Primary and secondary metabolites are substances that are always present in all plants. Proteins,

amino acids, carbohydrates, purines, and pyrimidines of nucleic acids, chlorophylls, etc. are examples of primary metabolites and participate in plant development and growth. Secondary phytochemicals encompass anything from acetogenins to terpenoids and alkaloids to various phenols are multifunctional metabolites that are typically involved in plant defense and environmental communication. Furthermore, they are associated with plant color, taste, and scent. Critically, they are also involved in the responses of plants to stress whether biotic- or abiotic-related (Akula et al., 2011; Jan et al., 2021).

It has been established that 656 species of flowering plants are used historically to treat diabetes (Verma et al., 2018).

Less than 2500 of an estimated 250,000 plants have been studied for pharmacological activity against diabetes in recent in vitro and in vivo research investigations on a variety of herbs that have been reported to lower blood glucose levels. Currently, the anti-diabetic medications

metformin (obtained from *Galega officinalis*) and acarbose (made via Actinoplanes utensil fermentation) are sourced from natural sources (Chinsembu, 2019; Verma et al., 2018).

Every civilization in the world has employed plants or their derivatives to treat or prevent sickness throughout history. Herbal remedies are widely utilized in Palestine by public healers, as they are in many other nations, to cure a variety of ailments (Jaradat et al., 2017). People's and scientists' interest in complementary medicine has grown recently, particularly in plants as a source of anti-diabetic effects. In our research, we will concentrate on the alternative treatments for diabetes mellitus, *Gundelia tournefortii* (*G. tournifortii*) and *Ocimum basilicum (O. basilicum).*

1.6.1 *G. tournifortii*

G. tournifortii is a member of the Asteraceae (Compositae) family and goes by the names Akub (Arabic) and Kanger (Kurdish). It is a naturally occurring plant that is indigenous to Asia's temperate regions, including Palestine, Cyprus, Egypt, Jordan, Azerbaijan, and Turkmenistan. *G. tournefortii* is a prickly, thistle-like plant that may be eaten (Kadan et al., 2018). Food sources include its stems, seeds, and leaves (Azeez and Kheder, 2012; Coruh et al., 2007). *G. tournefortii*, also known as "Akoob" in Arabic, is an uncultivated plant that local inhabitants pick and value highly owing to its alleged health advantages, especially in Palestine. (Coruh et al., 2007). Previously, it was listed as 1 of the 10 species with the highest mean cultural importance in northern Palestine. In Palestinian traditional medicine and ethnobotany, this plant is believed to possess nutritive and curing benefits for diabetes, epilepsy, stomach and intestinal diseases (Ali-Shtayeh et al., 2008).

The plant's stalk has a long history of usage in traditional medicine in the Middle East as a hepatoprotectant, blood purifier, and possible treatment for diabetes, chest pains, and heart problems (Jamshidzadeh et al., 2005). It has been shown in the literature to have antioxidant, hepatoprotective, and antibacterial properties (Coruh et al., 2007).

G. tournifortii is abundant in phenolic substances, mainly flavonoids, which are important for the biological action of the plant. These substances include saponins, limonene, zingiberene, and derivatives of caffeoylquinic acid (cynarin and chlorogenic acid), as well as quercetin and gallic acid (Hajizadeh-Sharafabad et al., 2016).

A few research on animals has shown that *G. tournifortii* might alter lipid profiles. It's possible that it may lower low-density lipoprotein- cholesterol (LDL-c) and total cholesterol, according to some studies of the effect of *G. tournifortii* on lipid profile on animal model (Asgary et al., 2008; Azeez and Kheder, 2012; Hajizadeh-Sharafabad et al., 2016).

Furthermore, in vivo tests on diabetic mice produced by dexamethasone, investigated the anti-diabetic efficacy of *G. tournifortii*. When given orally to diabetic mice, *G. tournifortii* dramatically lowered blood glucose levels (Azeez and Kheder, 2012). Concurrently, GLUT4 translocation to the PM was improved by *G. tournifortii* extracts, which demonstrated anti-diabetic action in vitro (Kadan et al., 2021; Kadan et al., 2018).

1.6.2 *O. basilicum*

In addition to being a medicinal plant belonging to the Lamiaceae family, *Ocimum basilicum L.* has long been used as a flavoring herb in cooking. Also known as sweet basil. It is a fragrant

plant with many beneficial qualities that are noteworthy due to its medicinal value and might be used as a cure for a variety of ailments (Faur et al., 2020; Kadan et al., 2016). A significant dose-dependent inhibition against pancreatic -amylase, intestinal sucrose, and maltose was found (El-Beshbishy et al., 2012).

Rats treated with *O. basilicum* extract had a greater hypoglycemic response than rats treated with metformin. It promoted glucose mobilization by promoting hepatic glycogen synthesis, improved oral glucose tolerance, and raised liver glycogen content in a dose-dependent manner (Ezeani et al., 2017).

The antidiabetic potential of *O. basilicum* aerial part extracts in methanol, hexane, and dichloromethane were examined in vitro. Particularly the methanol and hexane extracts promoted the translocation of the glucose transporter to the plasma membrane (Abu-Odeh and Talib, 2021; Kadan et al., 2016).

Due to the scent of the essential oils derived from basil, which have amazing biological qualities including antibacterial, antiviral, antioxidant, anticancer, larvicidal, and anthelmintic capabilities, basil is also utilized in perfumery, cosmetology, dental and oral goods, and sanitary products (Faur et al., 2020; Zarlaha et al., 2014). Finding the naturally occurring plant components may be useful to better understand the relationship between chemical components and biological properties of a medicinal plant, as the complexity and chemical diversity of the compounds present in medicinal herbs plays a significant role in the discovery and development of new compounds (Fathiazad et al., 2012).

According to previous reports on *O. basilicum's* phytochemical production, the plant primarily yields triterpenoids, polyphenols, steroids, and phenylpropanoids, some of which, like basilol, ocimol, basilimoside, rosmarinic acid, hydroxycinnamic acids, oleanolic acid, and betulinic acid,

have been demonstrated to have significant biological properties. In vitro studies have demonstrated that *O. basillicum* water and ethanol extracts have potent antioxidant properties (Ch et al., 2015; Kaurinovic et al., 2011; Teofilović et al., 2017). A possible herbal remedy for diabetes has recently been identified as *O. basillicum*. However, investigations did not identify the chemical molecules or compounds responsible for the purported anti-diabetic benefit (Kadan et al., 2016).

1.7 The Oral Glucose Tolerance Test (OGTT)

The glucose tolerance test (GTT) measures the clearance of a glucose load from the body. It is used to identify pathological variations in glucose metabolism which are linked to diabetes and metabolic disease. Animals are fasted and blood glucose levels are determined before a solution of glucose is administered (2g/kg BW). Subsequently, blood glucose concentrations are measured across 2-hours. It is the most common physiological test carried out in metabolic research, particularly for phenotyping transgenic mice and/or in response to metabolic challenges such as high-fat feeding or other stimuli. The procedure is simple to carry out and does not require specific technical training beyond confidence in basic animal handling and substance administration.

There are different ways to do a GTT utilizing the intraperitoneal, oral, voluntary oral, and intravenous methods of glucose delivery.

The oral administration of Glucose solution:

Oral administration of a glucose bolus can be achieved by intragastric delivery via a feeding needle (gavage). Although oral administration mimics physiological processes and activates the incretin response, the pace of stomach emptying can have a significant impact on glucose absorption and, thus, on glucose tolerance. The glucose bolus might be administered intravenously (IV) or intraperitoneally (IP) to exclude these characteristics from the interpretation. It should be noted that OGTT will result in lower peak plasma glucose levels than IP or IVGTT. Before starting an experiment, there are more factors to consider, such as age, nutrition, strain, sex, glucose dosage, protocol timings, etc.

The GTT is a straightforward test that may be used to quickly and accurately gather metabolic information on glucose tolerance. When combined with the measurement of plasma insulin during the GTT and knowledge of insulin sensitivity obtained from either an insulin tolerance test or clamp studies, its interpretation is most effective. (King et al., 2020; Luo et al., 2021; O'rielly et al., 2020).

2. Chapter Two: Aims of study

- In this research we try to evaluate in vivo antidiabetic activity of O. basillicum and G. tournefortii extract by measuring blood glucose levels.
- To determine the desired dose of plant extract that will be not toxic for mice.
- To study the effect of these extracts on antioxidant levels by measuring an MDA as a marker of the oxidative stress.
- To ass's pathological variation in glucose metabolism which are linked with diabetes via OGTT.
- Finally, to evaluate the therapeutic effect of the extract. Knowing that measuring differences in the basic parameters such as body weight, food intake, and water consumption, urination is one of the essentials aims of this study.

3. Chapter Three: Materials and Method

3.1. Study design

3.1.1 Plants collection

All data about the two plants including: scientific name, common name, family name, part of plant, month of collection, and region source are mentioned in table 1. below:

Table 1: Plants and part of the plant used for the extract preparation.

Scientific name	Common name	Family	Plant part	Date of collection	Region source
Ocimum basilicum	Great basil	Lamiaceae	Aerial parts (stem, leaves, flowers)	July- 2021	Tulkarm-Attil
Gundelia tournefortii	Galgal	Asteraceae (compositae)	Head and stem	February-March-2021	Nablus

The plants were gathered from the nearby local region. *O. basilicum* from Atill town/Tulkarm; *G. tournefortii* from Nablus city, and *O. basilicum* was dried in the shade (at room temperature) and *G. tournifortii* need additional drying in the oven (Drying Oven Venticell) at 40 °C.

3.1. 2 Plant extracts preparation

The dried aerial parts of *O. basilicum* and *G. tournifortii* were ground. About, 20 g of the powder was added to 100 ml of methanol (Sigma-Aldrich, Germany) solvent and incubated in a sonicator at 60°C for one hour. Both extracts were kept in a dark glass bottle at -20°C for later use (Kadan et al., 2016).

3.1.3 Induction of diabetes.

Streptozotocin (STZ), a substance that exhibits a preference for toxicity toward pancreatic cells, is used to cause diabetes in mice. Streptozotocin preparation was done daily for immediate injection by freshly dissolving in sodium citrate buffer in the morning. Mice get an injection of the diabetic medication at a dose of 50 mg/kg body weight for 5 days. During this time, the diabetic mice were fed on a (10%) sucrose diet that was added to their water (Bassalat et al., 2023).

3.1.4 Animals

C57BL/6, often referred to as "C57 black 6" males, 4-6 weeks old, 25-35(g) were used at the animal unit of Prof. Zaid at the Arab American University, Jenin. All mice were housed in (a 25×30×30 cm cage) under healthy conditions at 21-24 °C with 40-60% humidity and a 12 h light/dark cycle. All mice were fed standard rodent chow and filtered water and acclimated for 2 weeks before the beginning of the experiment. All procedures were approved under the principles of animal welfare according to Arab American University.

For the experiment, mice were divided into six groups (n=8/group) for each treatment as shown in table 2.

Table 2: Animal groups for each treatment.

Group1	Group 2	Group 3	Group 4	Group 5	Group 6
Control Normal mice	Normal mice+ *O. basilicum* extract	Normal mice+ *G. tournifortii* extract	Diabetic control mice	Diabetic mice+ *G. tournifortii* extract	Diabetic mice+ *O. bsilicum* extract

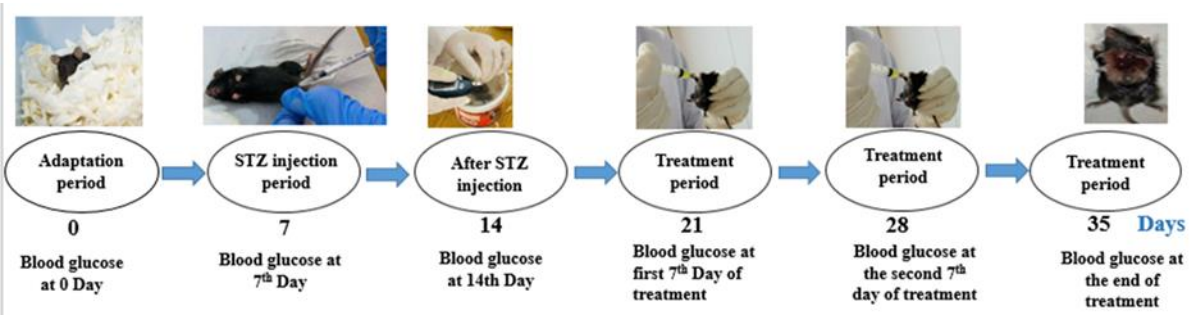


Figure B: The timetable of the experiment.

Group I: Control mice kept under normal diet conditions.

Group II: Mice fed (gavage) continuously once a day for 21 days with O. basilicum extract at a dosage of (100mg/Kg).

Group III: Mice fed (gavage) continuously once a day for 21 days with G. tournifortii extract at a dosage of (100mg/Kg).

Group IV: Mice made diabetic by daily IP injection for 5 days with 50mg streptozotocin/kg body weight.

Group V: Mice received daily IP injection of streptozotocin for 5 days and then fed (gavage) with G. tournifortii extract at the above-mentioned dosages for 21 days (Al-Attar and Zari, 2010).

Post STZ injection, ,on day 9, blood glucose level were monitored if it is > 200 mg /dl the mice are considered diabetic. Then plant extract will be given to the desired group with a concentration of 100mg/Kg. (Figure B)

3.1.5 Collecting blood samples.

At the end of the experiment, mice were sacrificed one day after the final dose of pant extract given via gavage and cardiac punctures were used to draw blood Samples from each mouse from all groups. All blood samples were then put in separate additive test tubes that were coated with heparin and contained EDTA. The heart, kidney, liver, and muscular gastrocnemius skeletal muscle tissues were taken right once after blood was drawn. They were then cleaned with a normal saline cold solution, dried with sterile gauze, and stored at -20°C for later use (Bassalat et al., 2020).

3.1.6 Blood glucose monitoring.

The blood glucose levels of all groups were checked first after the STZ, a diabetes agent, had been injected into mice for 5 days (on the 9th day) as show in table 3, then throughout the trial (10 days after the mice had been given the extracts), and lastly after the 21 days of plant extract gavage. The samples were taken from the tail vein using a digital glucometer and a glucose strip test (Bassalat et al., 2020).

3.1.7 Determination of antioxidant levels.

MDA levels were measured spectrophotometrically (Jenway spectrophotometer, France) in tissues according to the method described by (Ohkawa et al., 1979). In tissues (heart, liver, kidney and skeletal muscle), lipid peroxides formed a chromogen with 2-thiobarbituric acid (TBA) at 100^{o}C. N-butanol was added to this chromogen and the resulting color intensity was measured spectrophotometrically at 532 nm. (refer to Table: 3 for more details).

Calculation: Sample absorbance / Standard absorbance x Standard concentration

(100 mg/dL) = nmol/mg tissue (Ohkawa et al., 1979).

Table 3: MDA material with steps in order.

	Blank	Standard	Sample
	0.2 ml Distilled water	0.2 ml Standard	0.2 ml Homogenate
Sodium dodecyl sulphate	0.2 ml	0.2 ml	0.2 ml
Acetic acid	1.5 ml	1.5 ml	1.5 ml
Thiobarbituric acid (TBA)	1.5 ml	1.5 ml	1.5 ml
Distilled water	0.6 ml	0.6 ml	0.6 ml
Vortex- Boiling 1 hour			
Distilled water	1 ml	1 ml	1 ml
N- Butanol/Pyridine	5 ml	5 ml	5 ml
Vortex- (centrifuge 20 min/3000 rpm), measure absorbance at 532 nm			

3.1.8 Body weight and food consumption.

Body weight was measured weakly using a digital balance (Balance 3 Digit, Adam). These weights were determined at the same time during the morning, and every day the food consumption and water intake were observed using digital balance (Balance 3 Digit, Adam), and graduated cylinder respectively (Ramadan et al., 2017).

3.1.9 Oral Glucose Tolerance Test

At the end of treatment, all mice that fasted overnight were orally fed with 2 g/kg BW of glucose (Sigma-Aldrich Co.). Subsequently, the blood glucose level of the mice collected from the tail vein was measured at 0, 30, 60, 90, and 120 minutes by digital glucometer (Abbott, USA) and a glucose strip test (Luo et al., 2021).

3.1.10 Data analysis

The data was expressed as mean ± standard error (SE), the t-test was used for the statistical analysis and significance measures ($P<0.05$) were considered to be statistically significant. SPSS Program version 23 was used to analyze the results.

4. Chapter Four: Results

4.1 Effect of *O. basilicum* and *G. tournifortii* on mice blood glucose, weight, food and water consumption.

4.1.1 Blood glucose results:

As results show, the methanolic extract of the two plants doesn't have a positive effect on the side of lowering blood glucose. We notice in Figure 1 that the group of diabetic mice treated with *O. basilicum* extract with a concentration of (43mg/ ml) and a dose of (100mg/ kg of mice body weight) have higher level in blood glucose level compared with a diabetic control group, while diabetic mice treated with *G. tournifortii* methanolic extract with a concentration of (44mg/ ml) stay nearly in the same level with diabetic control group Figure2. Resulting in that *O. basilicum* has more aggressive effect than *G. tournifortii* on diabetic mice.

The same thing when we look at normal control mice receiving *O. basilicum* extract via gavage, and normal control mice receiving *G. tournifortii*, blood glucose measurements are higher than normal control Figure 1,2.

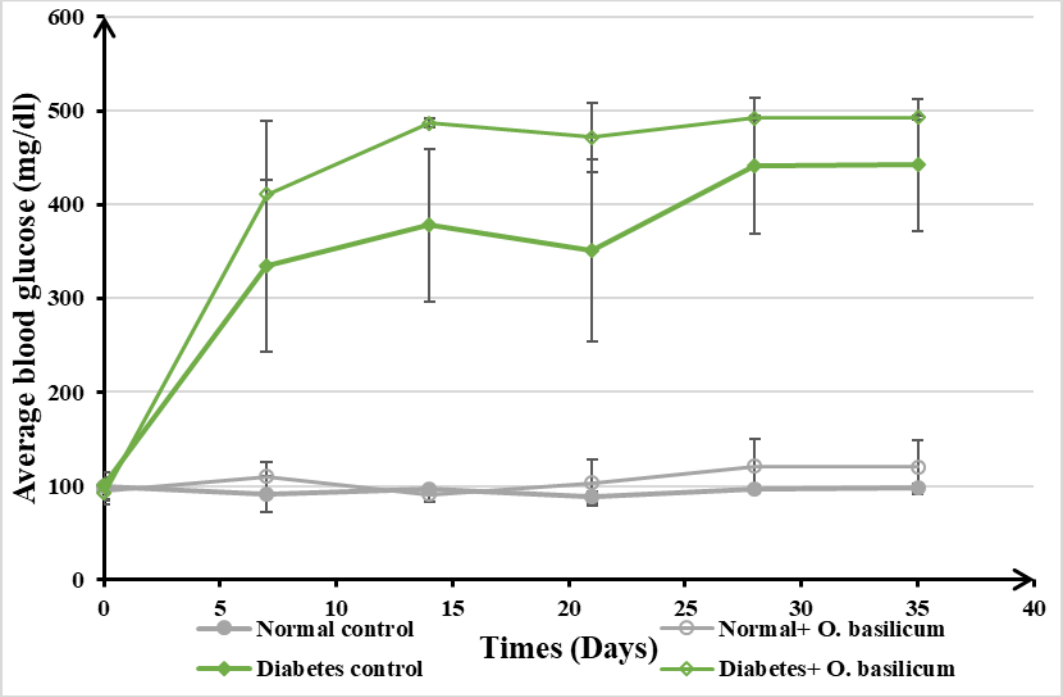


Figure 1: Blood glucose levels (mg/dl), for control and STZ- induced diabetic mice treated with and without *O. basilicum* extract for 3 weeks. Diabetes was induced by STZ injection at day 7 for 5 days. Extract treatment (O. basillicum) started at day 14 and continued until the end of the experiment.

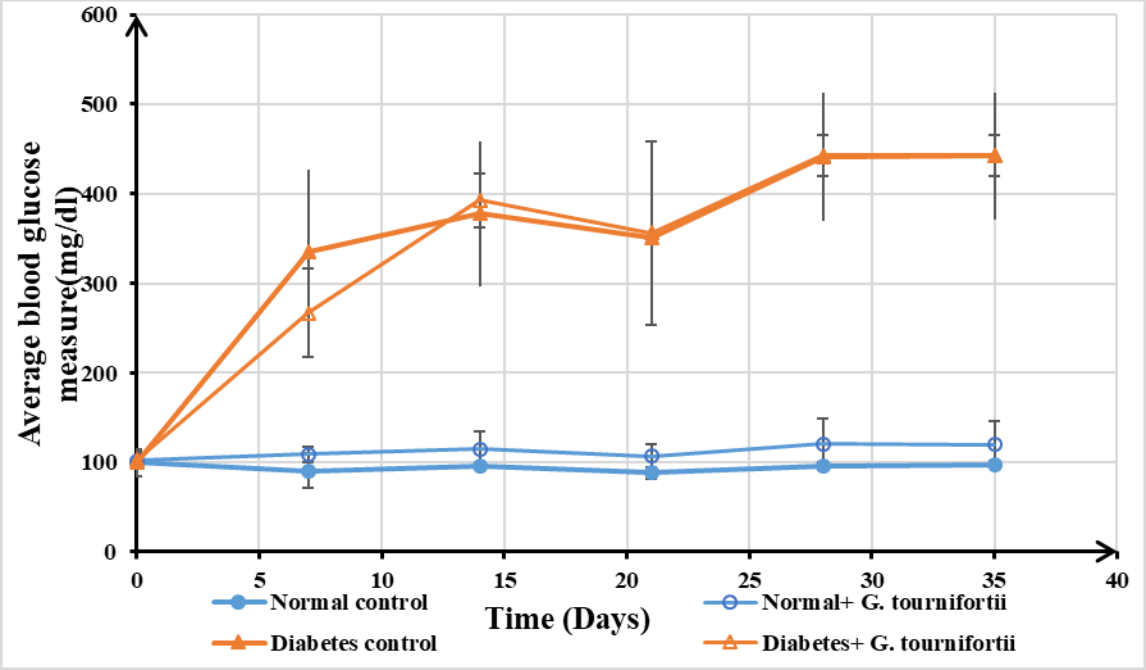


Figure 2: Blood glucose levels (mg/dl), for control and STZ- induced diabetic mice treated with and without *G. tournifortii* extract for 3 weeks. Diabetes was induced by STZ injection at day 7 for 5 days. Extract treatment (*G. tournifortii*) started at day 14 and continued until the end of the experiment.

4.1.2 Body weight of mice:

As anticipated prior to the experiment, Figures 3 and 4 show the results of the mice's body weight in the various groups. If we look at the weight of the mice in the normal control group, it increases during the course of the experiment over weeks, which is expected of normal mice, in contrast, mice of diabetic control group expected to decrease and this is what happened. Regarding diabetic mice treated either with *O. basilicum* or *G. tournifortii* methanolic extract, there is also a decrease in body weight which indicates the disability of the extracts to lower

diabetes effect. However, normal control mice treated with extracts stay in semi steady state nearly without decreasing.

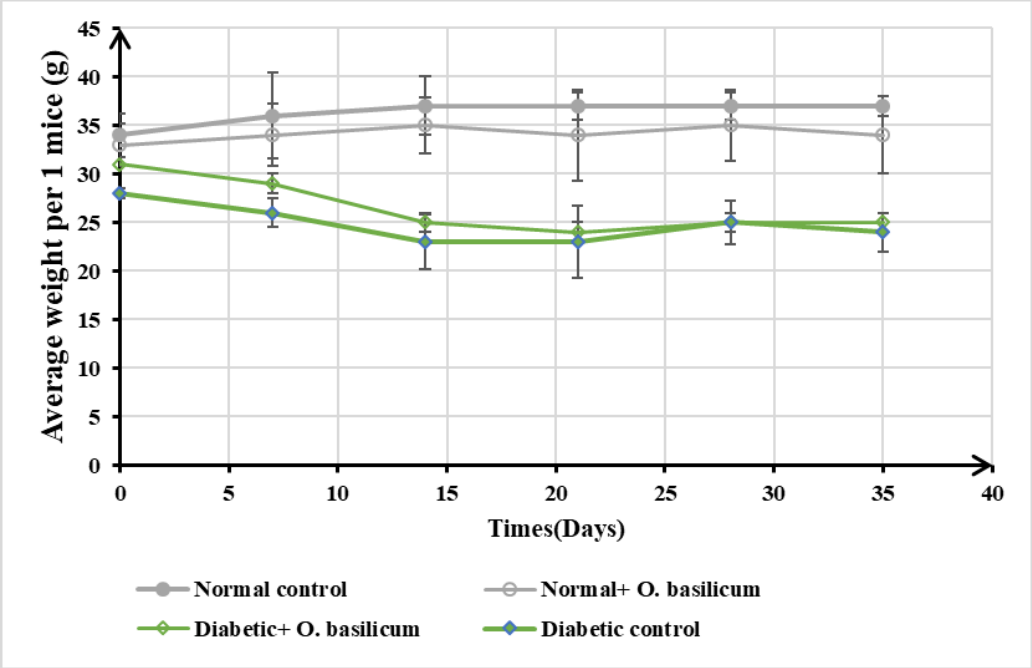


Figure 3: Average weight of mice (g) for normal and STZ-induced diabetic mice treated without and with *O. basilicum* extract.

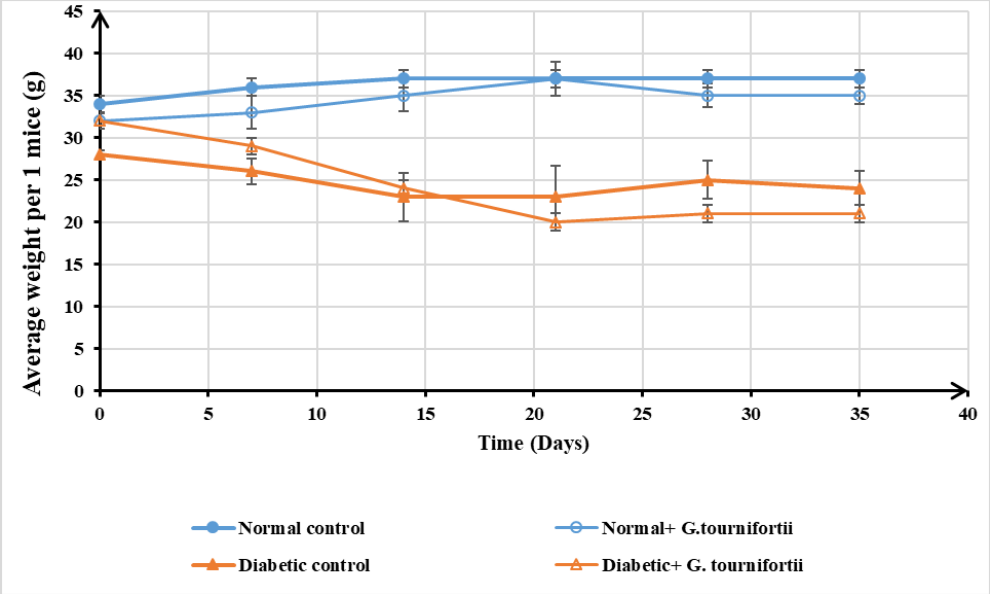


Figure 4: Average weight of mice (g) for normal and STZ-induced diabetic mice treated with and without *G. tournifortii* extract.

4.1.3 Food and water consumption:

The consumption of food and water was nearly the same with normal and diabetic mice; diabetic mice consumed more than normal ones. Diabetic ones treated with either *O. basillicum* or G. *Tournifortii* are the most in consumption, and this is for both food and water Figure 5, 6, 7 and 8.

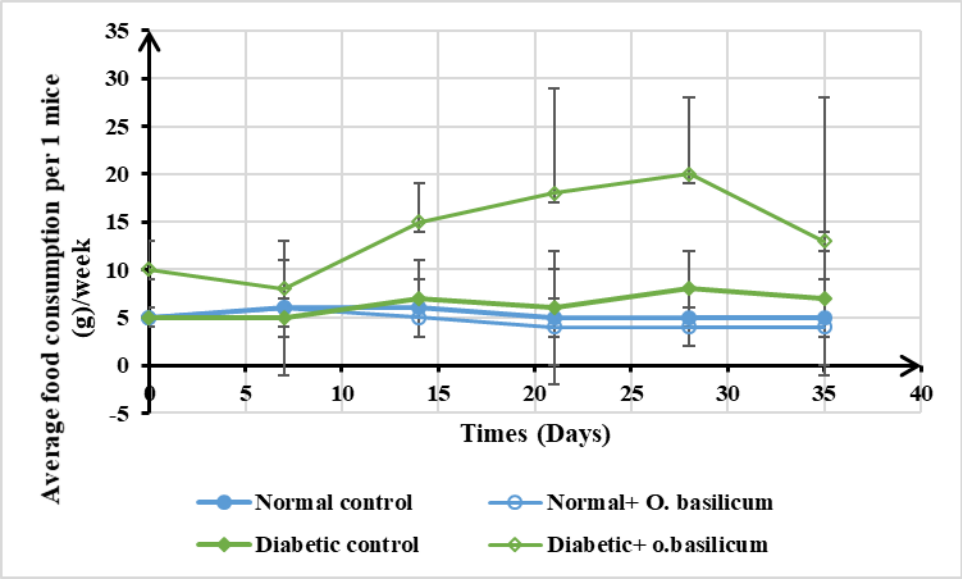


Figure 5: Food consumption average per mouse for normal and diabetic mice treated without and with *O. basilicum* extract.

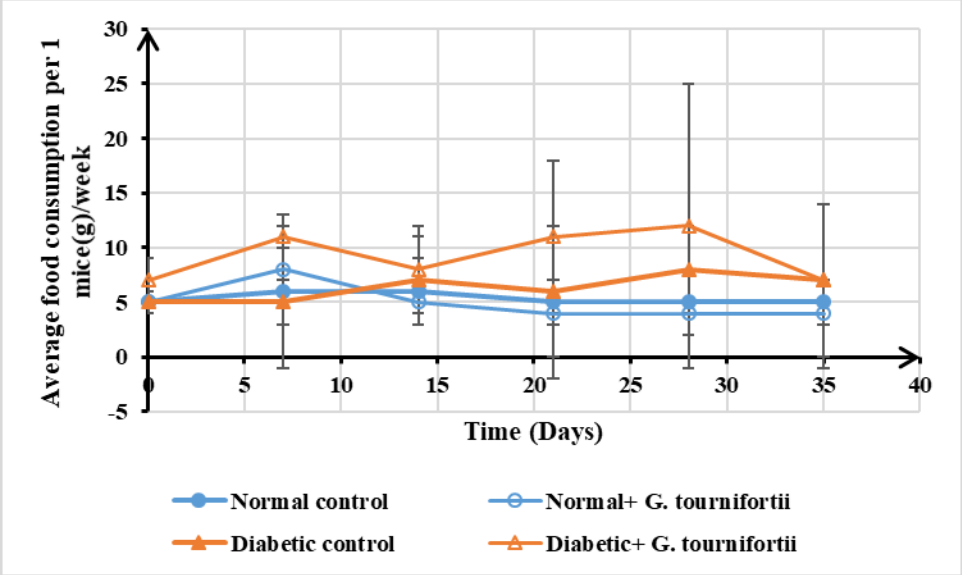


Figure 6: Food consumption average per mouse for normal and diabetic mice treated with and without *O. basilicum G. tournifortii* extract.

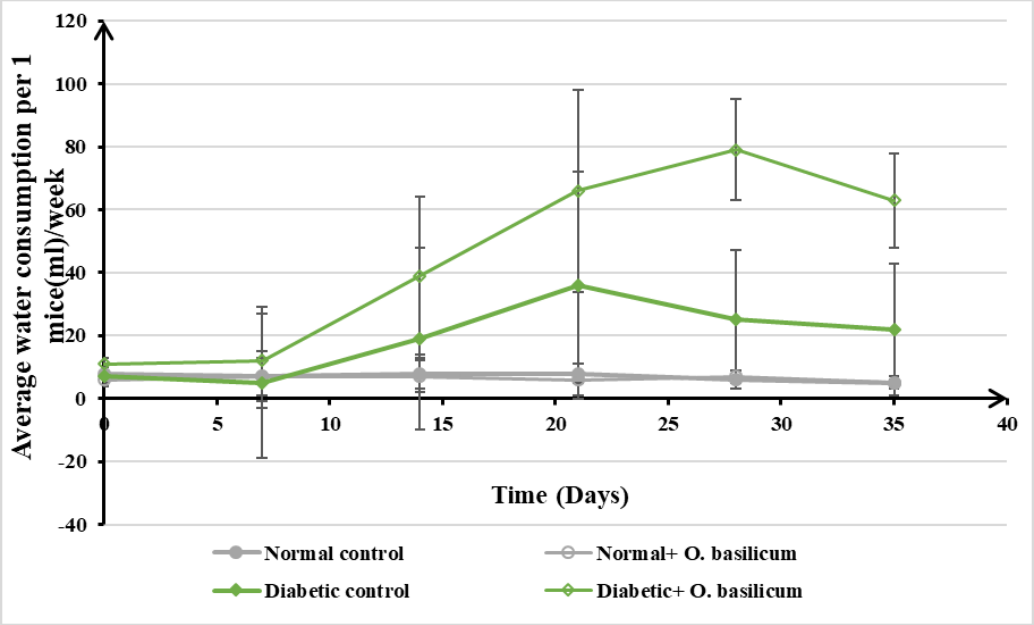


Figure 7: Water consumption average per mouse for normal and diabetic mice treated with and without *O. basilicum* extract.

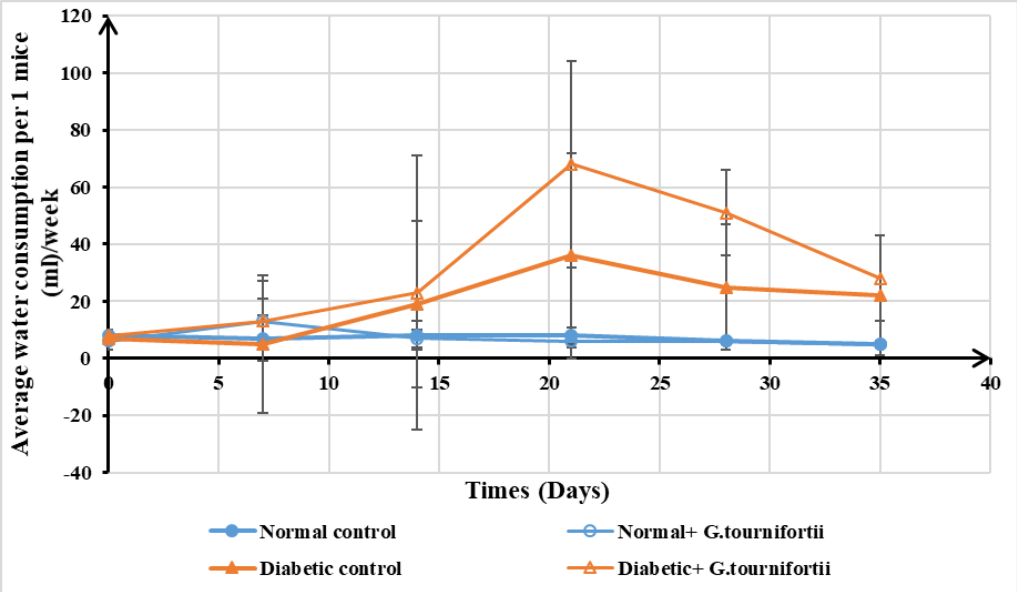


Figure 8: Water consumption average per mouse for normal and diabetic mice treated with and without *G. tournifortii* extract.

4.2 Result of Malondialdehyde (MDA) on heart, muscle, kidney, and liver.

As Figures 9, and 10 show, MDA levels are often greatest in the diabetic group for several organs. As we see in Figure (9. a, 9. b, 9. d) concerning to heart, muscle, and liver tissues in contrast to other groups treated with *O. basilicum* and normal control one. The same case is in Figure (10. b, 10. d) respectively for muscle and liver tissues in contrast to normal control and other groups treated with *G. tournifortii*. These results are in line with what we expect.

On the other hand, in the rest of Figures 9, and 10, there are no actual differences between diabetic, normal control, and diabetic-treated groups, it is nearly equal. It seems that these two extracts have a negative effect even in normally treated mice.

4.2.1 O. basillicum

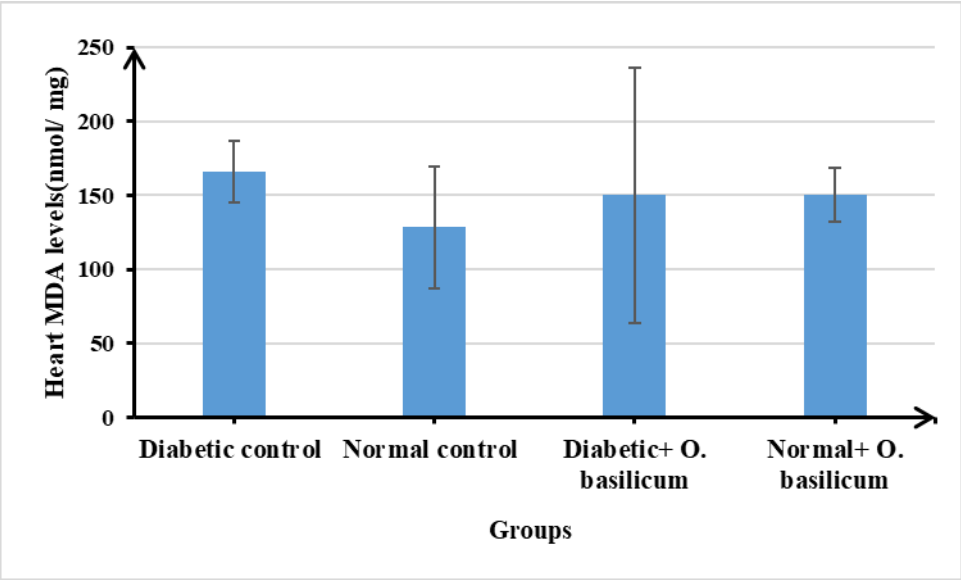


Figure 9. a: Heart MDA levels for *O. basilicum* groups. Diabetic control mice show higher oxidative stress on the heart, but also normal mice treated with extract have an oxidative stress nearly similar to diabetic treated ones.

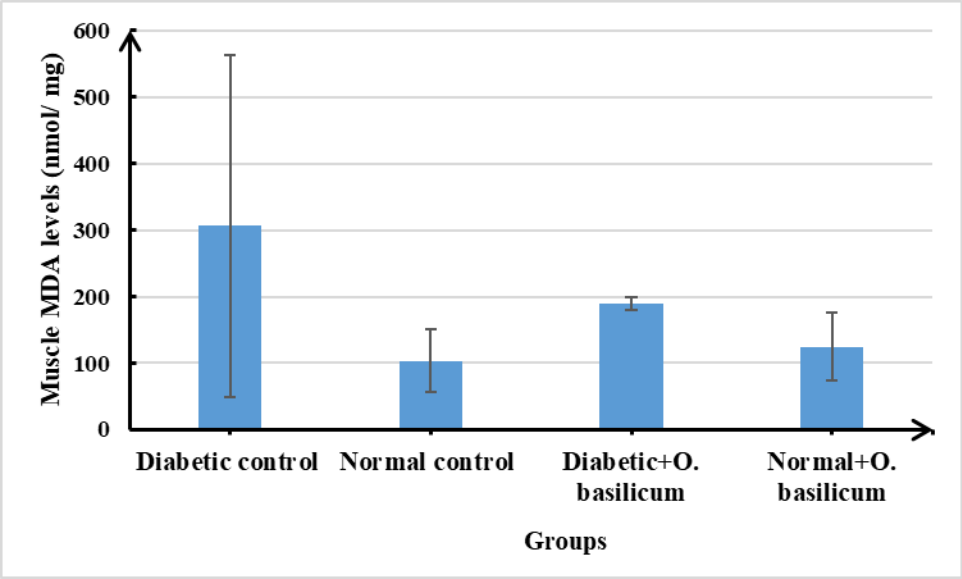


Figure 9. b: Muscle MDA levels for *O. basilicum* groups. With higher oxidative stress on muscle diabetic mice, and normal treated mice a bit higher than normal ones.

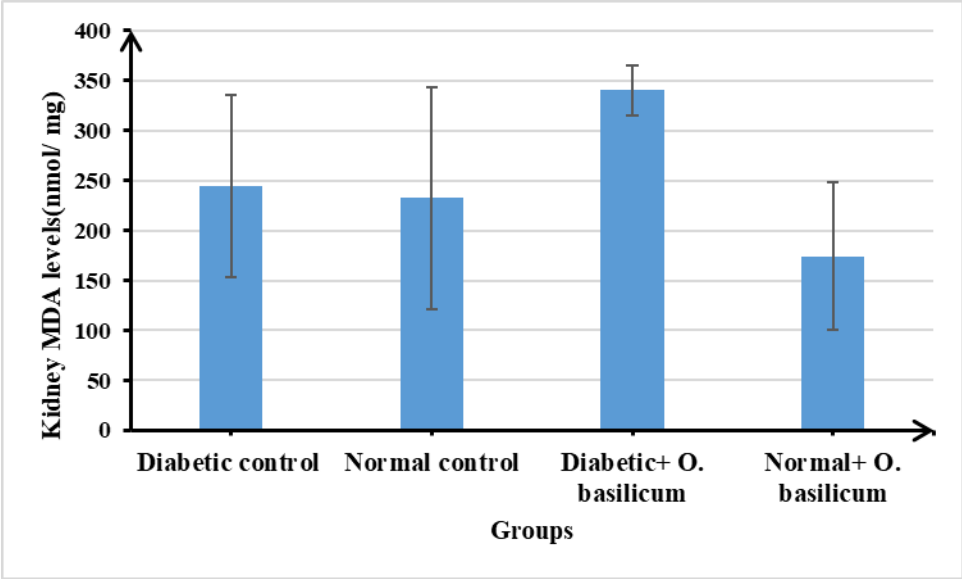


Figure 9. c: Kidney MDA levels for *O. basilicum* groups. With diabetic-treated mice are higher in oxidative stress levels.

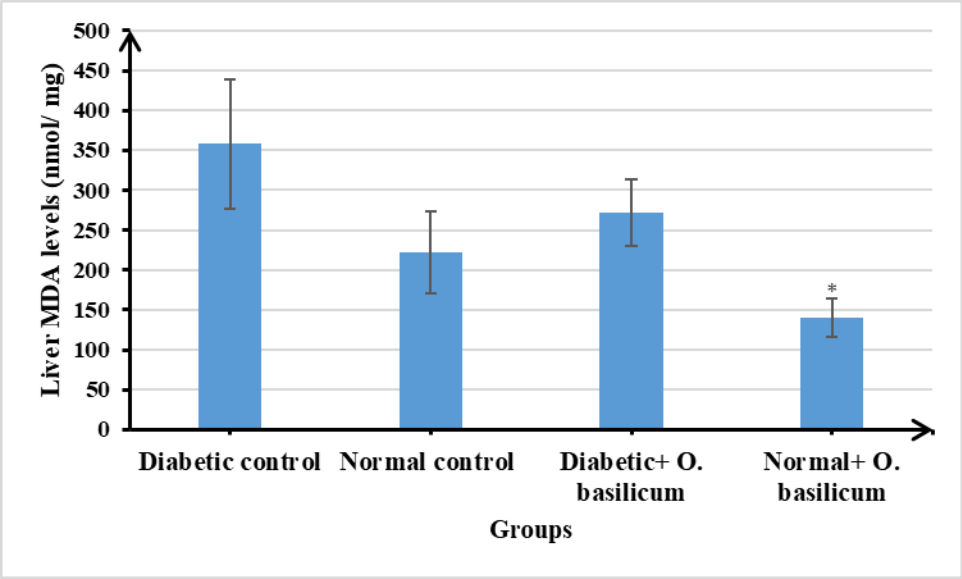


Figure 9.d: Liver MDA levels for *O. basilicum* mice. With higher oxidative levels in diabetic liver mice, and a significant difference in normal liver mice treated with *O. basilicum*.

4.2.2 *G. tournifortii*

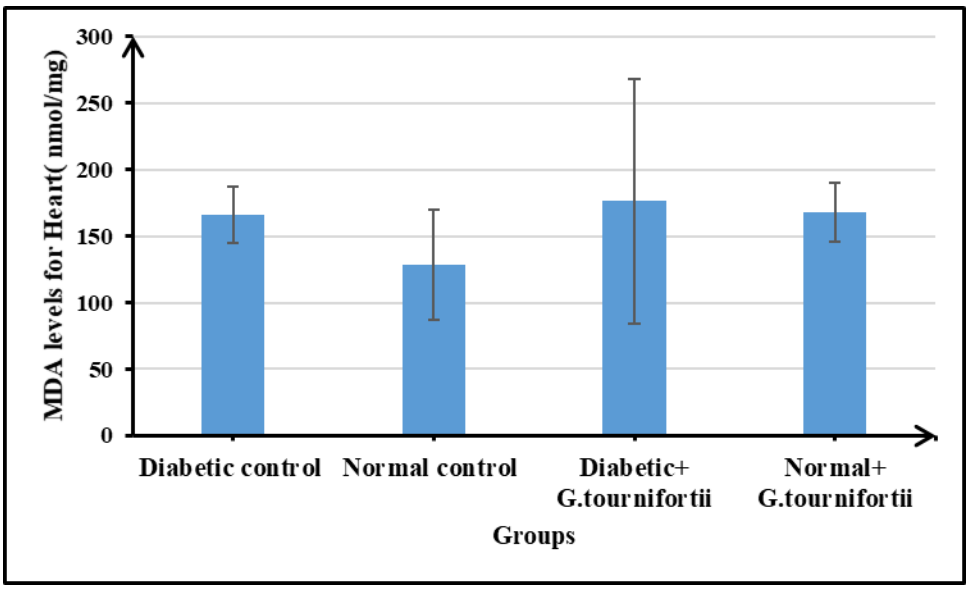


Figure 10.a: Heart MDA levels for *G. tournifortii* groups. Oxidative stress was nearly similar and higher in normal-treated mice and diabetic-treated mice than diabetic control ones.

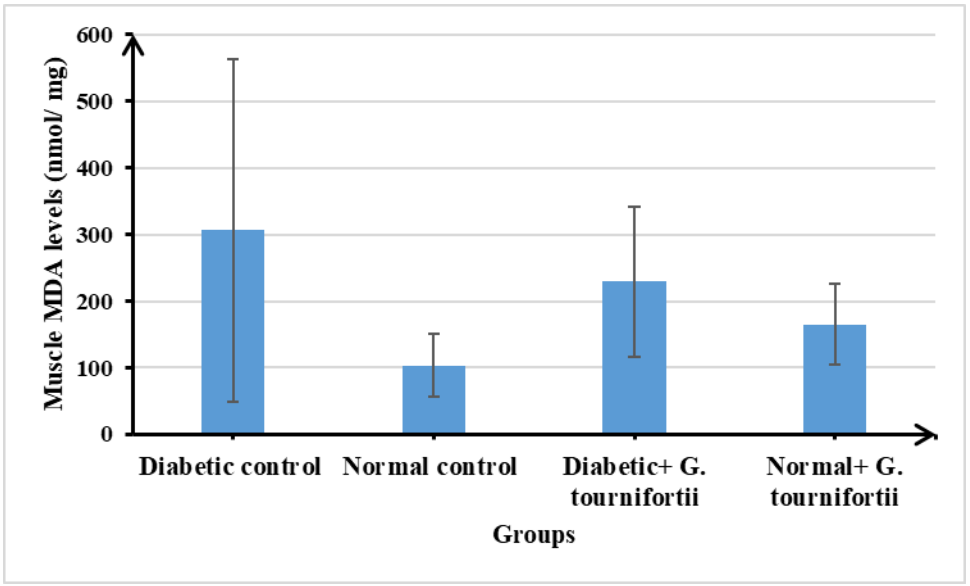


Figure 10.b: Muscle MDA levels for *G. tournifortii* groups. Normal-treated one has higher levels than normal-control.

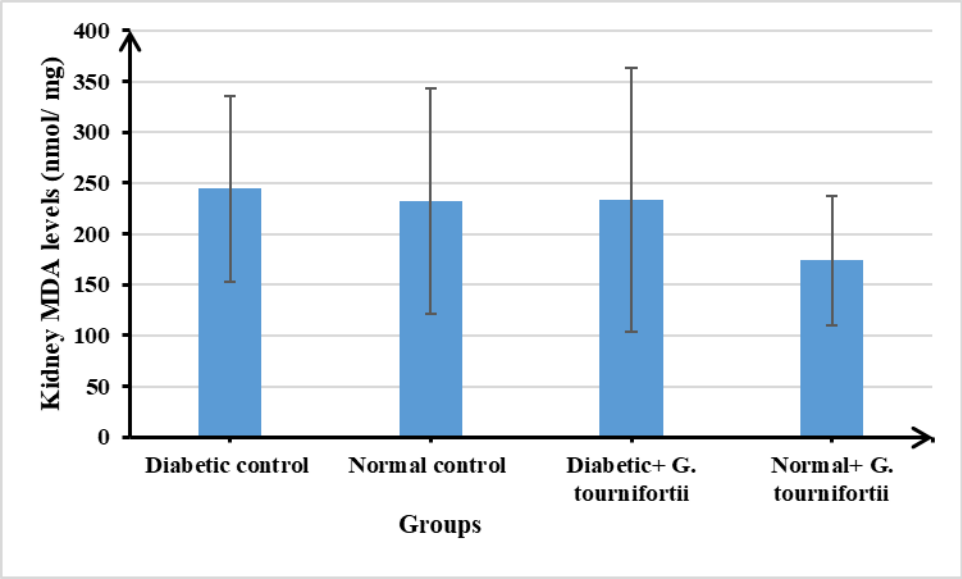


Figure 10.c: Kidney MDA levels on *G. tournifortii* groups. Diabetic mice treated have similar levels to normal ones.

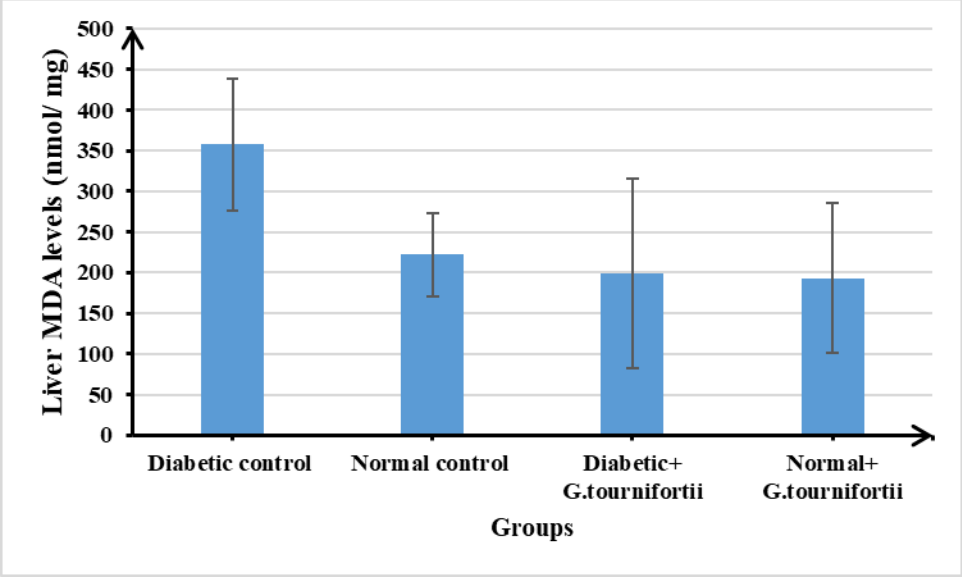


Figure 10.d: Liver MDA levels for *G. tournifortii* groups. Diabetic-treated mice have levels lower than normal ones.

4.3 Oral glucose tolerance test:

According to Figure 11.a. it seems that methanolic extract of *G. tournifortii* given to diabetic mice via gavage even for 1 day able to recover the ability of these mice to metabolize glucose in a sufficient way more than diabetic mice treated with *G. tournifortii* for a week contrary to the results in Figure 2 which shows no effect for *G. tournifortii* in lowering blood glucose levels in diabetic mice, which leads us to conclude that there is a temporary effect of this plant on blood sugar levels. Blood glucose levels in diabetic mice treated for a day reached their peak value after oral glucose administration at 30 minutes and this is expected to be because it is a short time for glucose to be absorbed, and then gradually decreased.

Normal mice treated with *G. tournifortii* methanolic extract for 1day show higher blood glucose measurements at 60, and 90 minutes and almost the same at 120 minutes in contrast to the normal control one as Figure 11. b shows, which reflects there is no significant difference between the normal control group and the normal one treated with the effect of *G. tournifortii* methanolic extract in OGTT.

According to Figure 11. c for OGTT for mice given O. basilicum extract, O. basilicum appears to significantly enhance the body's capacity to metabolize glucose after two hours of glucose

loading in diabetic mice given a one-day treatment.

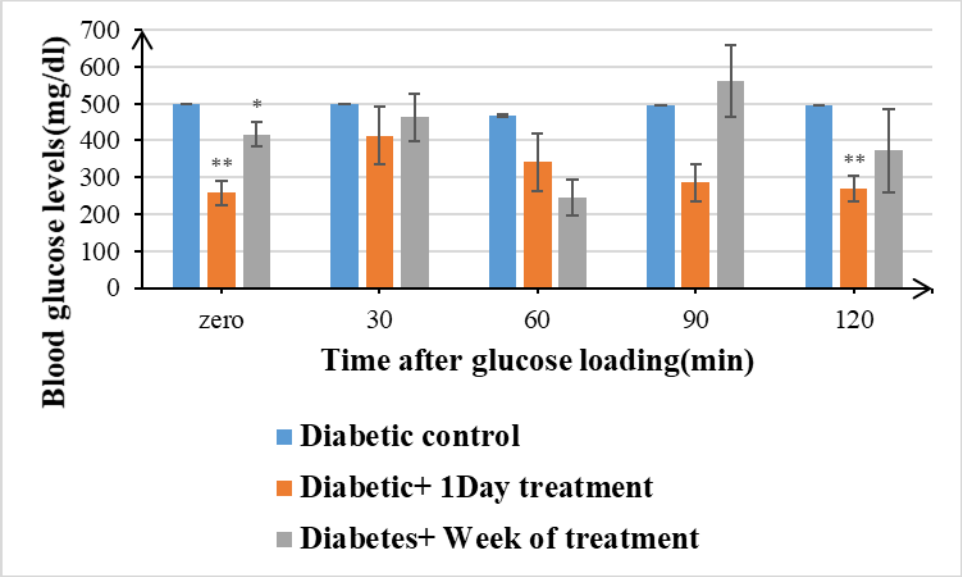


Figure11.a: Oral glucose tolerance test for diabetic groups treated with *G. tournifortii*. It seems that methanolic extract of *G. tournifortii* given to diabetic mice via gavage even for 1 day able to recover the ability of these mice to metabolize glucose in a sufficient way more than diabetic mice treated with *G. tournifortii* for a week.

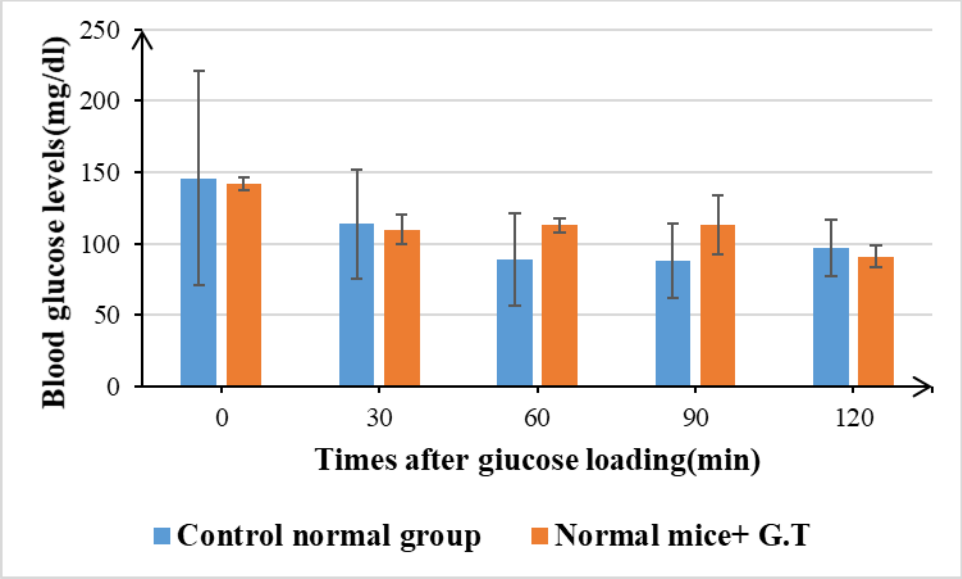


Figure 11.b: Oral glucose tolerance test for control groups treated with *G. tournifortii*. Normal mice treated with *G. tournifortii* methanolic extract for 1day show higher blood glucose measurements at 60, and 90 minutes and almost the same at 120 minutes in contrast to the normal control one, which reflects there is no significant difference between the normal control group and the normal one treated with the effect of *G. tournifortii* methanolic extract in OGTT.

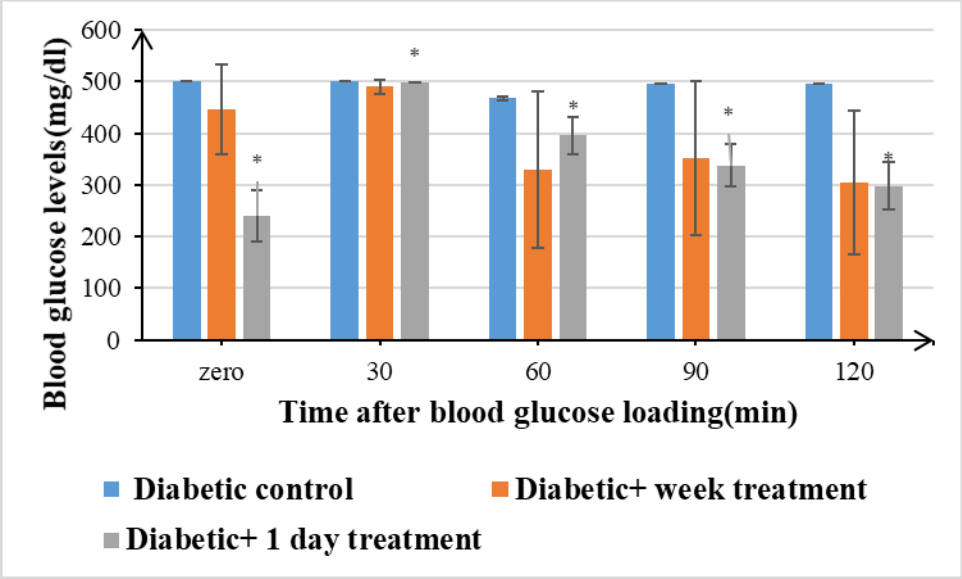


Figure 11.c: Oral glucose tolerance test for diabetic groups treated with *O. basilicum*. *O. basilicum* improves the body's ability to glucose in a positive significant way ($p \leq 0.05$) after 2 hours from glucose loading for diabetic mice treated for 1- day.

5. Chapter Five: Discussion and Conclusion

5.1 Discussion

Currently one of the most significant metabolic disorders in the world, diabetes which is defined by persistent hyperglycemia and is brought on by a lack of insulin secretion or insulin resistance. Patients with diabetes are more likely to experience acute metabolic complications like diabetic ketoacidosis, as well as chronic vascular complications (angiopathy) that include macrovascular diseases like cerebrovascular disease, that causes strokes and myocardial infarction, and microvascular diseases like diabetic retinopathy, diabetic peripheral neuropathy, diabetic nephropathy, and diabetic foot (Nie et al., 2021).

According to estimates, 25% of all prescriptions filled at community pharmacies in the United States contain plant extracts (Jan et al., 2021). Herbal remedies and plant-based medications are usually thought to be less harmful and have fewer adverse effects. According to the WHO, treating diabetes with hypoglycemic medications derived from plants is essential in traditional medicine (Jayakumar, 2010).

In this study, we use the methanolic extract of *O. basillicum* and *G. tournifortii* to evaluate their effects on T2DM in vivo after previous studies have proven their effectiveness at the level of in vitro tests. The results obtained from *G. tournifortii* extracts were incorporated into L6-GLUT4myc cells in the presence or absence of insulin, suggesting that in muscle L6-GLUT4myc cells. The translocation of GLUT4 to the plasma membrane is significantly enhanced in response to extracts of *G. tournefortii*, particularly the methanol extract, in both insulin-independent (basal) and insulin-dependent conditions. On the side of *O. basilicum* extract, noticed that its

increased GLUT4 translocation to the plasma membrane up to 7 times, using L6- GLUT4myc cells as a model to study (Kadan et al., 2016; Kadan et al., 2018; Saad et al., 2017).

Significant inhibition of a-glucosidase and a-amylase was observed in another in vitro study using *O. basilicum* aqueous extract (El-Beshbishy et al., 2012).

Our results show that there is no positive effect of the methanolic extracts of these two plants on lowering high blood glucose levels, furthermore, the group of diabetes mice receiving *O. basilicum* treatment have higher levels of blood glucose than diabetes control mice (Figure 1). For *G. tournifortii*, the group of diabetes mice that received *G. tournifortii* treatment had the same levels of blood sugar at the end of the experiment (Figure 2).

Previous in vivo experiments using extracts from *G. tournifortii* and *O. basilicum* were conducted in a variety of ways. In one study, all dosages of *G. tournefortii* aqueous extract therapy were able to significantly ($p \leq 0.05$) reduce the fasting blood glucose levels in mice with diabetes treated with alloxan monohydrate. (Goorani et al., 2018).

And in second one, *G. tournifortii* showed significant decrease in dexamethasone-induced hyperglycemic mice (Azeez and Kheder, 2012).

Also, aqueous extract of *O. basilicum* seeds showed significant decrease in blood glucose levels in Streptozotocin induced diabetic rats (Chaudhary et al., 2016).

Moreover, the extracts of plants fail to recover mice weight after induction of diabetes and receiving treatment, their weight was decreased at the same level of diabetic control (Figures 3-4).

Both treatment groups for diabetic mice showed increased consumption of food and water throughout the experiment, which spanned several weeks. In addition, their consumption is larger than in diabetic control mice (Figures 5- 8).

Malondialdehyde (MDA) is considered a biomarker of peroxidative cell membrane damage, which is typically brought on by chemical or physical oxidative stress. From previous study on *G. tournifortii* methanolic extract to evaluate its effect on biochemical parameter including MDA on obese experimental rat, it's found that MDA level variables between different kind of tissues, and that it was higher in liver, and kidney in obese mice (high calorie diet) received *G. tournifortii* via oral gavage compared to normal control group, and normal obese one. On the other hand, it was lower for example in lung tissue in obese rat (high calorie diet) in contrast with normal control one (Bati et al., 2021).

As for MDA results, it appears that these plants aren't able to reduce the effects of ROS and become a strong antioxidant agent, instead, they show severe bad effects in diabetic mice receiving treatment (Figures 9.c, 10 a) respectively for MDA result on the kidney of diabetes mice treated with *O. basilicum* and for heart in diabetes mice treated with *G. tournifortii*, in these two groups the oxidative stress was higher than diabetic control mice. However, in certain instances, the oxidative stress appears to be greater in normal mice that got therapy than in normal control mice (Figures 10 a, 10. b).

In the oral glucose tolerance test, it seems that both of the extracts have a significant decrease in blood glucose in (1day treatment groups), which leads us to conclude that these 2 extracts had temporary effects in blood glucose levels.

5.2 Conclusion

We have a proven evidence that methanolic extract of *O. basilicum* and *G. tournifortii* couldn't be anti-hyperglycemic agents and as well as they failed to reduce blood glucose levels in diabetic mice, and couldn't be an oxidative stress reducer. Further studies are needed to study the efficacy of methanolic extract of other parts of the plants such as seeds or other types of extracts.

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الملخص

تناقش هذه الرسالة مرض السكري المزمن وخصوصا النوع الثاني منه، حيث تم التعريف بالمرض وأنواعه في مقدمة الرسالة كذلك التعريف بنبتتي الريحان والعكوب اللتان تم استخدامهما لفحص فعالية عصارتيهما على مستوى سكر الدم من خلال استخدام فئران التجارب، وتقسيم الفئران الى مجموعات مختلفة (مجموعة سليمة، مجموعة سليمة تتلقى العصارة، مجموعة مريضة بالسكري، مجموعة مريضة بالسكري وتتلقى العصارة). تم مراعاة اتباع أخلاقيات التعامل مع الحيوانات وتوفير الظروف المناسبة لتربيتهم والقيام بالتجربة. تم احداث المرض من خلال استخدام مادة الستربتوزوتوسين المحدثة لمرض السكري من النوع الثاني من خلال الحقن في البطن وتدمير خلايا البنكرياس من نوع(ب). تلقت الفئران العصارة بوساطة أداة الكافاج من خلال الفم وصولا الى المعدة. وخلال فترة التجربة تم مراقبة وجود أية تغيرات سلوكية بالاضافة الى مراقبة الوزن، استهلاك الطعام والماء، ملاحظة الفرق في التبول بين المجموعات، حيث أنه يزداد في المجموعات المصابة بالسكري بشكل كبير مقارنة بالأخرى. كذلك تم تقييم النبتتين كنباتات مضادة للأكسدة من خلال فحص الميلانوألدهايد في عدة أعضاء. وأخيرا تقييم فعالية النبتة في التأثير على مستوى السكر في دم الفئران السليمه والسكريه. لم يكن هنالك تأثير ايجابي للنيتتين في خفض سكر الدم على المجموعتين المريضتين بالسكري و(اللتان تلقيتا العصارة)، كذلك استمر فقدان الوزن في المجموعتين المريضتين وتتلقى العصارة. فيما يتعلق في استهلاك الطعام والماء كانت مجموعات السكري والسكري التي تتلقى العصارة أعلى استهلاكا من المجموعات السليمة، بالاضافة لعدم نجاح النبتتين كمضادات للأكسدة حيث أوضح فحص الميلانوألدهايد مستويات عالية من الأكسدة في أعضاء مختلفة في المجموعات المريضة التي تتلقى العصارة مماثلة للمجموعات المريضة بالسكري وكذلك لم يقتصر التأثير السلبي لعصارات النبتتين فقط على المجموعات المريضة بالسكري بل على المجموعه الضابطه السليمةأيضا.نتائج هذه الدراسه مفاجأه ولم تكن موافقة للدراسات على النبتتين على مستوى الخلية حيث تم استخدام-L6

GLUT4myc muscle cells نموذجا للتجربة ونجحت عصارة النبتتين في تحفيزانتقال GLUT4 الى غشاء الخلية البلازمي. الا انه يبدو ان فعاليتها على مستوى الجسم الكامل مختلفه.