BAŞKENT UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHYSIOLOGY
MASTER'S PROGRAM

EVALUATION OF ELECTRODERMAL ACTIVITY IN DIABETIC RATS TREATED WITH VILDAGLIPTIN AND METFORMIN

BY

MUFTAH MOHAMED MUFTAH SHAWESH

MASTER'S THESIS

ANKARA - 2022

BAŞKENT UNIVERSITY
INSTITUTE OF HE ALTH SCIENCES
DEPARTMENT OF PHYSIOLOGY
MASTER'S PROGRAM

EVALUATION OF ELECTRODERMAL ACTIVITY IN DIABETIC RATS TREATED WITH VILDAGLIPTIN AND METFORMIN

BY

MUFTAH MOHAMED MUFTAH SHAWESH

MASTER'S THESIS

THESIS ADVISOR

PROF. DR. AYŞE ARZU YİĞİT

CO-ADVISOR

PROF. DR. NAZAN DOLU

ANKARA - 2022

BAŞKENT UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

This study, which was prepared by Muftah Mohamed Muftah Shawesh within the framework of the Department of Physiology Master's Program, was accepted as the Master's Thesis by the following jury.

Thesis Defense Date: 06 / 10 / 2022

Title of the Thesis: Evaluation of Electrodermal Activity in Diabetic Rats Treated with Vildagliptin and Metformin

Thesis Jury Members (Title, Name - Surname, Institution) **Signature**

APPROVAL

Institute Director
Date: / / 2022

BAŞKENT UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

MASTER'S / DOCTORAL THESIS STUDY ORIGINALITY REPORT

Date: 24 / 05 / 2022

Student Name, Surname: Muftah Mohamed Muftah Shawesh

Student Number: 21910599

Department: Physiology Program: Master

Supervisor's Title /Name, Surname:

Title of Thesis: Evaluation of Electro Dermal Activity in Diabetic Rats Treated with Vildagliptin and Metformin

According to the originality report, which was taken by the plagiarism detection program named Turnitin by the thesis supervisor on 23/05/2022 on the 34 page part of my Master's thesis study mentioned above, which consists of Introduction, Main Sections and Conclusion Section, the similarity rate of my thesis is 18% Applied filters:

1. Excluding bibliography
2. Excluding quotations
3. Excluding text parts that contain less than five (5) words of overlap

I have examined the Baskent University Institute Thesis Study Originality Report Obtaining and Using Procedures and Principles. I hereby declare that my thesis work does not include any plagiarism, that I accept all kinds of legal responsibilities that may arise in case the contrary is determined and that the information given above is correct

Student Signature:…….

APPROVAL

Date: 24 / 05 / 2022

Student Supervisor

ACKNOWLEDGMENT

This study was approved by Baskent University Ethical Committee for Experimental Resarch on Animals (Project no: DA 20/23, approved date: 11.23.2020) and supported by Baskent University Research Fund.

I would like to specially thank to my advisor · her support and assistance throughout the thesis. Also, I would like to thank to my co-advisor Prof. Dr. Nazan Dolu to help the initiate my MSc journey and gave her support.

I would not have been able to complete this thesis without their encouragement and support. Throughout this journey, they have mentored, encouraged, and believed in me. I am grateful to them for providing a critical evaluation of my studies as well as useful ideas for correcting my errors. It also brings me great pleasure to offer my deepest gratitude to all of the lecturers who helped me during my master's degree program.

Finally, I'd like to thank my family for their constant support and encouragement.

ÖZET

Muftah Mohamed Muftah SHAWESH, Vildagliptin ve metformin ile tedavi edilen diyabetik sıçanlarda elektrodermal aktivitenin değerlendirilmesi
Başkent Üniversitesi Sağlık Bilimleri Enstitüsü Fizyoloji Anabilim Dalı, Fizyoloji Tezli Yüksek Lisans Programı, Yüksek Lisans Tezi, 2022.

Elektrodermal aktivite (EDA), sempatik sinir sistemi ile innerve olan ekrin ter bezlerinin aktivitesini yansıtan derinin elektriksel iletkenliğinin ölçümüdür. Çalışmamızda, metformin, vildagliptin ve metformin+vildagliptin kombinasyonu uygulanan diyabetik sıçanlarda elektrodermal aktivitenin, dolayısıyla sempatik aktivitedeki değişikliklerin değerlendirilmesi amaçlandı.

Çalışmada 50 adet erkek Wistar sıçan kullanıldı. Sıçanlar 10'u kontrol, 40'ı deney grubu olacak şekilde ayrıldı. Deney grubundaki 40 hayvanda 45 mg/kg ip streptozotosin (STZ) uygulamasından sonra kan glikoz düzeyi 250 mg/dl' yi geçen hayvanlar diyabetli olarak kabul edildi. Sıçanlara 15 gün boyunca gavaj ile çeşme suyu, 500 mg/kg metformin, 50 mg/kg vildagliptin, ve 500 mg/kg metformin+50 mg/kg vildagliptin verilerek diyabetik kontrol (n=10) ve deney grupları (3 grup, n=10) oluşturuldu. 15 gün sonunda kan glikozluri tekrar ölçüldükten ve diyabetin devam ettiği görüldükten sonra Biopac MP35 cihazı kullanılarak, ses uyarısı verilmeden önce dinlenim durumundaki tonik, işitsel uyarıdan sonra gelişen cevap fazik deri iletkenlik seviyesi (DİS) olarak kaydedildi. Gruplar arasındaki farklılıklar one-way Anova testi ve Posthoc Tukey testi ile belirlendi. $P<0.05$ anlamlı olarak kabul edildi.

Hem tonik hem de fazik DİS diyabetli gruplarda arttı ($p<0.001$) ki bu diyabetli gruplarda sempatik aktivitenin arttığını gösterebilir. Metformin ve vildagliptin verilen gruplarda kan şekeri diyabetli gruba göre daha düşüktü. Tonik DİS, metformin+vildagliptin grubunda diğer deney gruplarına göre, fazik DİS ise metformin grubunda diyabetik gruba göre azaldı ($p<0.0001$). Vildagliptin ile metformin+vildagliptin gruplarının pik sayısı da tonik kayıtta diyabetik gruba göre azaldı (sırasıyla $p<0.0001$ ve $p<0.05$).

Çalışmamızda, metforminin, ve metformin vildagliptin kombinasyonlarının fazik DİS de azalma oluşturması, bu ilaçların ekrin ter bezi ve bu ter bezlerine etkiyen sempatik

aktiviteyi azalttığını gösterebilir. Sempatik aktivitede azalmanın diyabette olumlu etki oluşturup oluşturmayacağı ile ilgili daha ileri çalışmalara ihtiyaç bulunmaktadır.

Anahtar kelimeler: Diyabet, elektrodermal aktivite, metformin, sempatik aktivite, vildagliptin

Bu çalışma Başkent Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmış (Proje no: DA20/23) ve Başkent Üniversitesi Araştırma Fonunca desteklenmiştir.

ABSTRACT

Muftah Mohamed Muftah SHAWESH. Evaluation of electrodermal activity in diabetic rats treated with vildagliptin and metformin.
Baskent University Institute of Health Sciences, Department of Physiology, Master's Program of Physiology with thesis, Master's Thesis, 2022.

Electrodermal activity (EDA) is the measurement of the electrical conductivity of skin, which indicates the activation of sympathetically innervated eccrine sweat glands. Thus, sympathetic system activity that innervates eccrine sweat glands. In our study, it was aimed to evaluate electrodermal activity and thus changes in sympathetic activity in diabetic rats treated with vildagliptin, metformin and metformin+vildagliptin combination.

50 male rats were used in this study. Rats were divided into control (n=10) and experimental groups (n=40). Forty animals in experimental group were injected with 45 mg/kg streptozotocin as a single dose intraperitoneally. After 3 days, animals whose blood glucose level exceeded 250 mg/dl were considered as diabetic. Rats were divided into 4 groups as diabetes, vildagliptin, metformin and metformin+vildagliptin. Vildagliptin (50 mg/kg), metformin (500 mg/kg) and 500 mg/kg metformin+50 mg/kg vildagliptin were administered by gavage for 15 days. After that blood glucose were be measured again end of the 15 days and seeing that diabetes continues, tonic activity was recorded before sound stimulus, and phasic skin conductivity level (SCR) was recorded in response to the auditory stimulus by using Biopac MP35 device. Differences between groups were determined by one-way Anova test. Tukey test was used to determine the significance between the groups. $P<0.05$ was considered significant.

Both tonic SCL and phasic SCR were higher ($p<0.001$) in diabetic groups than the control group. These findings might explain the increase in the sympathetic activity in diabetic groups. In additiona, the blood glucose levels of the metformin and vildagliptin groups were lower than the diabetic group. Intrestingly, the metformin+vildagliptin group had a lower tonic SCL than the other experimental groups, whereas the metformin group had a less phasic SCR than the diabetes group ($p <0.0001$). Peak number of vildagliptin and metformin+vildagliptin groups decreased in tonic recording compared to diabetic group ($p<0.0001$, $p<0.01$ respectively).

Our study, the fact that diabetic drugs caused a decrease in SCL indicates that these drugs may reduce eccrine sweat gland and sympathetic activity affecting these sweat glands. Further studies are needed on whether a decrease in sympathetic activity will have a positive effect on diabetes.

Keywords: Diabetes, electrodermal activity, metformin, sympathetic, vildagliptin

This study was approved by Baskent University Ethical Committee for Experimental Research on Animals (Project no: DA20/23) and supported by Baskent University Research Fund.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

BIA	bioelectrical impedance analysis
CNS	central nervous system
DM2	type 2 diabetes
DPP-4	Dipeptidyl Peptidase-4
EDA	electrodermal activity
FPG	fasting plasma glucose
GDM	gestational diabetes mellitus
GSR	galvanic skin response
IDE	insulin-degrading enzyme
MDM	neonatal diabetes mellitus
PAD	peripheral artery disease
SCFr	skin conductivity fluctuation frequency
SCL	skin conductivity level
SCR	skin conductivity response
SPL	skin potential level
SSR	sympathetic skin response
T2DM	type 2 diabetes mellitus

1. INTRODUCTION

The metabolic disorder diabetes mellitus (DM) is characterized by increased blood glucose levels, which is caused by insulin production, action or both. It arises as a result of factors such as insulin deficiency, which causes autoimmune death of pancreatic cells, and abnormalities that induce insulin resistance and affect glucose, lipid, and protein metabolism. Insulin release abnormalities and a defective insulin mechanism frequently occur in the same patient, and it is not always evident which deficiency is the actual reason of hyperglycemia. It damages, malfunctions, and fails organs over time, including the eyes, lungs, brain, heart, and arteries (1).

Metformin is a widely recommended drug that's been demonstrated to help with glucose metabolism and diabetic problems. The mechanisms behind these benefits are complicated, and they are still not completely understood. Metformin has just been demonstrated to inhibit glucose production in the liver. The findings differed at the cellular scale relying on the metformin dosages and length of treatment, with evident variations of both acute and long - term administration (2). Vildagliptin decreases blood glucose levels by inhibiting dipeptidyl peptidase-4 (DPP-4), an enzyme that rapidly truncates and inactivates glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) after they are produced from intestinal cells. After the second amino acid from the N-terminus, dipeptidyl peptidase-4 cleaves oligopeptides. Its inhibition increases the quantity of active circulating incretin hormones by extending the half-life of GLP-1 and GIP (3). The duration of vildagliptin's DPP-4 inhibition is dose-dependent (4). Vildagliptin reduces fasting and postprandial glucose levels, as well as HbA1c levels. It stimulates glucose-dependent insulin release and improves alpha- and beta-cell glucose sensitivity. Fasting and postprandial glucose levels, as well as postprandial cholesterol and lipoprotein metabolism, are lowered (5, 6).

According to Gregor Gerak (2020) galvanic skin response (GSR) is another name for electrodermal activity (EDA), which is the electrical conductivity of the skin caused by ecrine sweat glands and nearby epidermal and dermal changes. It is also known as skin conductance level (SCL). When an individual's arousal level fluctuates, the EDA varies). Also, it reflects to the sympathetic activity. It is capable of recording with electrodes put on specific parts of the skin's surface (7).

Previous research has shown that EDA can be utilized to monitor the connection of autonomic nerves in diabetes and associated consequences (8,9). Furthermore, GSR has been reported to be reduced in male patients with type 2 diabetes (10). They did a preliminary investigation on neuropathy and electrodermal activity in diabetic patients and discovered that diabetic patients' skin conductance response (SCR) was less than those of the healthy control group (11).

This study was conducted to assess the impact of metformin and vildagliptin on EDA in streptozocin-induced diabetic rats.

2. GENERAL KNOWLEDGE

2.1. Diabetes mellitus

Diabetes is among the oldest human diseases. It was first mentioned 3000 years ago in Egyptian literature (12). In 1936, the distinction between type 1 and type 2 diabetes mellitus was established (13). For the first time in 1988, type 2 diabetes was recognized as a component of the metabolic syndrome (14). Diabetes has a variety of causes and etiologies, however, problems in insulin production or resistance, or perhaps both, are frequently present at some point throughout the disease's course. Type 1 diabetes is caused by an immunological response or is idiopathic, and it is insulin dependent. Insulin resistance and relative insulin insufficiency create type 2 diabetes, which is insulin independent. Type 2 diabetes is also the most frequent type of diabetes worldwide (15). Type 2 diabetes can be caused by the interplay of genetic, environmental, and behavioral risk factors (16,17). Diabetes may also be linked to pregnancy, genetic disorders, a variety of conditions, and certain medications (18).

2.1.1 Signs and symptoms

Since diabetes is a chronic condition, many people disregard its signs and symptoms. People may overlook this as a serious problem since, unlike most other illnesses, hyperglycemia symptoms may not present immediately. People aren't aware that damage might happen years until signs appear. This is unfortunate, because early detection of symptoms can help in the management of the disease and the prevention of vascular complications. Most common diabetes symptoms are polyuria, polydipsia, and polyphagia. Only type 1 or type 2 diabetes that has been undiagnosed for a long period causes significant weight loss. Diabetes undetected is also characterized by exhaustion, agitation, and physical pain. Mild symptoms or those that appear gradually may go overlooked at times. Likewise, its side effects are unexplained weight loss, regular fatigue, burnout, pain, numbness in the feet, delayed wound healing, recurrent infections in genital zones, urinary tract, skin, and oral cavity, reduced sight, impotence and Acanthosis nigricans is a condition in which glossy black areas appear on the neck, armpits, and groin, indicating insulin resistance (19).

2.1.2. Complications

Diabetes complications are prevalent among type 1 and type 2 diabetes patients, and they can result in significant morbidity and mortality. Diabetes' chronic implications are loosely categorized as microvascular and macrovascular, with the former being more prevalent than the latter (20). Microvascular problems include neuropathy, nephropathy, and retinopathy, whereas macrovascular complications include cardiovascular disease, stroke, and peripheral artery disease. Diabetic foot syndrome is characterized by the presence of a foot ulcer in conjunction with neuropathy, peripheral artery disease, and infection, and it is the main cause of lower limb amputation (21). Lastly, there are additional diabetic complications not addressed by the first two groups, such as dental disease, poor resistance to infections, and delivery issues in women with gestational diabetes (20).

2.1.3. Causes

2.1.3.1. Causes of type 1 diabetes mellitus

The immune system attacks the pancreas in type 1 diabetes, which is an autoimmune disorder. The following autoantibodies have been found in persons with type 1 diabetes, and measuring antibody production against some of them has become routine:

I. Autoantibodies to the cytoplasm of islet cells (ICAs).
II. Autoantibodies against insulinoma-2 (IA-2As).
III. Insulin autoantibodies (IAAs) are antibodies to insulin (more normal in youngsters than grown-ups).

The stronger the autoimmune attack, the more antibodies there are (22). Type 1 diabetes, often known as adolescent diabetes, is more common in adolescents; nevertheless, type 1 diabetes can progress in adults. Because the immune system has damaged the pancreatic beta cells that produce insulin in type 1 diabetes, the body is unable to produce insulin or adequate insulin (23).

It is not true that if you consume too much sugar, you will get diabetes. An individual with type 1 diabetes does not develop the disease because of excessive sugar consumption. This

is due to the immune system attacking insulin-producing beta cells, causing them to malfunction (24).

Monogenic diabetes is an uncommon kind of diabetes caused by single-gene mutations or alterations. The two most frequent kinds of monogenic diabetes are Neonatal Diabetes Mellitus (NDM) and Maturity-Onset Diabetes of the Young (MODY) (25). Diabetes that develops before the age of six months is nearly always NDM rather than autoimmune type 1 diabetes mellitus (T1DM). Maturity-Onset Diabetes of the Young is a cluster of autosomal dominants acquired disorders defined by early-onset moderate hyperglycemia. Rather than insulin resistance, it is caused by beta-cell dysfunction. Mutations in at least eight characteristics have been related to Maturity-Onset Diabetes of the Young. In addition to the typical youngsters with early-onset T1DM, there is a more mature population with a slower-onset disease. They may present in their forties with evident Type 2 Diabetes Mellitus (T2DM) yet show signs of autoimmunity as indicated by anti-glutamic acid decarboxylase (GAD neutralizer) estimates and eventually become insulin dependent. Adult Latent Autoimmune Diabetes (LADA) is the medical term for this condition (22).

2.1.3.2. Causes of type 2 diabetes mellitus

Type 2 diabetes, which accounts for approximately 85-95 percent of all diabetes diagnoses, has an inert, asymptomatic stage that typically lasts several years (19). Type 2 DM is a complicated metabolic and endocrine illness caused by the interaction of hereditary and environmental factors, which results in varying levels of alteration in insulin usefulness on peripheral tissues as well as in pancreatic cells. Hidden diseases, such as excess weight and obesity are the primary factors that support the improvement of T2DM (26, 27).

When muscle, fat, and liver cells do not efficiently respond to insulin and cannot utilize blood sugar as energy, the pancreas produces more insulin to compensate for the increased blood sugar levels and to break the islet cells' resistance. Blood sugar levels rise as a result of this phenomena. Insulin resistance is the medical term for this disorder (IR) (28).

Type 2 diabetes, sometimes known as adult diabetes, can affect individuals of any age, including children. It is significantly more prevalent among people in their forties and fifties. People who are overweight or inactive are also at risk of developing T2DM. Typically, insulin resistance is the initial stage. More insulin is necessary to allow glucose into the cells. In response to the increasing demand, the pancreas produces more insulin than before. In time,

when blood glucose levels rise, such as after meals, the pancreas does not produce enough when blood glucose levels rise, such as after meals, the pancreas does not produce enough insulin. Type 2 Diabetes Mellitus develops when the pancreas produces insufficient insulin. (23).

2.1.3.3. Type 3 diabetes mellitus

Type 3 diabetes mellitus (T3DM) is a form of diabetes that involves the brain. In recent years, it is reported that T3DM and dementia are almost certainly linked.

Diabetes individuals are at an increased risk of getting Alzheimer's disease. When diabetes is identified at a younger age, it has a greater impact on dementia patients who also have a hereditary propensity. Many hypotheses were proposed to describe the relation between diabetes and dementia, ranging from the effect of acute hyperglycemia (which might also impact working memory and attention) to the effects of chronic hyperglycemia (which might also cause and disrupt macrovascular disease, which is more typically associated with vascular dementia) (29,30). Chronic hyperglycemia can indeed result in increased oxidative stress, mitochondrial dysfunction, and the production of new glycation end products (30). Hyperinsulinemia and insulin resistance are linked to Alzheimer's disease because they cause neuron death and promote the formation of extracellular amyloid plaques (29, 30, 31). Excess amyloid may be removed under normal conditions by the lipoprotein receptor-related protein (which decreases if insulin resistance exists) or by a degradation cycle involving the insulin-degrading enzyme (IDE). However, it has been discovered that amylodine buildup raises the incidence of Alzheimer's disease in diabetics. Insulin transport via the blood-brain barrier is reduced in people with persistent peripheral hyperinsulinemia. This is significant because insulin, among other things, increases learning and long-term memory, activates the acetylcholinesterase enzyme responsible for acetylcholine, and reduces phosphorylation of tau proteins in the brain. insulin-degrading enzyme are activated by glucose, and when appealing insulin levels fall, IDE activation may be reduced, causing in more detrimental amyloid deposition (30). As a result, diabetes 3 could be defined as a condition in which hyperinsulinemia caused by insulin resistance produces a decline in cerebral insulin and insufficient IDE control. Amyloid would accumulate as a result of, among other things, slower IDE degradation (30, 32).

2.1.3.4. Other types of diabetes

Gestational diabetes mellitus (GDM) is a kind of diabetes that occurs in women who get the disease while pregnant. Symptoms frequently subside throughout the postpartum period (33). The majority of women who get GDM have it during their second or third trimester of pregnancy (34).

Maturity-onset diabetes of the young (MODY) is the different types of inherited diabetes that usually develop in adolescence or early adulthood. And it is a kind of monogenic diabetes caused by genetic abnormalities in pancreatic embryogenesis, beta-cell activity, or glucose detection. In addition, Latent autoimmune diabetes in adults (LADA) is similar to autoimmune type 1 diabetes. It affects 10% of diabetic patients. It is distinguished by the presence of glutamic acid decarboxylase antibodies (GADA) at the moment of diagnosis, it is dimorphic identical from Type 2 diabetes, but as time passes, it becomes more like Type 1 diabetes (34).

2.2. Diagnosis

Diabetes is diagnosed when blood glucose levels reach 200 mg/dl or higher and signs of hyperglycemia are present. It can also be diagnosed in asymptomatic persons if fasting plasma glucose (FPG) is 126 mg/dl or if plasma glucose is 200 mg/dl in the second hour of the Oral Glucose Tolerance Test (OGTT) or HbA1c is 6.5 percent(35). There is no agreement on which glycemic test is preferable in diagnosing patients at high risk of diabetes. Oral Glucose Tolerance Test is more sensitive in many populations, while FPG and HbA1c are more practical. The HbA1c criterion for diagnosing impaired glucose tolerance is defined as information from the 2005-2006 NHANES-National Health and Nutrition Review survey. The A1c threshold of 5.7 percent was found to have the best sensitivity (39%) and specificity (91%) in diagnosing cases with impaired fasting glucose(35).

There are a few advantages of using HbA1C to diagnose diabetes. It is more convenient than fasting plasma glucose or 2h plasma glucose (2hPG) in a 75 g oral glucose tolerance test OGTT since it may be calculated at any time of day. The HbA1c test replicates the average plasma glucose (PG) during the past two to three months, also eliminates the problem of daily variability in glucose tests (36). It must be calculated using a recognized metric that has been

standardized in accordance with the International Glycohemoglobin Standardization Program-Diabetes Control and Complications. In persons with hemoglobinopathies, iron insufficiency, hemolytic anemias, and severe hepatic and renal illness, HbA1c might be deceptive. Furthermore, HbA1c values of other ethnicities are up to 0.4 percent more than those of Caucasian patients at comparable values of glycemia. Clinical judgement is utilized to select the appropriate test for diabetes diagnosis. Each analytic test has advantages and disadvantages. If a single lab test result falls into the diabetic range, it is considered diabetes but there is no clinical hyperglycemia, a follow-up corroborative laboratory test (FPG, A1C, 2hPG in a 75 g OGTT) must be conducted on another day (37).

2.3 Management

2.3.1 Lifestyle

Diabetes can be avoided by altering the lifestyle. Diabetes patients' lack of understanding and hopelessness are influenced by the disease's progression and the emergence of issues that are frequently avoidable (38). Almost all DM patients are treated in clinics, where they are expected to closely comply to the medications and advice supplied by the clinic to achieve optimal glycemic control while also avoiding confusions and inabilities (39). Based on information from the American Diabetes Association (ADA), lifestyle management is an essential part of diabetes control and care because it contains six major essential topics: Dietary treatment, physical exercise, stop smoking counseling, and psychiatric care are all part of diabetic self-management education (DSME) (40). In any case, for the approaches to be applied at the population level, programs that are both socially and economically worthwhile and practicable must be devised, which are now lacking in a large percentage of developed and agricultural nations. Early detection and administration of suitable treatment techniques produce the desired glycemic outcomes while avoiding vascular complications (41). The programs are primarily focused on the development of individuals' knowledge, abilities, and capacities required for diabetes self-management and self-care, which encourages the support skills and behaviors needed to continue with management from the initial diagnosis throughout one's lifetime (42).

2.3.2 Medications

2.3.2.1 Oral hypoglycemic drugs

The most effective management with DM is the change of the lifestyle. Also, pharmacologic agents must be combined in the struggle with DM. Particularly in T2DM patients should encourage for these solution not to progress the beta cell destruction. Some of the oral hypoglysemic agents were given below:

A. Sulphonylureas: They reduce blood glucose levels by boosting insulin synthesis in pancreatic cells. It is very well absorbed after it is taken, and its half-life and duration of action can be modified. They include first-generation medications such as chlorpropamide (Diabinese), tolbutamide (Orinase), tolazamide (Tolinase), and acetohexamide (Dymelor), as well as second-generation drugs such as glyburide (DiaBeta, Micronase, PresTab, or Glynase) and glipizide (DiaBeta, Micronase, PresTab (Glucotrol or Glucotrol XL) (43), and third-generation agents like (Amaryl) (44). All of them produce mild hypoglycemia, but severe hypoglycemia is uncommon.

B. Biguanides: Biguanides are a class of medications, with metformin, one of them, being frequently given at the moment. Metformin has been used for decades to treat diabetes mellitus (DM), and its absorption profile is well-established. Type 1 diabetes (T1D), type 2 diabetes (T2D), and gestational diabetes (GD) are the three primary, generally defined, and frequently overlapping kinds of diabetes (45,46). DM is characterized by impaired glucose management and haemostasis, and inflammation is the pathogenic disruption that underpins all three kinds of DM. Glucose is digested and absorbed from foods containing tri, di, and mono polysaccharides, and glucose is released and absorbed into the circulation via the digestive system after digestion of the food. Excess glucose is then either stored in the liver, converted to fat, or stored in other bodily tissues (47,48).

C. Alpha-Glucosidase Inhibitors (α-Gis): α-glucosidase inhibitors, including such Acarbose and Miglitol, have been shown to be effective as monotherapy or in combination with sulfonylureas in the treatment of type II diabetes. These experts lower post-prandial glucose levels by blocking complicated carbohydrate digestion and delaying monosaccharide absorption from the gastrointestinal system. They postpone and lower postprandial blood glucose peaks by inhibiting the action of glucosidase, an enzyme required for carbohydrate digestion in the

intestine. In the colon, undigested sugar is transported and transformed into short-chain fatty acids, methane, carbon dioxide, and hydrogen (49).

2.4 Surgery

Microvascular problems such as diabetic retinopathy, neuropathy, and nephropathy are connected to chronic hyperglycemia. As a result, diabetes causes to vision loss, renal failure, and lower limb amputation (54). The most prevalent consequence of type 2 diabetes is diabetic nephropathy, resulting in a considerably diminished quality of life for those who suffer from it. It also costs a lot of money to the healthcare system, as indicated by the high cost of dialysis, among other things (55). Diabetes medicine, on the most basic level, can assure a long period of safe and effective blood glucose control. However, randomized controlled medical trials showing that surgery (along with increased healthcare treatment) treatment was indeed clearly superior to medication therapy have sparked a debate over whether metabolic surgery is a better strategy for treating T2DM, and if it can help reduce disease development and complications associated with diabetes. Surgery techniques are offered as information in the guidelines for the management of obesity through a step-by-step approach to therapy. In 2011, the International Diabetes Federation declared that metabolic surgery is an appropriate type 2 diabetes management method for persons with obesity-related type 2 diabetes who have yet to achieve their treatment targets (HbA1c 7.5%) using conservative measures (56).

2.5 Oral Antidiabetics Used in this Research

While metformin belongs to the biguanide class of drugs, vildagliptin belongs to the islet enhancer DPP4-I class of drugs.

2.5.1 Metformin

Metformin has become one of the most extensively used medications in the treatment of type 2 diabetes mellitus, with dosages ranging from 500 to 2500 mg/day. It was first approved in the United Kingdom in 1958 and the United States in 1995 (57).

Metformin is the only anti-diabetics, that approved by ADA as prediabetic agent (58). It is an antihyperglycemic drug that improves glucose tolerance in T2DM patients by lowering both baseline and postprandial plasma glucose. Other oral antihyperglycemic medications have

different pharmacologic modes of action. Unlike sulfonylureas, metformin does not cause hypoglycemia in T2DM patients or healthy persons, and it also does not cause hyperinsulinemia. Metformin has minimal effect on insulin production, however it can lower rising insulin sensitivity and the daylong plasma insulin response (59).

2.5.1.1 Mechanism of action

Metformin works in a different way to lower blood glucose levels. It decreases both the baseline and postprandial levels of plasma glucose, and its primary effect is to suppress gluconeogenesis in the liver while enhancing glucose intake to the muscles. It also boosts peripheral glucose take-up and utilization, which lowers intestinal glucose absorption and increases insulin sensitivity. Nonetheless, there are also lipid-lowering, anti-aging, and anti-inflammatory properties (60). Metformin may also improve peripheral glucose utilization whereas potentially lowering meal consumption and intestinal glucose absorption. Also, it can be use in the gestational diabetes as an alternative to insulin (58). Metformin does not produce hypoglycemia or hyperinsulinemia since it does not promote endogenous insulin secretion, which are common side effects of other diabetes medicines (61).

In a study where it was reported that the mixture of metformin and a DPP4 inhibitor, such as vildagliptin could be used for the treatment of T2DM, other effects were observed besides lowering plasma glucose (62). In addition to the synergistic effects of the medications, the intriguing possibility of this combination may be to obtain glucose reduction with positive impacts on ß-cell function, without promoting obesity or raising the risk of hypoglycemia, and without encouraging overweight or increasing the risk of hypoglycemia, and without intensifying metformin's glucose-dependent insulinotropic (GI) adverse effects. Ahren et al (63) were studied the combination of vildagliptin and metformin for 12-week phase II study, then in a 40-week double-blind, placebo-controlled extension study. They reported that when combined with metformin monotherapy, LAF237 significantly reduces glycemic control worsening in type 2 diabetic patients..

2.5.2 Vildagliptin

Despite having a half-life of two hours, 50 nM of vildagliptin is detectable in blood for at least ten hours following a 50 mg dose (64). Vildagliptin (Galvus®) was permitted in the

European Union at a dose of 50 mg. Vildagliptin is commonly used alone or in combination with other oral hypoglycemic medications. It can be used with metformin or thiazolidinedione, and 50 mg/day in conjunction with a sulfonylurea (SU). Vildagliptin/metformin fixed-dose combinations of 50 mg/850 mg and 50 mg/1000 mg have been approved for applicable dose. The mixture of vildagliptin and metformin improves glycosylated hemoglobin, fasting blood sugar, and random blood sugar considerably. An anti-diabetic's principal role is to reduce blood sugar levels (65).

Several clinical trials have indicated that vildagliptin and metformin combination treatment outperforms high-dose metformin alone in terms of efficacy and safety, with good gastrointestinal tolerability (66). A single-pill combination of vildagliptin/metformin has been approved for the treatment of persons with T2DM who have not been effectively treated with metformin alone in the European Union and many other countries around the world.

2.5.2.1. Mechanism of action

Vildagliptin is an orally administered incretin enhancer that functions by blocking DPP4-I., a protein that is responsible for the rapid deactivation of GLP-1 in the body (67). This event enhances the glucose-dependent activity of pancreatic islet β and α-cells in Type 2 diabetes. Vildagliptin boosts insulin production in both the postprandial and fasting phases by increasing β-cell sensitivity to glucose. Increased -cell activity is defined by the restoration of proper glucose-related glucagon control, which results in decreased endogenous glucose synthesis during both postprandial and fasting periods. The basic loss of -cell function in DM2 is stopped by long-term vildagliptin therapy.

Vildagliptin plus metformin may have a synergistic impact in dramatically raised GLP-1 levels, which may contribute to the long-term enhancements in -cell function found in type 2 diabetes patients who receive vildagliptin in addition to ongoing metformin treatment (68).

Extrapancreatic effects of vildagliptin have been linked to elevated peripheral insulin levels and postprandial triglyceride-rich lipoprotein metabolism. (69).

2.6. Diabetic Animal Models:

2.6.1 Rodent model of type 1 diabetes

2.6.1.1 Type 1 diabetes mellitus spontaneous autoimmune models

Japan created the Non-Obese Diabetic (NOD) mouse strain in 1974. These mice, as well as AKITA mice, bi-breeding (BB) rodents, LEW.1AR1 rodents, and others, are famous for their ability to spontaneously induce autoimmune diabetes (70). Diabetes' uncontrolled progression is frequently linked to a genetic mutation that affects T-lymphocyte selection and impairs auto tolerance regulating systems. Diabetic mice with immunological features similar to insulin dependent T1DM in humans have been frequently employed as models of spontaneous autoimmune T1DM for the past 25 years (71).

2.6.1.2 Diabetes caused by a genetic mutation

The AKITA mice were created in Japan by crossing C57BL/6NSIc mice with a random mutation in the ins 2 gene, which impairs normal proinsulin synthesis and causes endoplasmic reticulum stress. At the age of three months, mice with this mutation develop insulin-dependent diabetes, which is characterized by hyperglycemia, hypoglycemia, polyuria, and polydipsia. Because this model lacks β-cell mass, it is a feasible alternative to the STZ-induced model employed in transplantation investigations. AKITA mice are also employed in studies of macrovascular diseases and nerve injury as a T1DM model. This paradigm has been frequently used to look for ER-stress suppressors in pancreatic islet cells.

2.6.1.3 Diabetes mellitus caused by a chemical

Drug-induced T1DM is associated with the elimination of numerous endogenous β-cells, resulting in decreased endogenous insulin production, hyperglycemia, and weight loss. T1DM produced by chemicals (STZ, alloxan, dithizone) is suitable for testing medications or therapy strategies that lower blood glucose levels without relying on β-cells, such as innovative insulin designs. This method is also suitable for evaluating the efficacy of transplantation therapy, which lowers blood glucose levels. It is assumed to be required in transplantation to inhibit spontaneous regeneration of β-cells, as well as to perform a histological evaluation of the endogenous pancreas for detecting insulin-positive cells and quantifying insulin levels.

Regardless, the presence of β-cells has been demonstrated to be unrelated to their function in a chemically induced model of T1DM (72, 73).

2.6.2 Rodent model of type 2 diabetes

The Zucker diabetic fatty (ZDF) rat, the Otsuka Long Evans Tokushima fatty (OLETF) rat, (STZ, alloxan, dithizone), and the db/db mouse are the most extensively used animal models of T2DM. They all display obesity-associated insulin sensitivity and decreased cell, culminating in diabetes (74). While these animal studies have greatly aided our understanding of the pathophysiology, treatment, and consequences of T2DM, the essential pathways driving diabetes pathogenesis in these models do not correlate to what occurs in the vast majority of human T2DM patients. These etiological differences will almost certainly hinder effective translational research. Even though rats and mice are the most commonly used models for type 2 diabetes research, other rodents have also been found as viable models. These include the desert gerbil and, more recently, the Nile grass rat, both of which, when housed in captivity, are prone to obesity (70).

2.7. Electrodermal Activity

Electrodermal activity (EDA) is defined as the total of variations in electrodermal resistance and electrodermal potential. Electrodermal activity, also known as galvanic skin response (GSR), galvanic skin potential (GSP), sympathetic skin response (SSR), peripheral autonomic surface potential, or skin conductance, is the electrical resistance measured between two electrodes while a very small current is steadily passed between them (SC). EDAs are reactions that occur when the polysynaptic reflex arc is activated. The cholinergic eccrine sweat glands are the reflex arc's effectors (75). The electrodes are typically spaced about an inch apart, and the resistance recorded varies according to the subject's emotional state. It is the voltage obtained between two electrodes in the absence of any externally applied current. The electrodes are connected to a voltage amplifier to measure it. This voltage is also influenced by the subject's psychological condition. It is measured by miscosiemens or microohm (76).

Although the electrical activity doesn't really reveal which emotion was triggered, EDA demonstrates a relationship between emotional arousal and sympathetic activity. Sweat and blood flow are affected by autonomic sympathetic changes, which impact GSR. Sweat gland

density varies in the body, most of them in the arms and legs (200–600 sweat glands per cm^2). In reaction to external and internal stimuli, the conductivity of the skin and muscular tissue can vary by many microsiemens. A correctly calibrated equipment can record these changes and display minor deviations (76).

The electrical output of electrodermal activity is a depiction of sweat gland sensory activity. Since 1879, EDA has been used in psychophysiological (including subjective emotional stress) studies; however, experts have only recently begun using EDA for pathophysiological applications such as the evaluation of epilepsy, fatigue, sleepiness, pain, depression, exercise recovery, neuropathies, and other conditions. The adoption of new EDA systems and platforms has accelerated the development of novel signal processing methods, resulting in a growing pool of specific mathematical EDA measurements. For a long time, arousal was measured by computing the mean of EDA esteems over time. Scientists quickly understood that EDA contains information not only in the steady shifts (tonic segment) shown by the mean value, but also in the fast or phasic oscillations of the signal. With various analysts from science, health, sociology, and other fields recently working with EDA, the time has come to integrate and evaluate current achievements and provide an updated and synthesized framework for researchers interested in adopting EDA into their research (77). EDA measuring instruments of various types are currently used in a wide range of industries, including research, healthcare, education, and the entertainment industry. Regardless, despite their ubiquitous application, the nature of their estimating function (accuracy) is rarely discussed or investigated.

Autonomic arousal is increased transiently following a stimulus or event. Deconvolution accounts for the time delay between the commencement of sweat production and the rise in skin conductance seen, as well as overlapping sweat secretion responses. These SCRs have been used in a range of psychological studies, including classical conditioning, fear conditioning, emotional reactivity, attention, and cognitive effort, since they appear to represent the intensity of the sympathetic nerve response. Stimulus-evoked SCRs have been used as indication of task engagement and intensity in the applied experimental literature. It might be a sign of a lack of emotional or behavioral self-control. Nonspecific SCRs have also been linked to habituation, orienting response, negative emotional arousal, subjective arousal, and anticipatory stress, although the evidence is mixed and needs to be confirmed (78).

2.7.1 Units of EDA

Electrodermal activity measures the changes of eccrine sweat secretions, located in the extremities, particularly the fingertips, palms, and soles. Electrical conductivity of the skin rises in stimulated states due to increased sweat gland activity caused by sympathetic system stimulation. There are two parts to this reaction: skin conductivity level (tonic SCL) and skin conductivity response (SCR or Phasic SCR). Tonic skin conductivity level is the conductive activity of the skin in the absence of any external stimulation, whereas phasic SCR measures relate to short-term events that occur as a response to environmental stimuli such as sensory stimuli or cognitive processes (79). Electrodermal reactions (EDRs) can arise spontaneously in the absence of an exogenous trigger. These EDRs are known as "nonspecific SCR" (NS.EDR, NS.SCR) (80). One of the other tonic metrics is the frequency of the non-specific skin conductance response (NS.SCR freq), The NS.SCR freq is an additional measure in a

specified time (such as 30 to 60 seconds), and plays an important role in research on arousal, stress, and emotions. Tonic EDA may be useful in research on overall alertness and arousal (78). The phasic EDA can be used to investigate multidimensional attentional processes (81).

2.8 Electrodermal activity and diabetes mellitus

Diabetes mellitus is a metabolic condition that causes changes in several metabolic pathways, which affects the neurological system. The autonomic nerve system is also impacted by neuropathy in long-term diabetes mellitus. According to Mohanraj (2016) (82), sweat glands innervated by the autonomic nervous system would be impacted in individuals with type 2 diabetes mellitus who also had neuropathy symptoms. The sympathetic skin response (SSR) and galvanic skin resistance (GSR) in males with type 2 diabetes mellitus and controls were compared in this study. Thirty guys between the ages of 45 and 55 who had diabetes mellitus and a history of neuropathic symptoms were used as subjects, whereas thirty men in the same age range who had no history of diabetes mellitus or neuropathy were used as controls. In the noise and light reduced research laboratory, SSR and GSR were recorded utilizing Recorders and Medicare Systems 4 channel polygraph. All the recordings were place between 10 and 12 p.m. at room temperature. Deep inspiration was used to assess SSR, while supine and standing responses were used to quantify GSR. The percentage of reduction in galvanic skin resistance

was compared to the latency and amplitude of the sympathetic skin response. The SSR and GSR responses are greatly diminished in diabetics, and this study suggests that they can be utilized as a diagnostic tool for diabetic autonomic neuropathy.

In another study (83), it was investigated the connection between hyperglycemia and electrodermal activity (EDA) in diabetic rodents induced with streptozotocin (STZ). EDA was recorded 1h before the injection of (STZ) into the rats (beginning 0.day) 1 st day (one day after the injection) and 10^{th} day, the researchers examined the progression of neurophysiological changes in the peripheral sensory system in diabetic rats. The findings indicate that hyperglycemia influenced the peripheral nervous system and that blood glucose levels affect EDA boundaries. According to the results hyperglycemia affects the peripheral nerve framework, and blood glucose levels modify EDA characteristics, EDA is a noninvasive electrophysiological test that may aid in the early diagnosis of diabetic neuropathy.

According to Luis Bolanos (84), autonomic neuropathy may be associated with lower electrodermal activity (EDA).

Miranda (85), reported that metformin medication decreases superior vagal electrical activity while increasing sympathetic activity, resulting in an improvement in autonomic nervous system imbalance. Increased vagal activity has been shown to be a major underlying factor aftercontributing to obesity in monosodium glutamate-induced rats (MSG-rats). Furthermore, using parasympathetic neurotransmitter agonists and antagonists, it was demonstrated that metformin boosted insulin secretion regulation in MSG-rats. Also, it was reported that metformin improves glucose intolerance and hyperinsulinemia in MSG-rats, notwithstanding the difficulties of the intravenous glucose tolerance test. Rats respond to exogenous ACh and the non-selective cholinergic blocker atropine, according to this study. Many hormones, including enteric hormones and neurotransmitters, have a role in the body's regulation of blood glucose levels. Nonetheless, exogenous ACh elevates insulin levels, whereas atropine lowers insulin levels and has a clear effect on glycemia prior to glucose bolus administration. It was seen that MSG-treated rats responded better to ACh than MSG-untreated rats in this study. It's difficult to pinpoint the factors that influence insulin and glucose blood levels in the post-prandial phase. However, after a glucose infusion, catecholamines and ACh have been shown to decrease and enhance insulin production respectively in both animals and humans (86).

Oliveira and his group (87) demonstrated that chronic metformin had anti-inflammatory and antioxidant benefits, and it restored cardiac autonomic measures that are worsened in hypertension and are linked to end-organ damage and mortality, irrespective of glycaemia changes. These findings suggest that metformin might be used in novel ways in the future to treat cardiovascular disorders, including hypertension.

Vildagliptin improved glycemic control in T2DM patients who were not sufficiently managed with metformin or glimepiride. Vildagliptin is well tolerated, with just a minor risk of hypoglycemia and weight gain. This makes vildagliptin an alternative treatment option for people who have failed metformin with sulphonylurea, particularly those with a HbA1c of 8% or higher (88).

According to Trial Research Group for Diabetes Control and Complications (89), long-term diabetics show considerable autonomic dysfunction. They reported that diabetes therapy can postpone the beginning of the diabetes and make this process slower. Also, it was reported that evaluate of the sympathetic and parasympathetic functions can be used for the clinical trials for diabetics.

Sucharita et al. (90) used typical clinical autonomic tests like heart rate variability, and blood pressure variability to evaluate the autonomic nervous system function in type 2 diabetes. In this study, all participants performed a detailed clinical and autonomic symptoms questionnaire, also, anthropometric knowledge, a peripheral neural examination, and autonomic nervous system tests (heart rate and blood pressure variability) were gathered. The presence of symptoms, as well as the involvement of both autonomic nervous system components (sympathetic and parasympathetic), suggests that autonomic dysfunction has been present in these diabetics for some time.

According to Patel et al. (91), there are three major mechanisms affecting endothelial health and erectile dysfunction caused by metformin: (I) endothelial-dependent vasodilatory impairment; (II) sympathetic nerve activity elevation; and (III) atherosclerotic luminal narrowing. They further stated that these findings were linked to insulin resistance, which is linked to obesity, dyslipidemia, diabetes, and hypertension. Metformin has a beneficial effect on two of three pathways, notably endothelium-dependent vasodilation, and sympathetic nerve activity attenuation. However, no effect on hypertension control, according to the researchers.

In the literature, there are no studies concerning the effects of metformin and vildagliptin on EDA in diabetic rats. Therefore, the aim of this thesis was to evaluate the electrodermal activity and thus the changes in sympathetic activity in diabetic rats treated with vildagliptin, metformin and metformin+vildagliptin combination.

3. MATERIAL AND METHODS

3.1. Experimental Animal:

In this study, 50 male Wistar rats with 3 months aged were selected. Animals were provided by Baskent University Experimental Animal Breeding Center. They were held in regular cages with unrestricted access to water and food. They exposed to 12:12 dark and light cycle. All treatments and experiments were carried out in the Baskent University Animal Research Laboratory. The experiments were conducted with the agreement of Başkent University's Ethical Committee for Animal Experimentation (20/23).

3.2 Experimental Groups:

Before start to trial, animals held the laboratory for one week for adaptation.

The animals were divided into five groups in equally and randomly (n = 10).

- Sham group (C): Each rat was administered oral gavage of normal tap water during the trial
- Treatment groups: Firstly, 45 mg/kg IP streptozotocin was injected to 40 of rats for induce diabetes. Three days later, their blood glucose was measured and 250 mg/dl and above have accepted as diabetes. Then, divide into four groups and treated in different ways once a day for 15 days as:
    - Diabetic control group (D group): Received normal tap water by gavage.
    - Metformin group (DM group): 500 mg /kg gavage was used to deliver metformin.
    - Vildagliptin medicine (DV group): 50 mg / kg day vildagliptin was administered by oral gavage.
    - Metformin + vildagliptin group (DMV group**):** 500 mg / kg day metformin and 50 mg /kg day following that, vildagliptin was given by oral gavage.

After 15th days, EDA records were taken. Figure 3.1 shows the schedule of study.

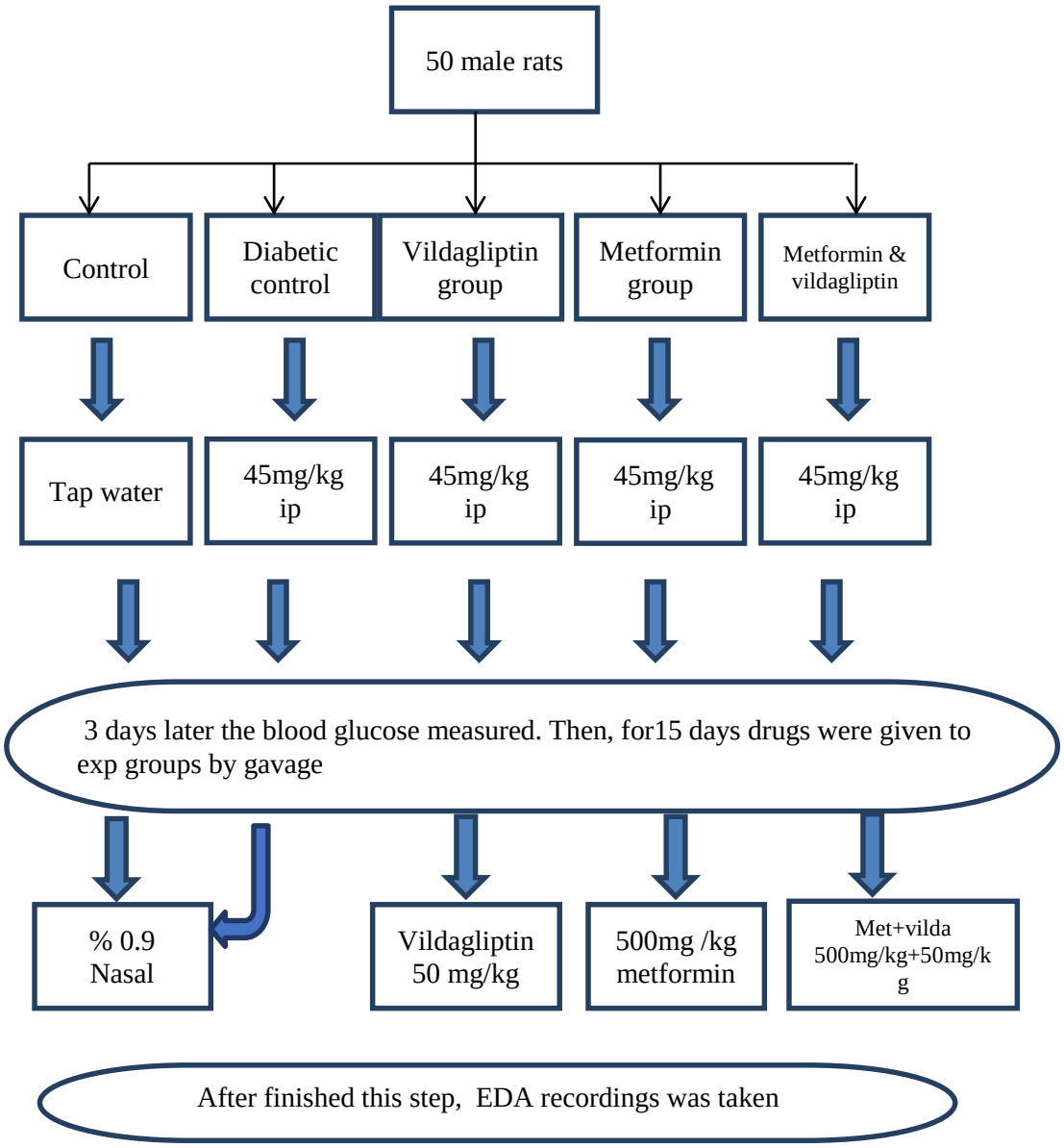

Figure 3. 1 Schedule of study

3.3. EDA Recording:

EDA was recorded following the completion of the drugs administration cycle (after two weeks). The tail and hind limbs of rats were not mounted in the immobilizer. 0.8 cm diameter

Silver/Silver Chloride (Ag/AgCl) electrodes were used to measure galvanic skin between the paw pads of both hindlimbs. GSR (Galvanic Skin Response) transducer was used to attach electrodes to the MP35 device (BIOPAC). Electrode paste was applied to the paw to improve conductivity and reduce the resistance between the skin and the electrodes (Figure 3.2). After the electrodes were implanted, the animal was able to relax in a dark and quiet space for 5 minutes. Then, without any sound stimulus, a 2-minute tonic record was taken (tonic SCL). Then, phasic record was taken after the tonic record for 10-minutes, in which 15 sound stimuli were randomly provided at intervals ranging from 30-80 seconds without pausing (phasic SCR). For sound stimuli that trigger electrodermal responses, a stimulus generator (sound amplifier) was used. The stimulus generated sounds with a frequency of 1000Hz and a volume of 90 dB. Sound was played to the animals with two speakers installed in the experimental room (Figure 3.3).

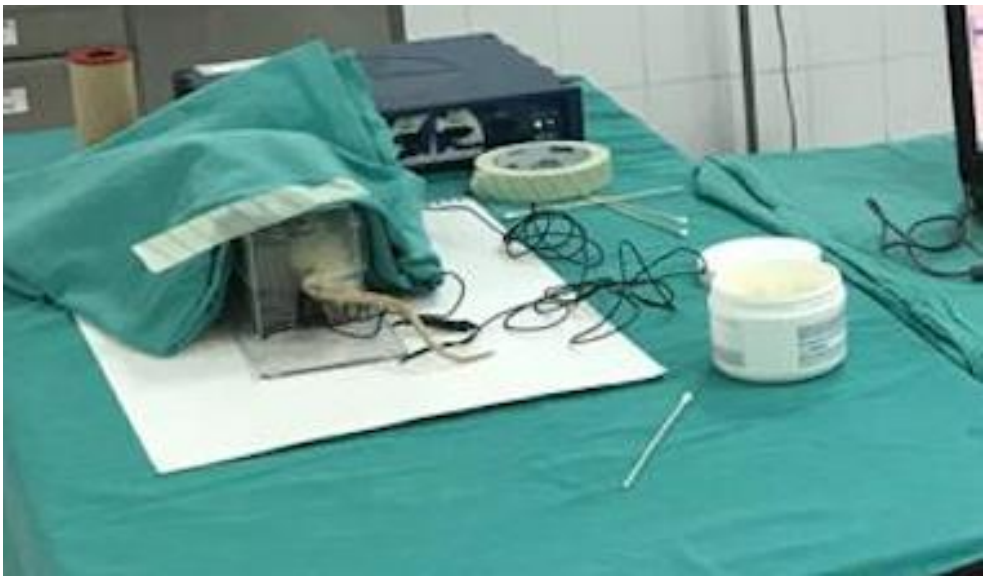

Figure 3. 2 The immobilizer, electrode paste and electrodermal electrodes of EDA

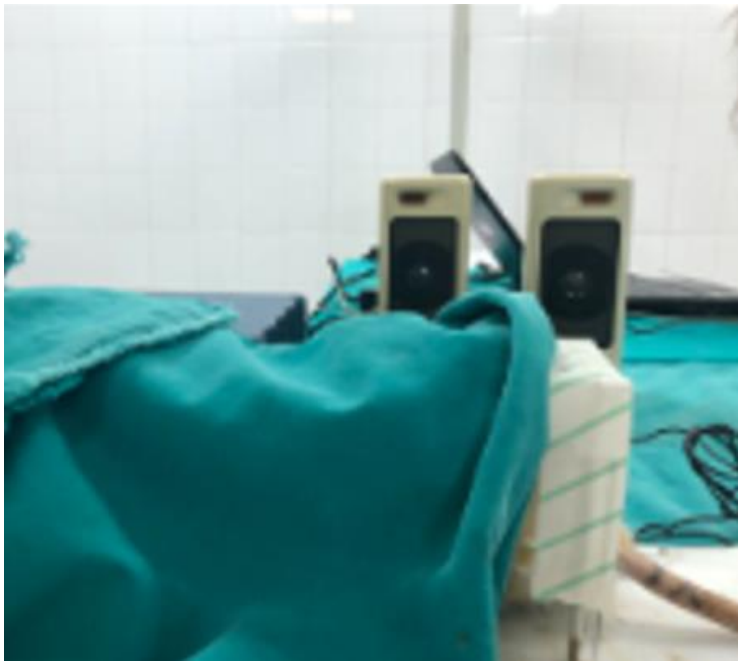

Figure 3. 3 The animals were taken by the sound stimuli by 2 speakers

Skin conductivity signals captured with the MP35 device were converted to digital signals, which were then processed in a computer environment for data analysis. (Figure 3.4).

Figure 3. 4 Recordings were taken by MP35 record device

The skin conductivity unit and the MP35 software system were calibrated just before the recordings. The calibration buttons on the conductivity unit were pressed for this reason, and the values to be read were inserted into the program in the appropriate positions. After the calibration, firstly SCL, then SCR were recorded. An increase in SCR corresponds to a rise in sympathetic behavior and anxiety. Experiment was carried out in two stages:

Tonic EDA: It was registered for the first 2 minutes (120 seconds) without any stimulus, then a cursor was used to pick it and the mean electrodermal response was measured.

Phasic EDA: It was made immediately after tonic EDA recording for 10 minutes with auditory stimulus.

Also, NS-SCR freq is counted as a number of EDRs in a 2 min window in tonic phase.

The animal was taken out of the immobilizer and returned to its cage after the recording process was finished. After each animal, the immobilizer and electrodes were washed with alcohol and clean water.

3.4. Data Analysis of EDA:

Skin conductance measurements are measured in micromho (mho). The records were analyzed using the Biopac software. The mean of SCL was determined after selecting waves with the cursor (Figure 3.5 and Figure 3.6).

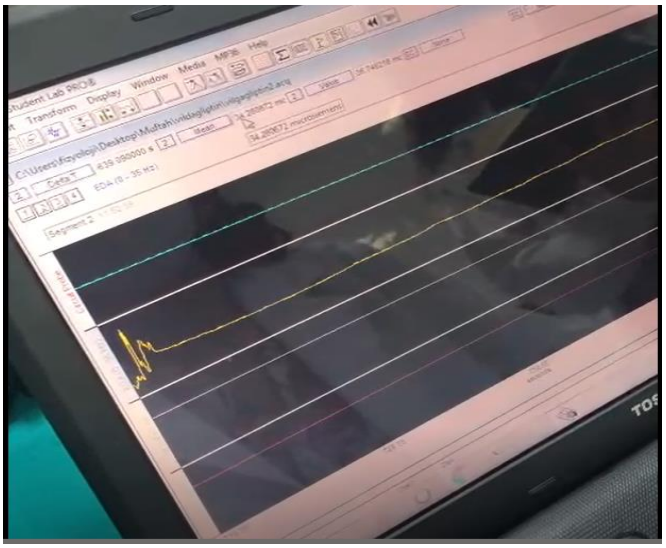

Figure 3. 5 Analyze of electrodermal recordings

3.5. Statistical Evaluation:

The one-way analysis variance (ANOVA) test and the Post-hoc Tukey test were used in Graphpad Prism 8 (La Jolla, California) for statistical analysis. Results were given as mean ± SE. Significance was assessed as $p < 0.05$. Tonic SCL and phasic SCR and NS-SCR freq values evaluated by paired t test of all groups compared to each other.

4. RESULTS

4.1. Blood glucose levels of the rats

As shown in Table 4.1. Three days after STZ injection, blood glucose levels were tested, and those exceeding 250 mg/dl were deemed diabetic. All the blood glucose values of the diabetic groups (D, DM, DV, DMV) were higher than control group (*** $p<0.001$).

Table 4. 1 Blood glucose values of the streptozocin-induced diabetic rats (mg/dl)

Glucose levels of control group	**Fasting glucose levels of rats 3 days after STZ injection**			
75	700	456	600	481
80	720	700	700	700
83	750	750	720	750
92	501	259	484	435
97	585	485	530	596
77	710	582	700	730
86	700	594	533	464
81	720	432	510	564
82	463	391	268	345
98	437	286	277	385

4.2 Tonic Changes in Skin Conductance Level (SCL)

When the groups were compared, it was found that the D group had a greater SCL than the C group, as shown in Figure 4.1. There was no significant difference between both the D and DM groups, or the D and DV groups, SCL was decreased in DMV group compared to D group and only DV group. All the significance was the level of $p<0.001$.

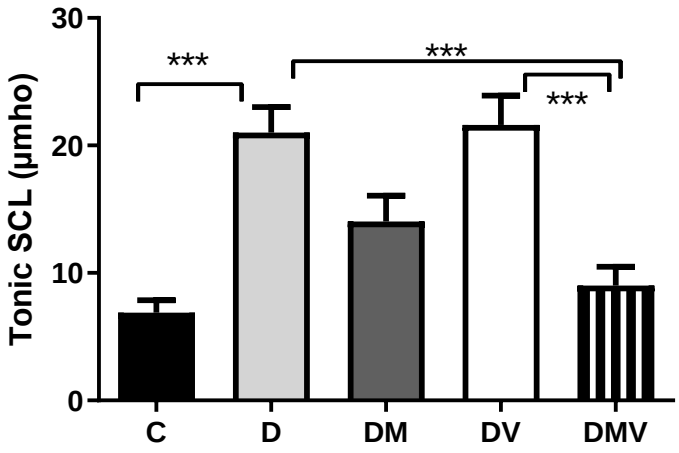

Figure 4. 1 Comparison Tonic SCL's of the groups (C:Control, D:Diabetic, DM:Metformin, DV: Vildagliptin, DMV: Metformin+ Vildagliptin)
***P < 0.001

4.3 Phasic Changes in Skin Conductance Level (SCR)

As shown in figure 4.2, phasic skin conductance response was higher in D group than C group ($p<0.001$). It decreased in DM group compared to the D group ($p<0.001$). Also, it decreased in DMV group compared to only DV group ($p<0.01$). Although SCR in DMV group was lower than the D group, statistically differences was not shown.

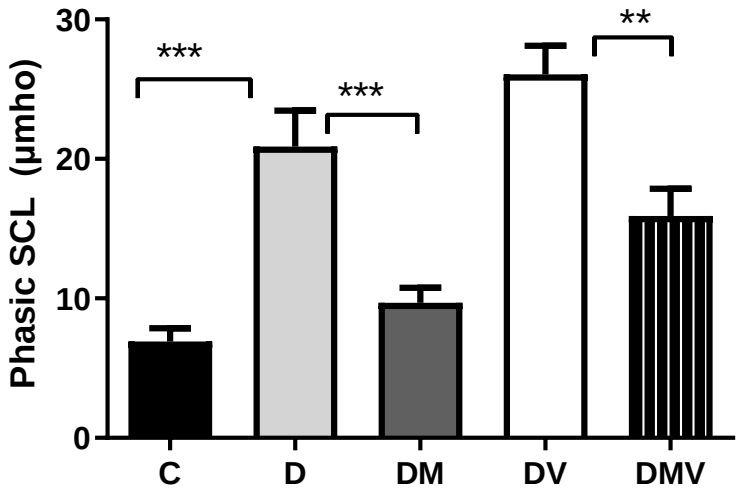

Figure 4. 2 Comparison of the Phasic SCR's of the groups (C: Control, D:Diabetic, DM: Metformin, DV: Vildagliptin, DMV: Metformin+ Vildagliptin)
P < 0.01,*P < 0.001

4.4. Frequency of the Nonspecific Skin Conductance Response (NS-SCR freq)

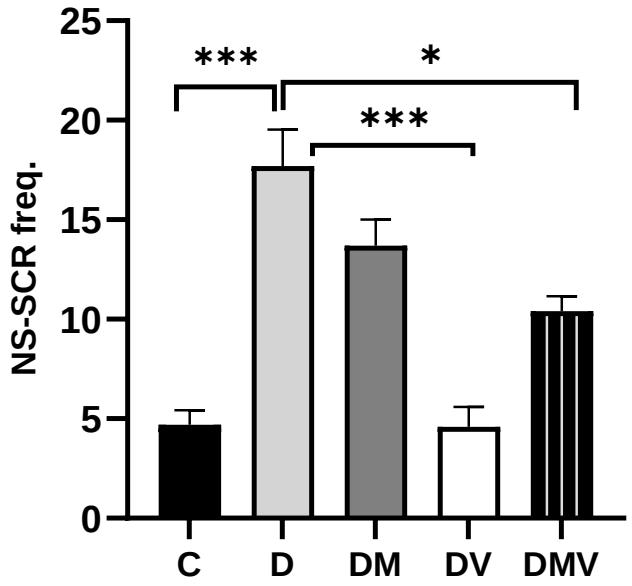

Figure 4. 3 Comparison of NS-SCR freq of the groups (C: Control, D: Diabetic, DM: Metformin, DV: Vildagliptin, DMV: Metformin+ Vildagliptin)

As shown in figure 4.3, NS-SCR freq. was higher than in diabetic control group than the control group. This value was decreased in DV and DMV groups compared to D group ($p<0.001$ and $p<0.05$ respectively).

5. DISCUSSION

The nerves of the sweat glands are sympathetic cholinergic fibers, which are unmyelinated C-type fibers that account for the majority of sweat gland activity (92,93). But limbic system (particularly amygdala and hippocampus), basal nuclei (striatum and pallidum), thalamus, and cortical temporal lobe (B6th area) are also involved in the emergence of EDA. Auditory stimuli specifically stimulate the limbic-hypothalamic pathway. Especially, palmar and plantar regions are important for the recordings of the electrodermal activity, because they have plenty of arterioles and arteriovenous anastomosis. These arterioles are innervated by sympathetic adrenergic vasoconstrictor fibers (94). Although the postganglionic pathways are different, sympathetic activity pathways of the skin involves both sudomotor and vasoconstrictor impulses. Vasoconstrictor fibers, which are mainly involved in executing thermoregulatory reflexes, are also affected by mental and emotional events. As a result, these events are the emotional stimulus that causes changes in both skin blood flow and EDA. Because sweat gland ducts run along the stratum corneum. This behavior is essential for palmar and plantar regions, that is preferred in electrodermal recordings due to high sweat gland density. It is mostly accepted that skin conductivity rises with an increase in the sweat in the duct (95).

Changes in peripheral autonomic activity are associated with emotional, cognitive, and physical activities. EDA is mostly used as a marker for attention, cognitive activity, or emotional arousal (78). It is a measure of stimuli's psychological processing properties, such as value, originality, or affective significance, as well as effortful processing (96). This approach has also been used to assess autonomic function in individuals with dysautonomia, as well as psychophysiological and cognitive problems. Defective sweating is a symptom of autonomic dysfunction in the lower limbs, which is caused by the degradation of cholinergic sympathetic unmyelinated fibers (97). While the absence of the external stimuli shows the tonic EDA (SCL); stress, emotions sensory stimuli and cognitive processes affect the phasic EDA (SCR), which one of the components of EDA. While tonic EDA increases with high arousal as a result of increased attention and motor readiness, and indicative for hippocampal information processing, phasic EDA indicates the orienting or defensive response and emerges at the end of the interaction with hippocampus and amygdala (78). It is accepted that when the activity of the eccrine sweat glands is raised because of increased sympathetic activity, both components of

EDA are increased (98). While tonic SCL recordings are accepted to be useful for measuring more generalized arousal, SCR was accepted as a marker of phasic arousal response. The frequency of the nonspecific SCR in a certain time interval gives an extra tonic EDA measurement, that emerges the substantial role in study into arousal, stress, and emotions (78).

Many physiological and cognitive markers are affected by hyperglycemia. Hyperglycemia is a symptom of diabetes mellitus (DM). One of the most prevalent consequences of diabetes is autonomic neuropathy, which can induce distinct autonomic symptoms. Diabetic polyneuropathy (DP) is a duration, and its claimed incidence varies widely, most likely due to discrepancies in clinical and electrophysiological criteria for its classification. The use of electrophysiological tests to diagnose DP is an extension of the clinical assessment. Sensory and motor nerve conduction investigations, late response recordings such as the F wave, and needle electromyography are among them (99). In peripheral neuropathies, EDA is also commonly utilized to diagnose functional impairment of unmyelinated postganglionic sudomotor sympathetic fibers (100).

Animal models of diabetes, notably rats, have been shown to exhibit neurophysiological changes. The temporal course of neurophysiological alterations in both motor and sensory nerve conduction velocity was examined in the peripheral nervous system of diabetic rats. EDA has not been employed to quantify the role of peripheral adrenergic and cholinergic sympathetic activity in diabetic neuropathy in rats or to determine the influence of hyperglycemia on them (101).

In this study, electrodermal activity was investigated in streptozocin-induced diabetic rats treated with antidiabetic drugs metformin, vildagliptin, and combination of both. Results show that both tonic and phasic EDA of the diabetic group higher than the control group. While tonic SCL and phasic SCR were reduced in the combined treatment group (DMV) compared to DV group, tonic SCL was decreasing in DMV group compared to D group and phasic SCR was decreasing in DM group compared to the D group. Although it was not seen a statistically significant in DM group compared to D group in SCL, it was seen that a reduction of the tonic SCL activity of the DM Group. The same situation was seen in SCR level in D and DMV groups. The findings showed that while diabetes increases the tonic and phasic skin conductance, treatment of metformin and metformin-vildagliptin combination can be reduced these activities.

Biessels et al. (1999) evaluated the progression of neurophysiologic alterations in the central and peripheral nervous systems in STZ-diabetic rats given a dosage of 40 mg/kg body mass dissolved in saline. Conduction velocities of the sciatic nerve, as well as auditory and visual evoked potentials, were assessed in this study. They reported that peripheral deficits appeared weeks after diabetes induction, whereas central impairments appeared months later (102). In addition, Fadli et al. (2021) found no sympathetic cutaneous response in diabetics with neuropathy (103). As seen in the literature results, our results showing an increase in sympathetic arousal in rats with STZ-induced diabetes are consistent with the results of the literature, since the cause of diabetic neuropathy is the decrease in blood flow to the palmar and plantar regions as a result of sympathetic arousal.

In contrast to our findings, Dolu et al. (2006) found that while the SCL level did not change in rats exposed to 60 mg/kg streptozocin, it was lower in diabetic rats subjected to 80 mg/kg streptozocin on both the 1st and 10th days than the control group. On the 10th day, the diabetic group's SCL and skin conductance fluctuation rate (SCFr) were considerably lower than on the first day. After 10 days of injection, both STZ dosages resulted in peripheral nerve deficits, and SCL was dramatically reduced. SCL can be a relevant an indicator for determining nerve degeneration in the early stages of diabetes, according to this finding (83). Contrary to the results of Dolu et al. (2006), the fact that tonic SCL did not decrease in 60 mg/kg STZ application in our study, but the similar results with the group exposed to 80 mg/kg of the same study suggest that the similarity may be due to a decrease in skin conductivity caused by diabetes-induced neurodegeneration, even if the dose is different. Similar with our results, Hirai et al. (2000) declared that nerve fibers of epidermis almost disappeared in insulin independent diabetic patients because of the diabetic sensory neuropathy. Also, they reported that sweat gland innervation was reduced in most tissue sections of the diabetic patients in comparison to placebo group skins (104).

The SCL is determined by a number of parameters, including epidermal structure and hydration, electrical membrane effects, and the morphology and degree of eccrine sweat gland activity, and it is a function of sympathetic sudomotor fibers. Hirai et al. (2000) investigated diabetic sensory neuropathy in non-insulin dependent diabetic patients with biopsied calf skins. They measured cutaneous nerves immunohistochemically to assess diabetic neuropathy. The

epidermis was devoid of nerve fibers, with only a few fibers visible in the dermis. In comparison to placebo group skins, sweat gland innervation was reduced in most tissue sections(104).

Metformin therapy can reduce circulating free fatty acids (FFAs) (105). Since FFAs are known to stimulate sympathetic outflow, Manzella et al. (2004) showed that metformin can decrease sympathetic activation by decreasing norepinephrine (NE) secretion. The ability of metformin to reduce serum FFAs may be due to less food consumption. Metformin has also been reported to increase lipid absorption and induce lipolytic and thermogenic processes in brown adipose tissue. The fat-burning properties of metformin may also have led to the efficient use of fatty acids, thereby reducing circulating FFA levels (106).

Chine et al. (2021) also reported that metformin significantly improved HPA axis function in diet-induced obese (DIO) rats. In the study, adult male DIO rats were fed a normal or HF diet for 7 weeks, and low-dose (60 mg/kg) or high-dose (300 mg/kg) metformin was given to drinking water from 4 weeks onwards. The function and relevance of metformin's effects on body weight and eating were examined and showed that oral metformin could effectively ameliorate HPA axis dysfunction associated with reduction of circulating free fatty acids in DIO rats, eliciting a unique effect of metformin in the treatment of obesity (105). In some other studies, metformin has been found to successfully reduce body weight and adiposity (107-112). Another reason for this is that metformin is thought to help people lose weight by increasing their energy expenditure. A recent study in HF-fed mice found that metformin treatment increased energy expenditure (113). In our study, the reason for the decrease in tonic SCL and phasic SCR levels in diabetic groups given metformin can be attributed to the decrease in sympathetic activation due to metformin reducing FFA intake.

Singh et al. (114) showed that metformin is a very effective drug for improving glycemic management, reducing peripheral nerve damage in T2DM patients, and may have antineuropathic and neuroprotective benefits unrelated to glycemic.

On the other hand, Klempfner et al. (115) observed that in combinations of vildagliptin and metformin, especially vildagliptin can minimize hypoglycemia attacks by reducing sympathetic activations. In our study, the level of skin conductivity did not change in the groups given only vildagliptin.

Bell et al. (116) reported that although metformin is one of the first drugs that comes to mind when it comes to treatment of type 2DM, its effect on reducing vitamin B12 depending on dose and time is well known. It is also known that the main symptoms of B12 deficiency are anemia and neuropathy. In this study, it is thought that the decrease in skin conductivity in the metformin group and metformin+vildagliptin group compared to the diabetic group may be due to neuropathy.

However, Yang et al. (117) reported no association between long-term metformin treatment and the degree of neuropathy in diabetic patients in a metaanalytic study. Al Omari et al. (2016) investigated the efficacy and safety of vildagliptin in combination with metformin in patients with type 2 diabetes who were not adequately controlled with metformin alone. In a 12-week prospective observational study, 1700 mg of metformin and 50 mg of vildagliptin twice daily were administered to T2DM patients with Hba1c levels of 7-10%. It has also been reported that vildagliptin in addition to metformin increases glycemic control and is more effective in lowering blood sugar compared to patients given metformin alone (118).

Studies have also proven that vildagliptin alone (119,120) as well as in combination with metformin (121-123), or with insulin and other antidiabetic agents (124-126) improves glycemic control in patients with T2DM. In addition, vildagliptin has been shown to be well tolerated, neutral in weight, and generally safe with low blood glocuse formation (122,123,127,128). Furthermore, vildagliptin, as a sulfonylurea or pioglitazone, is equally effective at lowering Hba1c and causes less hypoglycemia (128).

Although there are many studies (129,130) about the neuroprotective effect of vildagliptin, the fact that the vildagliptin dose used in our study was not different from the diabetic group in tonic SCL and phasic SCRs and shows that this dose has no effect on neuropathy caused by diabetes. The decrease in tonic and phasic SCR values of metformin and vildagliptin combinations in our study is in line with studies (130) showing the use of combinations of these drugs for therapeutic purposes in diabetic neuropathy. Although vildagliptin alone was not effective in diabetic neuropathy, its combination with metformin is thought to be effective in diabetic neuropathy.

NS-SCR freq. was higher in the diabetic group than in the control group. This value was decreased in the DV, and DMV groups compared to the D group ($p<0.001$ and $p<0.05$, respectively). We could not find any study with metformin and vildagliptin related to this information. An increase in the number of peaks in this parameter may interpreted as an increase in alertness, stress and attention (80).

In conclusion, no study was found on the effects of metformin, vildagiptin or their combinations on electrodermal activity in cases with diabetes in the literature reviews, and this study may be the first study to evaluate the electrodermal activity of diabetic rats receiving vildagliptin and metformin. The results showed increased sympathetic skin response in streptozocin-induced rats with diabetes. In addition, the fact that metformin and the combination of metformin and vildagliptin caused a decrease in tonic and phasic SCL indicates that this combination reduces sympathetic activity by affecting eccrine sweat glands. Further studies are needed on the effects on different treatment durations and whether the reduction in sympathetic activity will have a positive effect on diabetes. As an alternative method, EDA can be considered to be advantageous in determining sympathetic changes in diabetes treatments because it is an easy, inexpensive and non-invasive method.

REFERENCES

1 . Mellitus D. Diagnosis and classification of diabetes mellitus. Diabetes care. 2005 Jan 1;28(S37):S5-10.

2. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017 Sep;60(9):1577-85.

3. Ahren B. DPP-4 inhibitors. Best Practice & Research Clinical Endocrinology & Metabolism. 2007 Dec 1;21(4):517-33

4. He YL. Clinical pharmacokinetics and pharmacodynamics of vildagliptin. Clinical pharmacokinetics. 2012 Mar;51(3):147-62.

5. Croxtall JD, Keam SJ. Vildagliptin. Drugs. 2008 Nov;68(16):2387-409.

6. Mathieu C, Degrande E. Vildagliptin: a new oral treatment for type 2 diabetes mellitus. Vascular health and risk management. 2008 Dec;4(6):1349.

7. González-Correa CA. Clinical applications of electrical impedance spectroscopy. InBioimpedance in Biomedical Applications and Research 2018 (pp. 187-218). Springer, Cham.

8. Vlckova E, Srotova I, Bednarik J. Sympathetic Skin Response in the Diagnosis of Small Fibre Neuropaty. CESKA A SLOVENSKA NEUROLOGIE A NEUROCHIRURGIE. 2016 Jan 1;79(1):52-60.

9. Khalfallah K, Ayoub H, Calvet JH, Neveu X, Brunswick P, Griveau S, Lair V, Cassir M, Bedioui F. Noninvasive galvanic skin sensor for early diagnosis of sudomotor dysfunction: application to diabetes. IEEE Sensors Journal. 2010 Dec 30;12(3):456-63.

10.Mohanraj S, Narayanan K. Sympathetic skin response and galvanic skin resistance in males with type 2 diabetes mellitus. Journal of Evidence Based Medicine and Healthcare. 2016;3(50):2544-9.

11.Bolanos L, Vicente JM, Vivas OA, Sabater-Navarro JM. Electrodermal activity in relation to diabetes, autonomic neuropathy and aging: a preliminary study. Ingeniería e Investigación. 2019 Dec;39(3):27-33.

12.Ahmed AM. History of diabetes mellitus. Saudi medical journal. 2002 Apr 1;23(4):373-8.

13.Canivell S, Gomis R. Diagnosis and classification of autoimmune diabetes mellitus. Autoimmunity reviews. 2014 Apr 1;13(4-5):403-7.

14.Patlak M. New weapons to combat an ancient disease: treating diabetes. The FASEB Journal. 2002 Dec;16(14):1853e-

15.Vinay K, Abbas AK, Fauston N, Aster JC. Robbins and Cotran pathologic basis of disease. New Delhi, India. 2005:628-36.

16.Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care. 2004 May 1;27(5):1047-53.

17.Gastaldelli A. Role of beta-cell dysfunction, ectopic fat accumulation and insulin resistance in the pathogenesis of type 2 diabetes mellitus. Diabetes research and clinical practice. 2011 Aug 1;93:S60-5.

18.Mellitus—Evaluating D. Guidance for Industry. Center for Drug Evaluation and Research (CDER). 2008 Dec.

19.Ramachandran A. Know the signs and symptoms of diabetes. The Indian journal of medical research. 2014 Nov;140(5):579.

20.Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. Physical therapy. 2008 Nov 1;88(11):1254-64.

21.Tuttolomondo A, Maida C, Pinto A. Diabetic foot syndrome as a possible cardiovascular marker in diabetic patients. Journal of diabetes research. 2015 Mar 26;2015.

22.Kousar S. Type 1 Diabetes: Causes, Symptoms and Treatments, Review with Personal Experience. Current Research in Diabetes & Obesity Journal. 2019;11(4):77-83.

23. Parchman ML, Noel PH, Culler SD, Lanham HJ, Leykum LK, Romero RL, Palmer RF. A randomized trial of practice facilitation to improve the delivery of chronic illness care in primary care: initial and sustained effects. Implementation Science. 2013 Dec;8(1):1-7.

24.https://www.niddk.nih.gov/

25.Hattersley A, Bruining J, Shield J, Njolstad P, Donaghue KC. The diagnosis and management of monogenic diabetes in children and adolescents. Pediatric diabetes. 2009 Sep;10:33-42.

26.Stumvoll M, Goldstein BJ, Van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. The Lancet. 2005 Apr 9;365(9467):1333-46.

27.Taylor R. Type 2 diabetes: etiology and reversibility. Diabetes care. 2013 Apr 1;36(4):1047-55.

28.Durruty P, Sanzana M, Sanhueza L. Pathogenesis of type 2 diabetes mellitus. In Type 2 Diabetes-From Pathophysiology to Modern Management 2019 Feb 1.

29.Domínguez RO, Pagano MA, Marschoff ER, González SE, Repetto MG, Serra JA. Alzheimer disease and cognitive impairment associated with diabetes mellitus type 2: associations and a hypothesis. Neurología (English Edition). 2014 Nov 1;29(9):567-72.

30.Formiga F, Reñe R, Pérez-Maraver M. Dementia and diabetes: Casual or causal relationship?. Medicina Clínica (English Edition). 2015 Feb 20;144(4):176-80.

31.Doña PJ, Artigas PA, Guerra ML, Monfort CA. Factores de riesgo vascular y enfermedad de Alzheimer. Revista Española de Geriatría y Gerontología. 2009 Mar 1;44(2):98-105.

32.Accardi G, Caruso C, Colonna-Romano G, Camarda C, Monastero R, Candore G. Can Alzheimer disease be a form of type 3 diabetes?. Rejuvenation research. 2012 Apr 1;15(2):217-21.

33.Tayade S, Gangane N, Kore J, Kakde P. Surveillance of surgical site infections following gynecological surgeries in a rural setup–Lessons learnt. Indian Journal of Obstetrics and Gynecology Research. 2019 Jan;6(1):58-62.

34.Bhatt AJ, Vyas AS, Shah DP. New Frontier in the Treatment of Diabetes. Journal of Drug Delivery and Therapeutics. 2019 Oct 15;9(5-s):178-87.

35.Uygur MM, Yavuz DG. Diyabet tanısı ve sınıflandırılması. Turkiye Klinikleri J Nutr Diet-Special Topics. 2017;3(3):120-9.

36.American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2013 Jan;36(Suppl 1):S67.

37.Goldenberg R, Punthakee Z. Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. Canadian journal of diabetes. 2013 Apr 1;37:S8-11.

38.Ali A, Iqbal F, Taj A, Iqbal Z, Amin MJ, Iqbal QZ. Prevalence of microvascular complications in newly diagnosed patients with type 2 diabetes. Pakistan journal of medical sciences. 2013 Jul;29(4):899.

39.Sosale A, Kumar KP, Sadikot SM, Nigam A, Bajaj S, Zargar AH, Singh SK. Chronic complications in newly diagnosed patients with type 2 diabetes mellitus in India. Indian journal of endocrinology and metabolism. 2014 May;18(3):355..

40.Lambrinou E, Hansen TB, Beulens JW. Lifestyle factors, self-management and patient empowerment in diabetes care. European Journal of Preventive Cardiology. 2019 Dec;26(2_suppl):55-63.

41.Abdul-Ghani MA, DeFronzo RA. Pathophysiology of prediabetes. Current diabetes reports. 2009 Jun;9(3):193-9.

42.Eigenmann C, Colagiuri R. Outcomes and indicators for diabetes education: a national consensus position. NDSS, National Diabetes Services Scheme, Diabetes Australia; 2007.

43.Karmoker JR, Priya RJ, Sarkar S, Islam S. Comparative in vitro equivalence evaluation of some local Gliclazide brands of Bangladesh. The Pharma Innovation. 2017 Mar 1;6(3, Part C):152.

44.Sylvia LM, DiPiro JT. Allergic and pseudoallergic drug reactions. Pharmacotherapy: A pathophysiologic approach. 8th ed. New York: McGraw-Hill. 2011.

45.Wang GS, Hoyte C. Review of biguanide (metformin) toxicity. Journal of intensive care medicine. 2019 Nov;34(11-12):863-76.

46.Negrulj R, Mooranian A, Al-Salami H. Potentials and limitations of bile acids in type 2 diabetes mellitus: applications of microencapsulation as a novel oral delivery system. Journal of Endocrinology and Diabetes Mellitus. 2013;1:49-59.

47.Gromova LV, Fetissov SO, Gruzdkov AA. Mechanisms of glucose absorption in the small intestine in health and metabolic diseases and their role in appetite regulation. Nutrients. 2021 Jul;13(7):2474.

48.Adeva-Andany MM, Pérez-Felpete N, Fernández-Fernández C, Donapetry-García C, Pazos-García C. Liver glucose metabolism in humans. Bioscience reports. 2016 Dec 1;36(6).

49.Piero NM, Murugi NM, Mwiti KC, Mwenda MP. Pharmacological management of diabetes mellitus. Asian Journal of Biochemical and Pharmaceutical Research. 2012;2(2):375-80.

50.Landgraf R. Meglitinide analogues in the treatment of type 2 diabetes mellitus. Drugs & aging. 2000 Nov;17(5):411-25.

51.Perley MJ, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. The Journal of clinical investigation. 1967 Dec 1;46(12):1954-62.

52.Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. The Journal of Clinical Endocrinology & Metabolism. 1973 Nov 1;37(5):826-8.

53.Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. The Lancet. 2006 Nov 11;368(9548):1696-705.

54.Pfeiffer AF, Klein HH. Therapie des diabetes mellitus typ 2. Dtsch Arztebl Int. 2014 Jan 31;111(5):69-82.

55.Billeter AT, Kopf S, Zeier M, Scheurlen K, Fischer L, Schulte TM, Kenngott HG, Israel B, Knefeli P, Büchler MW, Nawroth PP. Renal function in type 2 diabetes following gastric bypass: a prospective cohort study in mildly obese insulin-dependent patients. Deutsches Ärzteblatt International. 2016 Dec;113(49):827.

56.Dixon JB, Zimmet P, Alberti KG, Rubino F, International Diabetes Federation Taskforce on Epidemiology and Prevention. Bariatric surgery: an IDF statement for obese type 2 diabetes. Surgery for Obesity and Related Diseases. 2011 Jul 1;7(4):433-47.

57.Scarpello JH, Howlett HC. Metformin therapy and clinical uses. Diabetes and Vascular Disease Research. 2008 Sep;5(3):157-67.

58.Corcoran C, Jacobs TF. Metformin. InStatPearls [Internet] 2021 Jul 19. StatPearls Publishing.

59.https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021748s005lbl.pdf

60.Mahmood K, Naeem M, Rahimnajjad NA. Metformin: the hidden chronicles of a magic drug. European journal of internal medicine. 2013 Jan 1;24(1):20-6.

61.Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenetics and genomics. 2012 Nov;22(11):820.

62.Zander M, Taskiran M, Toft-Nielsen MB, Madsbad S, Holst JJ. Additive glucose-lowering effects of glucagon-like peptide-1 and metformin in type 2 diabetes. Diabetes Care. 2001 Apr 1;24(4):720-5.

63.Ahrén B, Gomis R, Standl E, Mills D, Schweizer A. Twelve-and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. Diabetes care. 2004 Dec 1;27(12):2874-80.

64.Foley J. Vildagliptin-mode of action. Med chem. 2014;4:439-40.

65.Salahuddheen K. Emerging Role of Mono & Combination Therapy of Vildagliptin in Assessing the Safety & Efficacy in Type-2 Diabetic Patients (Doctoral dissertation, College of Pharmacy, JKK Munirajah Medical Research Foundation, Komarapalayam).

66.Bosi E, Dotta F, Jia Y, Goodman M. Vildagliptin plus metformin combination therapy provides superior glycaemic control to individual monotherapy in treatment-naive patients with type 2 diabetes mellitus. Diabetes, Obesity and Metabolism. 2009 May;11(5):506-15.

67.Drucker DJ. Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes: preclinical biology and mechanisms of action. Diabetes care. 2007 Jun 1;30(6):1335-43.

68.Mathieu C. The scientific evidence: vildagliptin and the benefits of islet enhancement. Diabetes, Obesity and Metabolism. 2009 May;11:9-17.

69.Bellary S. For type 2 diabetes poorly controlled by metformin monotherapy, the addition of any non-insulin antidiabetic drug reduces HbA1c to a similar extent, but with differing effects on weight and hypoglycaemic risk. BMJ Evidence-Based Medicine. 2011 Apr 1;16(2):39-40.

70.King AJ. The use of animal models in diabetes research. British journal of pharmacology. 2012 Jun;166(3):877-94.

71.Niens M, Grier AE, Marron M, Kay TW, Greiner DL, Serreze DV. Prevention of "Humanized" diabetogenic CD8 T-cell responses in HLA-transgenic NOD mice by a multipeptide coupled-cell approach. Diabetes. 2011 Apr 1;60(4):1229-36.

72.Gvazava IG, Rogovaya OS, Borisov MA, Vorotelyak EA, Vasiliev AV. Pathogenesis of type 1 diabetes mellitus and rodent experimental models. Acta Naturae (англоязычная версия). 2018;10(1 (36)):24-33.

73.Novikova L, Smirnova IV, Rawal S, Dotson AL, Benedict SH, Stehno-Bittel L. Variations in rodent models of type 1 diabetes: islet morphology. Journal of diabetes research. 2013 May 13;2013.

74.Chang AY. Spontaneous diabetes in animals. General Pharmacology: The Vascular System. 1978 Jan 1;9(6):447-50.

75.Kucera P, Goldenberg Z, Kurca E. Sympathetic skin response: review of the method and its clinical use. BRATISLAVSKE LEKARSKE LISTY.. 2004 Feb;105(3):108-16.

76.Pflanzer R, McMullen W. Galvanic skin response and the polygraph. BIOPAC Systems, Inc. Retrieved. 2013 May;5.

77.Posada-Quintero HF, Chon KH. Innovations in electrodermal activity data collection and signal processing: A systematic review. Sensors. 2020 Jan;20(2):479.

78.Boucsein W. Electrodermal activity. Springer Science & Business Media; 2012 Feb 2.

79.Esen F, Esen H. Effect of electromagnetic fields emitted by cellular phones on the latency of evoked electrodermal activity. International journal of neuroscience. 2006 Jan 1;116(3):321-9.

80.Boucsein W, Fowles DC, Grimnes S, Ben-Shakhar G, Roth WT, Dawson ME, Filion DL. Society for psychophysiological research ad hoc committee on electrodermal measures. Publication recommendations for electrodermal measurements. Psychophysiology. 2012 Aug;49(8):1017-34...

81.Cacioppo JT, Tassinary LG, Berntson G, editors. Handbook of psychophysiology. Cambridge university press; 2007 Mar 5.

82.Mohanraj S, Narayanan K. Sympathetic skin response and galvanic skin resistance in males with type 2 diabetes mellitus. Journal of Evidence Based Medicine and Healthcare. 2016;3(50):2544-9.

83.Dolu N, ÖZesmi Ç, Comu N, SÜEr C, Gölgeli A. Effect of hyperglycemia on electrodermal activity in diabetic rats. International journal of neuroscience. 2006 Jan 1;116(6):715-29.

84.Bolanos L, Vicente JM, Vivas OA, Sabater-Navarro JM. Electrodermal activity in relation to diabetes, autonomic neuropathy and aging: a preliminary study. Ingeniería e Investigación. 2019 Dec;39(3):27-33.

85.Miranda RA, Agostinho AR, Trevenzoli IH, Barella LF, Franco CC, Trombini AB, Malta A, Gravena C, Torrezan R, Mathias PC, de Oliveira JC. Insulin oversecretion in MSG-obese rats is related to alterations in cholinergic muscarinic receptor subtypes in pancreatic islets. Cellular Physiology and Biochemistry. 2014;33(4):1075-86.

86.Ahrén B. Autonomic regulation of islet hormone secretion–implications for health and disease. Diabetologia. 2000 Apr;43(4):393-410.

87.Oliveira PW, de Sousa GJ, Birocale AM, Gouvêa SA, de Figueiredo SG, de Abreu GR, Bissoli NS. Chronic metformin reduces systemic and local inflammatory proteins and improves hypertension-related cardiac autonomic dysfunction. Nutrition, Metabolism and Cardiovascular Diseases. 2020 Feb 10;30(2):274-81.

88.Lukashevich V, Del Prato S, Araga M, Kothny W. Efficacy and safety of vildagliptin in patients with type 2 diabetes mellitus inadequately controlled with dual combination of metformin and sulphonylurea. Diabetes, Obesity and Metabolism. 2014 May;16(5):403-9.

89.DiabetesControl and Complications Trial Research Group. The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT). Diabetologia. 1998 Mar;41(4):416-23.

90.Sucharita S, Bantwal G, Idiculla J, Ayyar V, Vaz M. Autonomic nervous system function in type 2 diabetes using conventional clinical autonomic tests, heart rate and blood pressure variability measures. Indian journal of endocrinology and metabolism. 2011 Jul;15(3):198.

91.Patel JP, Lee EH, Mena CI, Walker CN. Effects of metformin on endothelial health and erectile dysfunction. Translational andrology and urology. 2017 Jun;6(3):556.

92.Donadio V, Incensi A, Vacchiano V, Infante R, Magnani M, Liguori R. The autonomic innervation of hairy skin in humans: an in vivo confocal study. Scientific reports. 2019 Nov 18;9(1):1-7.

93.Farrell MJ, Trevaks D, Taylor NA, McAllen RM. Control of Autonomic Function: Insights from Neurophysiological Studies in Conscious Animals (Including Humans): Regional brain responses associated with thermogenic and psychogenic sweating events in humans. Journal of Neurophysiology. 2015 Nov;114(5):2578.

94.Lagopoulos J. Electrodermal activity. Acta Neuropsychiatrica. 2007 Oct;19(5):316-7.

95.Lambrinou E, Hansen TB, Beulens JW. Lifestyle factors, self-management and patient empowerment in diabetes care. European Journal of Preventive Cardiology. 2019 Dec;26(2_suppl):55-63.

96.Uncini A, Pullman SL, Lovelace RE, Gambi D. The sympathetic skin response: normal values, elucidation of afferent components and application limits. Journal of the neurological sciences. 1988 Nov 1;87(2-3):299-306.

97.Mishra V, Nayak P, Sharma M, Albutti A, Alwashmi AS, Aljasir MA, Alsowayeh N, Tambuwala MM. Emerging Treatment Strategies for Diabetes Mellitus and Associated Complications: An Update. Pharmaceutics. 2021 Oct;13(10):1568.

98.Dolu N, Keloglan S, Bitiktas S, Cug S. The effects of the enriched environment on sympathetic skin response in pentylenetetrazol-kindled rats. Biomed Environ Sci. 2013 May 1;26(5):394-7.

99.Wang Y, Wang C, Li K, Song X, Yan X, Yu L, He Z. Recent advances of nanomedicine-based strategies in diabetes and complications management: Diagnostics, monitoring, and therapeutics. Journal of Controlled Release. 2021 Feb 10;330:618-40.

100.Kucera P, Goldenberg Z, Kurca E. Sympathetic skin response: review of the method and its clinical use. BRATISLAVSKE LEKARSKE LISTY.. 2004 Feb;105(3):108-16.

101.Maleki H, Khoshnevisan K, Sajjadi-Jazi SM, Baharifar H, Doostan M, Khoshnevisan N, Sharifi F. Nanofiber-based systems intended for diabetes. Journal of nanobiotechnology. 2021 Dec;19(1):1-34.

102.Biessels GJ, Cristino NA, Rutten GJ, Hamers FP, Erkelens DW, Gispen WH. Neurophysiological changes in the central and peripheral nervous system of streptozotocin-diabetic rats: course of development and effects of insulin treatment. Brain. 1999 Apr 1;122(4):757-68.

103.Fadli N, Hakim M, Safri AY, Antono D. P-PN027. Sympathetic skin response in diabetic neuropathy with and without peripheral arterial disease. Clinical Neurophysiology. 2021 Aug 1;132(8):e114.

104.HIRAI, Akinori, et al. Evaluation of diabetic neuropathy through the quantitation of cutaneous nerves. Journal of the neurological sciences, 2000, 172.1: 55-62.

105.Chin AC, Balasubramanian P, Suryadevara P, Zyskowski J, Herdt TH, MohanKumar SM, MohanKumar PS. Metformin effectively restores the HPA axis function in diet-induced obese rats. International Journal of Obesity. 2021 Feb;45(2):383-95.

106.Manzella D, Grella R, Esposito K, Giugliano D, Barbagallo M, Paolisso G. Blood pressure and cardiac autonomic nervous system in obese type 2 diabetic patients: effect

of metformin administration. American journal of hypertension. 2004 Mar 1;17(3):223-7.

107.Apolzan JW, Venditti EM, Edelstein SL, Knowler WC, Dabelea D, Boyko EJ, Pi-Sunyer X, Kalyani RR, Franks PW, Srikanthan P, Gadde KM. Long-term weight loss with metformin or lifestyle intervention in the diabetes prevention program outcomes study. Annals of internal medicine. 2019 May 21;170(10):682-90.

108.Lentferink YE, Knibbe CA, Van Der Vorst MM. Efficacy of metformin treatment with respect to weight reduction in children and adults with obesity: a systematic review. Drugs. 2018 Dec;78(18):1887-901.

109.Warnakulasuriya LS, Fernando MM, Adikaram AV, Thawfeek AR, Anurasiri WM, Silva RR, Sirasa MS, Rytter E, Forslund AH, Samaranayake DL, Wickramasinghe VP. Metformin in the management of childhood obesity: a randomized control trial. Childhood obesity. 2018 Dec 1;14(8):553-65.

110.Rouru J, Huupponen R, Pesonen U, Koulu M. Subchronic treatment with metformin produces anorectic effect and reduces hyperinsulinemia in genetically obese Zucker rats. Life sciences. 1992 Jan 1;50(23):1813-20.

111.Rouru J, Pesonen U, Koulu M, Huupponen R, Santti E, Virtanen K, Rouvari T, Jhanwar-Uniyal M. Anorectic effect of metformin in obese Zucker rats: lack of evidence for the involvement of neuropeptide Y. European journal of pharmacology. 1995 Jan 24;273(1-2):99-106.

112.Seifarth C, Schehler B, Schneider HJ. Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. Experimental and clinical endocrinology & diabetes. 2013 Jan;121(01):27-31.

113.Schommers P, Thurau A, Bultmann-Mellin I, Guschlbauer M, Klatt AR, Rozman J, Klingenspor M, de Angelis MH, Alber J, Gründemann D, Sterner-Kock A. Metformin causes a futile intestinal–hepatic cycle which increases energy expenditure and slows down development of a type 2 diabetes-like state. Molecular metabolism. 2017 Jul 1;6(7):737-47.

114.Singh AK, Maurya AK, Kant S, Umrao J, Kushwaha RA, Nag VL, Dhole TN. Rapid detection of drug resistance and mutational patterns of extensively drug-resistant strains by a novel GenoType® MTBDRsl assay. Journal of postgraduate medicine. 2013 Jul 1;59(3):179.

115.Klempfner R, Leor J, Tenenbaum A, Fisman EZ, Goldenberg I. Effects of a vildagliptin/metformin combination on markers of atherosclerosis, thrombosis, and inflammation in diabetic patients with coronary artery disease. Cardiovascular Diabetology. 2012 Dec;11(1):1-6.

116.Bell DS. Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. Southern medical journal. 2010 Mar 1;103(3):265-7.

117.Yang W, Cai X, Wu H, Ji L. Associations between metformin use and vitamin B12 levels, anemia, and neuropathy in patients with diabetes: a meta-analysis. Journal of diabetes. 2019 Sep;11(9):729-43.

118.Al Omari M, Khader Y, Dauod AS, Beni Yonis OA, Khassawneh AH. Vildagliptin efficacy in combination with metformin among Jordanian patients with type 2 diabetes mellitus inadequately controlled with metformin. Journal of Drug Assessment. 2016 Jan 1;5(1):29-33.

119.Pi-Sunyer FX, Schweizer A, Mills D, Dejager S. Efficacy and tolerability of vildagliptin monotherapy in drug-naive patients with type 2 diabetes. Diabetes research and clinical practice. 2007 Apr 1;76(1):132-8.

120.Schweizer A, Couturier A, Foley JE, Dejager S. Comparison between vildagliptin and metformin to sustain reductions in HbA1c over 1 year in drug-naïve patients with Type 2 diabetes. Diabetic Medicine. 2007 Sep;24(9):955-61.

121.Ahrén B, Gomis R, Standl E, Mills D, Schweizer A. Twelve-and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. Diabetes care. 2004 Dec 1;27(12):2874-80.

122.Bosi E, Camisasca RP, Collober C, Rochotte E, Garber AJ. Effects of vildagliptin on glucose control over 24 weeks in patients with type 2 diabetes inadequately controlled with metformin. Diabetes care. 2007 Apr 1;30(4):890-5.

123.Bolli G, Dotta F, Rochotte E, Cohen SE. Efficacy and tolerability of vildagliptin vs. pioglitazone when added to metformin: a 24-week, randomized, double-blind study. Diabetes, Obesity and Metabolism. 2008 Jan;10(1):82-90.

124.Garber AJ, Foley JE, Banerji MA, Ebeling P, Gudbjörnsdottir S, Camisasca RP, Couturier A, Baron AM. Effects of vildagliptin on glucose control in patients with type 2 diabetes inadequately controlled with a sulphonylurea. Diabetes, Obesity and Metabolism. 2008 Nov;10(11):1047-56.

125.Garber AJ, Schweizer A, Baron MA, Rochotte E, Dejager S. Vildagliptin in combination with pioglitazone improves glycaemic control in patients with type 2 diabetes failing thiazolidinedione monotherapy: a randomized, placebo-controlled study. Diabetes, Obesity and Metabolism. 2007 Mar;9(2):166-74.

126.Fonseca V, Schweizer A, Albrecht D, Baron MA, Chang I, Dejager S. Addition of vildagliptin to insulin improves glycaemic control in type 2 diabetes. Diabetologia. 2007 Jun;50(6):1148-55.

127.Karagiannis T, Paschos P, Paletas K, Matthews DR, Tsapas A. Dipeptidyl peptidase-4 inhibitors for treatment of type 2 diabetes mellitus in the clinical setting: systematic review and meta-analysis. Bmj. 2012 Mar 12;344.

128.Schweizer A, Dejager S, Foley JE, Kothny W. Assessing the general safety and tolerability of vildagliptin: value of pooled analyses from a large safety database versus evaluation of individual studies. Vascular Health and Risk Management. 2011;7:49.

129.Jin H.Y, Liu W.J, Park J.H, Baek H.S,ParkT. S. Effect of dipeptidyl peptidase-IV (DPP-IV) inhibitor (Vildagliptin) on peripheral nerves in streptozotocin-induced diabetic rats. Archives of medical research,2009 Oct40(7), 536-544

130.Shigematsu N, Kawashiri T, Kobayashi D, Shimizu S, Mine K, Hiromoto S, Shimazoe T. Neuroprotective effect of alogliptin on oxaliplatin-induced peripheral neuropathy in vivo and in vitro. Scientific Report.2020 Apr.10(1):1-8

APPENDIX 1: PROJECT APPROVAL

Evrak Tarih ve Sayısı: 09.12.2020-35643

1993
BAŞKENT ÜNİVERSİTESİ
Tıp ve Sağlık Bilimleri Araştırma Kurulu

Sayı : 94603339-604.01.02/
Konu : Proje Onayı

09.12.2020

SAĞLIK BİLİMLERİ ENSTİTÜSÜ MÜDÜRLÜĞÜNE

Fizyoloji Anabilim Dalında görev yapmakta olan Prof. Dr. Nazan Dolu'nun danışmanlığında, Sağlık Bilimleri Enstitüsü / Fizyoloji Tezli Yüksek Lisans Programı öğrencisi Muftah Mohamed Muftah Shawesh'in sorumluluğunda yürütülecek olan DA20/23 nolu "Evaluation of electrodermal activity in diabetic rats treated with vildagliptin and metformin" başlıklı araştırma projesi Kurulumuz ve Hayvan Deneyleri Yerel Etik Kurulu Kurulu'nun 23/11/2020 tarih ve 20/21 sayılı kararı ile uygun görülmüştür. Projenin başlama tarihi ile çalışmanın sunulduğu kongre ve yayımlandığı dergi konusunda Kurulumuza bilgi verilmesini rica ederim.

e-imzalıdır
Prof. Dr. Hakan ÖZKARDEŞ
Kurul Başkanı

Not: Çalışma bildiri ve/veya makale haline geldiğinde "Gereç ve Yöntem" bölümüne aşağıdaki ifadelerden uygun olanının eklenmesi gerekmektedir.

— Bu çalışma Başkent Üniversitesi Hayvan Deneyleri Etik Kurulu tarafından onaylanmış (Proje no:…) ve Başkent Üniversitesi Araştırma Fonunca desteklenmiştir.

— This study was approved by Baskent University Ethical Committee for Experimental Research on Animals (Project no:…) and supported by Baskent University Research Fund.

DAĞITIM
Sağlık Bilimleri Enstitüsü Müdürlüğüne
Fizyoloji Anabilim Dalına

APPENDİX 2 . ETHİCAL APPROVAL

1993
BAŞKENT UNIVERSITY

LOCAL ETHICS COMMITTEE FOR ANIMAL EXPERIMENTS DECISION		
SESSION NO	DECISION NO	DATE OF DECISION
11	20/21	23/11/2020

Project DA20/23 no entitled "Evaluation of electrodermal activity in diabetic rats treated with vildagliptin and metformin" pending to be conducted by Nazan Dolu with the Department of Physiology has been reviewed and unanimously approved by the Local Ethics Committee for Animal Experiments.