BASKENT UNIVERSITY
INSTITUTE OF HEALTH SCIENCE
DEPARTMENT OF PHYSIOLOGY
MASTER'S PROGRAM

EFFECT OF VILDAGLIPTIN AND METFORMIN ON ANXIETY IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

BY

MOHAMMED ALSHAREEF

MASTER'S THESIS

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

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ÖZET

Mohammed ALSHAREEF, Streptozotosin ile diyabet oluşturulan sıçanlarda vildagliptin ve metforminin anksiyete üzerine etkisi. Başkent Üniversitesi Sağlık Bilimleri Enstitüsü Fizyoloji Anabilim Dalı, Fizyoloji Tezli Yüksek Lisans Programı Yüksek Lisans Tezi, 2021.

Diabetes mellitus, insülin sekresyon eksikliğinden veya etkisindeki bir bozukluktan kaynaklanan metabolik bir hastalıktır. Metformin ve vildagliptin, tip 2 diabetes mellitus tedavisinde kullanılan en bilinen ilaçlardan ikisidir. Çalışmanın amacı, streptozosin ile diyabet oluşturulmuş sıçanlarda metformin, vildagliptin ve bunların kombine kullanımının anksiyete üzerine etkilerini ortaya koymaktır. Bu amaçla, 50 erkek Wistar sıçanın 10'u kontrol grubu (K) olarak ayrıldıktan sonra diğerlerinde streptozotosin ile diyabet oluşturuldu. Diyabetli hayvanların da 10'u diabetic kontrol grubu (D) olarak ayrıldıktan sonra kalanları 3'e ayrılarak 500 mg/kg metformin (DM), 50 mg/kg vildagliptin (DV) ve her ikisinin kombinasyonu (DMV) ile 15 gün boyunca tedavi edildi. Sonrasında hayvanlar açık alan ve yükseltilmiş artı labirent testine tabi tutuldu.

Açık alan testinde, diyabetli grupta artan ayağa kalkma sayısı ($p<0.001$), metformin, vildagliptin ve kombine tedavi uygulanan grupta diyabetik kotrol grubuna göre anlamlı oranda azaldı (sırasıyla $p<0.001$, $p<0.01$ ve $p<0.001$). Merkezde zaman geçirme süresi D ve DM grubunda K grubuna gore azalırken ($p<0.05$), kombine tedavi grubunun merkezde geçirdiği süre diğer bütün diyabetli gruplara gore daha çok oldu ($p<0.01$). Periferde geçirilen süre de DMV grubunda diyabetli diğer gruplara göre azaldı ($p<0.05$). Diyabet, DM ve DV gruplarında kontrol grubuna göre artan idrar yapma sayısı, DMV grubunda diyabetli gruplara göre azaldı. Defekasyon sayısı ise D grubunda K grubuna göre artarken ($p<0.01$), DMV grubunda D grubuna gore azalma görüldü ($p<0.05$).

Yükseltilmiş artı labirent testinde de kapalı alanda geçirilen süre diyabetli grupta K grubuna gore artarken ($p<0.05$), tedavi gruplarında D grubuna gore bir farklılık görülmedi.

Sonuç olarak araştırmamız, vildagliptin ve metforminin tek başlarına kullanımlarında diyabetik hayvanlarda oluşan kaygıya karşı etkileri belirgin değilken; kombine kullanıldığı tedavilerin diyabetin oluşturduğu kaygıyı azaltmada etkili olabileceğini gösterdi.

Anahtar Kelimeler: Açık alan testi, anksiyete, diabetes mellitus, metformin, vildagliptin

Bu çalışma Başkent Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmış (Proje no: DA20/22) ve Başkent Üniversitesi Araştırma Fonunca desteklenmiştir.

ABSTRACT

Mohammed ALSHAREEF, Effect of Vildagliptin and Metformin on Anxiety in Streptozotocin-induced Diabetic Rats. Başkent University, Institute of Health Sciences, Department of Physiology. Master's Program of Physiology with thesis, Master's Thesis, 2021.

Diabetes mellitus is caused by insulin secretion deficiency or a disorder in its action. Vildagliptin and metformin are two of the most recognized drugs that are used in the treatment of type 2 diabetes mellitus. The study aimed to compare the effects of metformin, vildagliptin, and their combined effect in altering behaviours of streptozocin-induced diabetic rats. For this purpose, after 10 of 50 male Wistar rats were separated as the control group (C), diabetes was induced in the others with streptozotocin. After separation of 10 diabetic control group (D), other diabetic animals were divided into 3 and treated with 500 mg/kg metformin (DM), 50 mg/kg vildagliptin (DV) and a combination of both (DMV) for 15 days. The animals were then subjected to open field and elevated plus maze testing.

In the open field test, while the number of rearing increased in the group with diabetes ($p<0.001$), it was significantly decreased in the metformin, vildagliptin and combined treatment group compared to the diabetic control group ($p<0.001$, $p<0.01$, and $p<0.001$, respectively). While the time spent in the center decreased in the D and DM groups compared to the C group ($p<0.05$), the time spent in the combined treatment group in the center was longer than in all other diabetes groups ($p<0.01$). The time spent in the periphery was also decreased in the DMV group compared to the other groups with diabetes ($p<0.05$). The number of urinations increased in the D, DM and DV groups compared to the control group, and decreased in the DMV group compared to the diabetic groups. While the number of defections increased in the D group compared with the C group ($p<0.01$), it was decreased in the DMV group compared to the D group ($p<0.05$).

In the elevated plus maze test, while the time spent in enclosed arms increased in the group with diabetes compared with the C group ($p<0.05$), there was no difference in the treatment groups compared to the D group.

As a result, our study showed that the effects of vildagliptin and metformin on anxiety in diabetic animals were not evident when they were used alone; also, it showed that combined treatments can be effective in reducing anxiety caused by diabetes.

Key words: Open field maze, anxiety, diabetes mellitus, metformin, vildagliptin

This study was approved by Baskent University Ethical Committee for Experimental Research on Animals (Project no: DA20/22) and supported by Baskent University Research Fund.

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AD	anno domini
BBDP	bio breeding diabetes prone
BCE	before the common era
DCCT	diabetes control and complication trial
DKA	diabetic ketoacidosis
DNA	deoxyribonucleic acid
DPP	dipeptidyl peptidase
GLP	glucagon-like-peptide
GT	Goto Kakizaki
HLA	human leukocyte antigen
MHC	major histocompatibility complex
RGP	rehmannia glutinosa polysaccharide
STZ	streptozotocin
WHO	world health organization
ZDF	Zucker diabetic fatty

1. INTRODUCTION

Diabetes mellitus is caused by insulin secretion deficiency or a disorder in its action. There are defined three main types of the diabetes as type 1, type 2, and gestational diabetes. There are many risk factors that can lead to diabetes mellitus, and they are mainly of genetic or environmental nature. The complications of the disease can lead to several damages in body systems, including vascular, nephrological, and neural systems. The effects of the disease and its complications can lead to several psychological and behavioral issues, besides physiological damage.

Metformin and vildagliptin are two of the most recognized drugs that are used in the treatment of diabetes mellitus type 2. While metformin is used for type 2 and gestational diabetes and it inhibits glucose hepatic production and improves insulin sensitivity; vildagliptin works by inhibiting the degradation of glucagon-like-peptide-1 (GLP-1) and keeps glycemia leading to control of body weight. It also stimulates insulin secretion and at the same time blocks glucagon secretion in glucose-dependent states. Moreover, it prohibits the production of glucose from the liver by the mechanism of changing in hormone secretion of the pancreatic cells and developing the sensitivity to insulin.

There are studies in the literature that showed high levels of anxiety in correlation with diabetic subjects (1-3). It is indicated that the use of long-term treatment of metformin (16 weeks) reduced high fat diet-induced anxiety (4) and vildagliptin significantly decreased the diabetes-induced memory loss and improves the learning skills (5). Another study showed a combination of these drugs was effective on the reduce of blood glucose in patient with type 2 diabetes mellitus (6). However, there is no research on these drugs comparatively and the combined effect on anxiety in streptozotocin-induced diabetes.

The current research investigated the effects of metformin and vildagliptin on anxiety behaviors of diabetic male Wistar rats, when used solely or in combination. To evaluate anxiety behavior, open-field maze and elevated plus maze were used. The results of the study will show whether these drugs, which have antidiabetic effects, also have anxiolytic effects when used alone or in combination.

2. GENERAL KNOWLEDGE

2.1. History of Diabetes Mellitus

Diabetes mellitus is defined as a metabolic disorder and is characterized as hyperglycemia caused by impaired insulin secretion, impaired insulin action or both. It can be recognized by frequent urination, thirsty, raised appetite, which are common symptoms. Diabetes was discovered 3000 years ago by the Egyptians as there were similar features as is in nowadays (7). Ancient Indians recognized what they called "madhumeha" , which means honey "sweet" like urine. Sushruta and Charaka two Indian physicians in the fifth century AD differentiated two types of diabetes mellitus according to body weight & age of individuals. They noticed that diabetic slim individuals, unlike overweight people, had diabetes when they were young, while those who were overweight had an onset of diabetes later and were diabetic for a long time after diagnosis (8).

Ibn-Sina published (Canon Avicenna) the canon of medicine is an encyclopedia of medicine in five books & he described diabetes in detail in his medical textbook in 1025 AD. Some current understanding of diabetes goes back to a period of European discoveries from the sixteenth to eighteenth centuries. Paracelsus (1494–1541) a Swiss physician vaporized the collected urine from the diabetic patients to get remains, but he was mistaken when he thought those remains were salt as a cause of being thirsty (the sign for diabetes) or the kidney contained salt. In 1776, Matthew Dobson a British physiologist was the first to notice the sweetness in the taste of both urine & blood as a mark that they contained sugar. Before Dobson, diabetes was indicated as kidney disease, but he raised the theory that it was a systemic disease (8).

Diabetes mellitus is indicated as a syndrome rather than a disease having many diseases and types that point it with the same symptoms, signs & complications, but the causes are not the same. Diabetes mellitus is considered one of the oldest diseases and this syndrome is getting widespread globally more common in well-progressing regions. In 2008, WHO reported that there were 347 million patients with diabetes mellitus around the world. Some predictions indicate the number will be twice more before the year 2030. The increasing of blood sugar is known as hyperglycemia and remarkable character of diabetes mellitus (9).

2.2. Types of Diabetes

There are defined three main types of the diabetes as type 1, type 2, and gestational diabetes.

2.2.1. Type 1 diabetes

In this type, pancreatic B cells are destructed due to an autoimmune response, which leads to insufficient insulin production that's why it is known as insulin-dependent diabetes mellitus (10). There are many factors that can trigger this issue which is divided into (11, 12):

- Genetic: several connections were found between the diseases and issues in HLA genetic region.
- Environmental: bacterial and viral infections that cause issues in the immune system.

2.2.2. Type 2 diabetes

The reason for this type of diabetes is insulin resistance. Type 2 diabetes mellitus is also known as insulin-independent diabetes mellitus. Although insulin production can be normal, the issue may arise due to possible genetic abnormalities in insulin receptors or related to lifestyle. Most people with overweight and don't have exercise habits tend to have type 2 diabetes. The relation between insulin and its cellular receptor is disrupted (13) and emerges insulin resistance along with decreased insulin secretion (14).

Type 2 has several risk factors that are related to genetics, diet, and age. Research showed strong correlations between the type and higher age, obesity, and the increased levels of HDL, TG, and cholesterol (15). Family history of diabetes was also found to be influential in the prevalence of the disease amongst next generations (16).

2.2.3. Gestational diabetes

Gestational diabetes is very similar to type 2 diabetes mellitus in terms of having a mixture of insufficient insulin production and response to insulin relatively. It occurs during pregnancy and can develop or disappear after giving birth, it might be diagnosed during the period of second and third months of pregnancy (13). This type is more prevalent among certain ethnicities, including Middle Eastern and Asian ones. It is also related to family history, dietary habits, and

aging (14). In genetics, the research identified pregnancy as an environmental stressor and associated gestational diabetes with several genetic issues (15).

2.3. Symptoms of diabetes mellitus

Several symptoms are seen as indications got diabetes mellitus such as hyperglycemia, polyuria, and glycosuria (to maintain the normal level of glucose in the blood, kidneys extract excessive glucose via urine). Due to insulin shortage, body energy is provided by the metabolization of fatty acids, this condition causes lethargy, tiredness, and weight loss. Subsequently, the excessive use of fatty acids as a source of energy results into ketonuria noticeable through the breath of the patient due to the production of ketone bodies. These metabolic reactions cause further damage to the body in a phenomenon called acidosis, as renal and respiratory systems work to excrete ketones through their channels.

The rise of glucose level in blood increases osmotic pressure in blood vessels around the eye, which causes changes in its shape and exerts pressure on the lens that affects the vision quality. Thus, patients with diabetes may have blurred visions or can temporarily become short-sighted as long as glucose levels are not balanced. Furthermore, the high glucose levels form a thriving environment for bacteria that can cause genital soreness, thrush, and skin infections.

2.4. Complications of Diabetes Mellitus

There are three types of complications, and each may differ, although some factors are common to all. These complications are neurologic, microvascular, and macrovascular.

2.4.1. Macrovascular complications

Its complications related to the large blood vessels which are located in the heart, the brain & legs. Thus, macrovascular diseases occur more commonly in coronary arteries & legs, especially most people with diabetes at a young age have atherosclerosis of coronary arteries, which may lead them to death. Atherosclerosis occurs in the same mechanism for diabetic and nondiabetic individuals, only the rate of development is different. Retinopathy can develop with diabetes as blood vessels around the eye are weakened or damaged. Hypertension, obesity,

sedentary lifestyle, smoking, and cholesterol are all factors that increase the risk of coronary artery disease. Increased glucose levels harm the mechanism of clotting, so blood glucose levels must be controlled for preventive purposes from the disease in diabetic people (17). Figure 2.1 shows the effects of diabetes on macrovascular system.

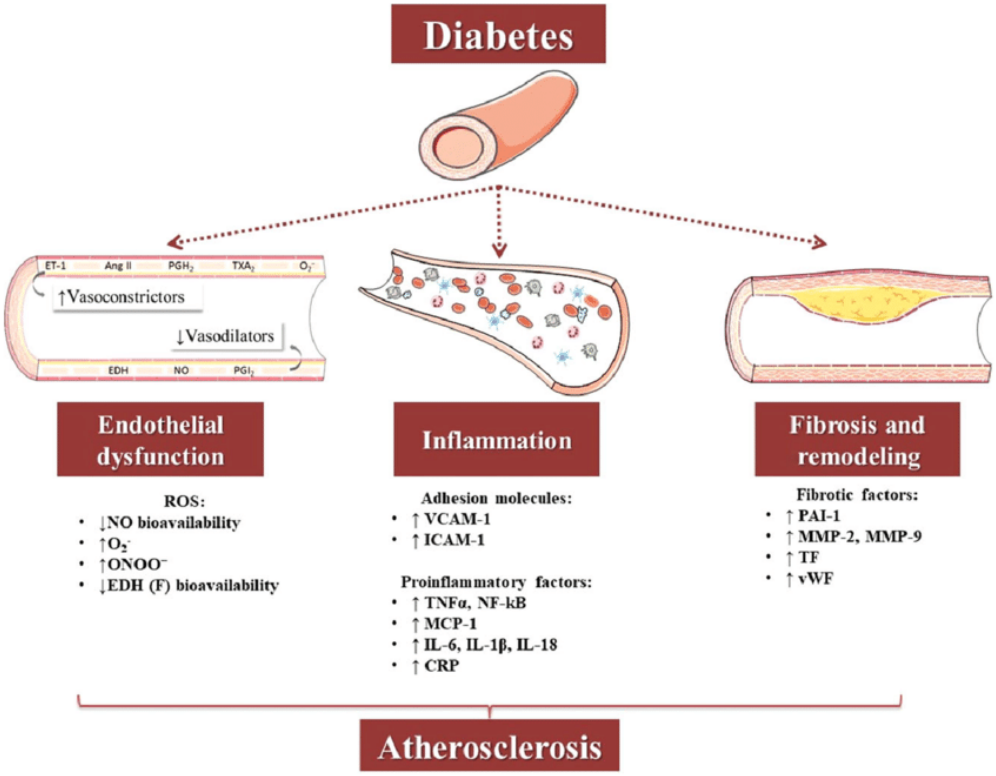

Figure 2. 1. Effects of diabetes on macrovascular system (17)

2.4.2. Microvascular and nephrological complications

Because of the well understanding of the pathophysiology of small blood vessels diseases, it can be easier to understand their related diseases in people with diabetes rather than the large vessel diseases in people with diabetes mellitus. Capillaries in different parts of the body are mostly affected by microvascular diseases such as capillaries in the eyes & kidneys. Diabetic retinopathy has four mechanisms: AGE formation, hexosamine pathway, PKC pathway, and polyol pathway (18). For instance, blindness in adults is caused mainly by diabetic retinopathy, and the number of people, who are administered dialysis or kidney transplantation is almost less

than half of the diabetic nephropathy people (19). Figure 2.2 shows the direct and indirect effects of diabetes on the microvascular system.

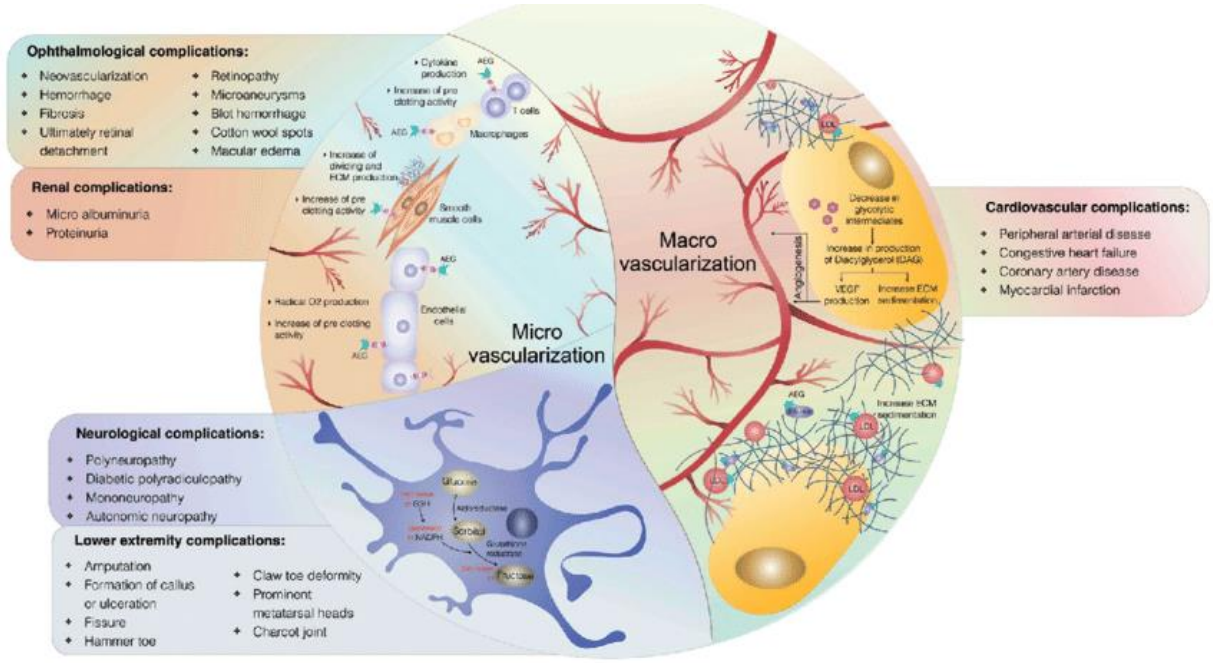

Figure 2. 2. Effects of diabetes on microvascular and nephrological systems (19)

2.4.3. Neurological complications

Neuropathies develop in more than 50% of diabetes patients. Both autonomic and somatic sections of the peripheral nervous system are related the neuropathic syndromes of diabetes. In addition to minor neurologic damages, higher central nervous system and spinal cord can be affected causing more severe issues. Cardiovascular dysfunctions, erectile dysfunctions, and impaired wound healings are all complications that can be caused by diabetes neuropathy. Moreover, neuropathies of this nature can progress to vascular abnormalities, such as endothelial hyperplasia and capillary basement membrane thickening (20). Figure 2.3 shows diabetic neuropathy.

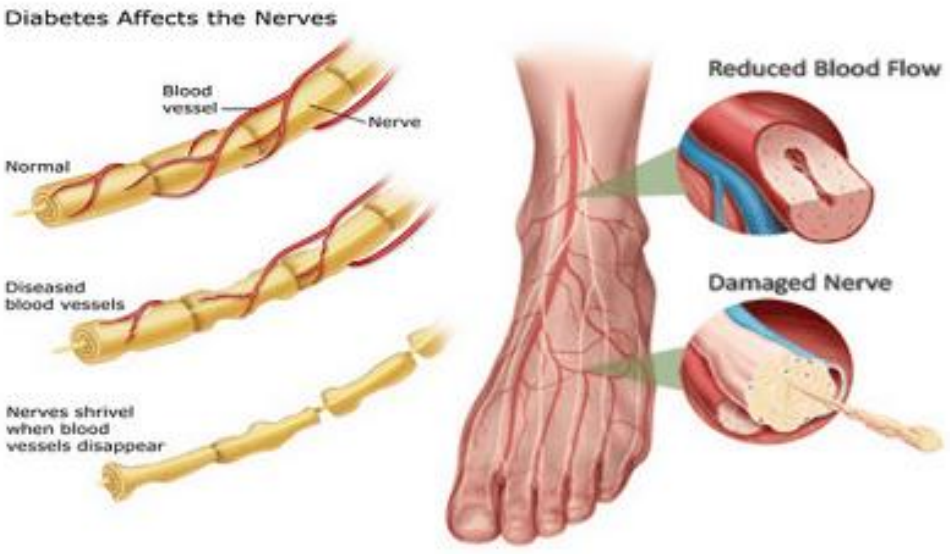

Figure 2. 3. Neuropathy of diabetes (21).

2.4.4. Diabetes and Anxiety

The relationship between diabetes and adverse psychological issues, especially anxiety, is addressed through several studies in the literature. Woon et al. (22) conducted a study on 300 patients with diabetes, where the psychological evaluation showed that 9% of the patients showed clear signs of anxiety. Moreover, at least 20% of the sample showed anxiety and depression comorbidity. Tovilla-Zarate et al. (23) showed the prevalence of anxiety with type 2 diabetes through investigating the case on more than 800 patients using Hamilton anxiety scale. The findings of the research showed that more than 55% of the patients had anxiety, and anxiety increased with the increase of diabetic complications. The prevalence of anxiety among elderly patients was also found high with type 2 patients with diabetes. A study in Tunisia found that anxiety is prevailed in more than 40% of patients above 60 years old using hospital anxiety and depression scale (24). Another research had also found that long-term experience with type 2 diabetes negatively affects patients' quality of life, where the evaluations of the patients were negatively affected by the duration of disease duration and the increase in anxiety prevalence (25).

2.5. Diagnosis of Diabetes Mellitus

As diabetes mellitus is characterized by rising in blood glucose, its diagnosis is done by monitoring blood glucose using specific analyses depending to the collected samples. Type 1 diabetes is diagnosed if he/she has a high level of glucose level in the blood, as well as ketonuria that occurs due to the lack of insulin. Usually, these symptoms are present in a short-term basis. Type 2 is diagnosed if the case lacked ketonuria, and the symptoms of high glucose levels were observed over a longer period. Blood glucose level ranges from normal to above normal, which helps to decide whether the condition diabetic or not (26):

- Fasting blood sugar:
  - Normal level: < 110 mg/dl.
  - Cannot be defined as diabetic state: 110–126 mg/dl for fasting condition and <126 mg/dl for glucose tolerance test.
  - Diabetes mellitus level: ≥ 126 mg/dl.
- One hour glucose tolerance non-fasting (rapid)/ Oral glucose tolerance test:
  - Normal level: < 140 mg/dl.
  - Cannot be defined as diabetic state: ≥ 140 mg/dl for glucose tolerance test.
  - Diabetes mellitus level: ≥ 200 mg/dl.
- Hb1Ac:
  - The normal level: < 6% Diabetes Control and Complications Trial (DCCT).
  - Cannot be defined as diabetic state: 6.0%-6.4% for both fasting and glucose tolerance test.
  - Diabetes mellitus level: ≥6.5.

2.5.1. Procedures of the diagnosis of diabetes mellitus

The level of glycated hemoglobin (HbA1c) is tested through a venous blood sample to diagnose and monitor diabetes as its count signifies its bounding to erythrocytes. Thus, the HbA1c level is proportionally the main indicator of the level of glucose. The threshold level of

HbA1c is 48 mmol/mol (over 6%) and it facilitates the diagnosis and treatment plan for the diabetic condition over the next few months. Diabetes can also be diagnosed using a glucose-level reading for a venous plasma in fasting conditions for at least eight hours prior obtaining the sample. A diabetes diagnosis is made if the glucose level in that sample exceeds 7 mmol/liter or 130 mg/dl (27).

2.5.2. Oral glucose tolerance

This test has a prerequisite of a fasting condition with only water allowed at least 9 h prior obtaining the sample. The first step of the test is obtaining a venous blood sample to check glucose level. Then, the patient is asked to intake 75 grams of glucose with water after 10 min. After two hours from the first sample, another venous blood sample is obtained to check glucose level. The timing of the sample taking is crucial for the reliability of the test, which has a tolerance timing of only 5 min of the 120 minutes (28).

2.6. Most commonly used oral antidiabetics

2.6.1. Metformin

Metformin is used orally as a treatment for type 2 diabetes mellitus, especially in overweighted patients and for polycystic ovary syndrome treatment. Metformin is safe as it has not been causing hypoglycemia. The mechanism of action of metformin is through inhibiting hepatic glycogenolysis to reduce glucose production (29). It also improves insulin sensitivity, decreases insulin resistance and, augments peripheral glucose uptake in type1 diabetes patients. Additionally, it is used for gestational diabetes and has the same effects of vildagliptin including inhibiting glucose hepatic production and improving insulin sensitivity (30).

2.6.2. Vildagliptin

Vildagliptin (dipeptidyl peptidase-4 (DPP-4) inhibitor is used orally as monotherapy and combination therapy for type 2 diabetes mellitus. The basis mechanism of vildagliptin is the prohibition of the degradation of glucagon-like-peptide-1 (GLP-1) and minimizes glycemia in patients with type 2 diabetes mellitus with lower risk of hypoglycemia and keeping the body

weight stable. It has been estimated that vildagliptin stimulates insulin secretion and at the same time blocks glucagon secretion in glucose-dependent states. Also, vildagliptin prohibits the production of glucose from the liver by the mechanism of changing in hormone secretion of the pancreatic cells and developing the sensitivity to insulin (31).

2.6.3. Usage of Metformin and Vildagliptin in Anxiety Treatment

There are findings from different studies on the usage of metformin and vildagliptin as anti-anxiety treatments. Zemdegs et al. (4) performed an experiment on insulin-resistant mice and found that a mechanism of metformin reduces the circulation of branched-chain amino acids, and subsequently cause tryptophan uptake regulation in brain. Thus, the authors suggested the drug as a complementary treatment for anxiety. The mechanism was further described by Fan et al. (32), who confirmed the potential of using metformin in anxiety treatment. The authors found that the drug upregulated $GABA_A$ receptors responsible for fast inhibition in the basal ganglia in cell membranes, which increased miniature inhibitory postsynaptic currents and produced anxiolytic effects. For vildagliptin, not many studies adopted the same direct approach. However, experimental results can indicate similar effects. Ma et al. (33) concluded that vildagliptin targeted HepG2 cells to reduce endoplasmic reticulum stress in rats. Maeda et al. (34) stated that vildagliptin reduced oxidative stress in streptozotocin-induced diabetic rats, and reduction of vascular damage in the same subjects was unclear. Swain et al. (5) reported that chronic treatment of 5, 10 and 20 mg/kg vildagliptin significantly decreased the diabetes-induced memory loss and improves the learning skills in rats. decreased circulating and brain oxidative stress levels. Thus preventing brain and hippocampal mitochondrial dysfunction and improving learning behaviours impaired by HFD.

Since vildagliptin decreased in oxidative stress in brain, it (they suggested that) enhance the learning by preventing brain and hippocampal mitochondrial dysfunction.

2.7. Laboratory animal models for diabetes

2.7.1. Laboratory animals for spontaneous diabetes and impaired glucose tolerance models

2.7.1.1. Zucker diabetic fatty (ZDF) rats

A cross between two types of rats: Sherman and Merck resulted in the discovery of this type in 1961. The rats developed obesity by the fourth week due to an induced hyperphagia by a mutation of the leptin receptor. The rats were also characterized with glucose intolerance, hypertensive, hyperlipidemic, and hyperinsulinemia. By the eighth week of age rats had glucose intolerance and high insulin resistance, which caused their diabetic conditions with glucose levels of 500 mg/dL by the tenth week.

2.7.1.2. Biobreeding (BB) rats

In 1974, the identification of autoimmune diabetes in Canada led to deriving this type of rat from outbred Wistar rats. The created colonies included two strains: Biobreeding Diabetes-Prone (BBDP) rats (outbred) and Diabetes-Prone/Worcester (BBDP/Wor) (inbred) through Biobreeding. By the sixteenth week, more than 90% of BB rats develop diabetes with equal incidents between females and males. The diabetes in BB rats is characterized with ketonuria, hyperinsulinemia weight loss, hyperglycemia, which makes insulin treatment imperative for their survival. BB rats also have a near absence of CD4+ and CD8+ T cells, which makes them lymphogenic, despite their insulitis case with the existence of natural killer cells, macrophages, B cells, and T cells. Thus, the use of BB rats to model type 1 human diabetes with biobreeding is considered disadvantageous.

2.7.1.3. Lieu 1ARA1/-iddm rats

A mutation in the lieu.1AR1 caused ZDF rats to become diabetic, which make them favorable to simulate Type 1 human diabetes. By the 90^{th} day, these rats develop diabetes characterized by a destruction of β cells caused by the quick progression of insulitis. The β cell destruction is caused by islet infiltrating immune cells that produce proinflammatory cytokines.

2.7.1.4. Goto-Kakizaki rats

A nonobese model was used to develop Goto-Kakizaki (GK) rats with mild hyperglycemia at an early age. Wistar (W) rats are used for developing GK rats to model Type 2 diabetes. An inbreeding technique is used with glucose tolerant and intolerant rats, which target the impairment of cells and inheritance of polygenic rats.

2.7.1.5. Nonobese Diabetic Mouse

This type of mouse was first developed at Shionogi Research Laboratories in Osaka, Japan, in 1974. Insulitis (disease of the pancreas caused by infiltrating lymphocytes) appears at around 3rd or 4th week of age. During this prediabetic stage, the islets of the pancreas become infiltrated by CD4+ and CD8+ lymphocytes, though natural killer (NK) and B cells are also present. Non-obese diabetic (NOD) mouse is one of the most commonly used models to study type 1 diabetes (T1D). Unlike other models used in autoimmunity studies, this model can develop spontaneous disease similar to humans.

2.7.1.6 Akita Mice

The Akita mouse was initially developed in Akita, Japan, from a C57BL/6NSlc mouse due to a spontaneous mutation in insulin 2 gene leading to incorrect proinsulin processing. This mutation caused the aggregation of misfolded proteins and led, subsequently, to endoplasmic reticulum (ER) stress. These alterations resulted in pronounced insulin-dependent diabetes with an onset of 3 to 4 weeks of age (35).

The resulting rodent model exhibits characteristic signs, including hyperglycemia, hyperinsulinemia, polyuria, and polydipsia (thirsty). This model is usually used to investigate potential alleviators of ER stress in the islets. In this regard, some of the pathological manifestations of type 2 diabetes (T2D) are also visible in the Akita mouse model (36).

2.7.2 Chemically Induced Diabetes

Alloxan and streptozotocin (STZ) are considered the most potent diabetogenic chemicals in diabetes research so far. Both chemicals are employed as cytotoxic glucose analogs that tend to accumulate in pancreatic beta cells through glucose transporter 2 (GLUT2).

2.7.2.1. Alloxan induced diabetes

Alloxan is the derivative of pyrimidine. It dissolves easily in water, the powder form should be stored at 2–8 C and the solution form below 4 ^{o}C. Alloxan produces selective necrosis of beta cells. It induces diabetes in different animals, including dogs, rats, and mice. The animal species used in the experiment is a main determinant for the alloxan dosage used.

Diabetes is induced in mice through:

- Alloxan dissolved in distilled water or saline (0.9% NaCl) at a dose of 150 mg/kg for mice fasted for 18 h is administered intraperitoneally.
- This application is repeated three times in 48 h.

Diabetes is induced in rats through the following:

- Alloxan dissolved in distilled water or saline is administered to rats intraperitoneally at a dose of 120 mg/kg for three consecutive days.
- Three days after the last alloxan application, the rats are fasted overnight, and fasting blood sugars are measured in the morning.
- Those with blood sugar levels above 250 mg/dL are considered diabetes.
- According to another method used to create experimental diabetes, dissolved alloxan in saline solution is administered once intraperitoneally at a dose of 150 mg/kg.
- Depending on the application of Alloxan, dense insulin is secreted from the pancreas.
- Therefore, there is a possibility of developing fatal (lethal) hypoglycemia.
- To prevent this, 15–20 mL of 20% glucose solution is administered intraperitoneally in rats after 4–6 hours.

2.7.2.2. Streptozotocin (STZ) induced diabetes

Streptozotocin is naturally occurring alkylating chemical that particularly produces a toxin to beta cells of the pancreas. STZ acts as a metabolite, DNA synthesis inhibitor, antimicrobial agent, and antineoplastic agent. It comes as an off-white powder form and has a melting point of 115 °C. Two hours after injection, the hyperglycemia occurs due to decrease in blood insulin levels. Streptozotocin impairs glucose oxidation and decreases insulin synthesis and release. It changes the DNA in pancreatic β cells. The β cell death due to alkylation of DNA by STZ. Thus, it damages the pancreatic β cells, causing both insulin dependent and insulin independent diabetes:

- Insulin dependent diabetes of intravenous STZ administration in a single dose (40–60 mg/kg) in adult rats.
- It has been reported that administration of 100 mg/kg STZ to new-born rats by a single dose intraperitoneally or intravenously causes diabetes independent of insulin.
- The dosage can also be administered multiple times and in small doses (5 mg / kg / day) for 5–6 consecutive days, this is called sub diabetic dosing.

Diabetes is induced in mice through the following:

- STZ dissolved in citrate buffer (pH: 4.5) is administered intraperitoneally in a single dose (200 mg / kg). Diabetes mice are considered to occur on the same day.
- It has been reported that STZ administered to mice at a dose of 150 mg/kg (single dose) intraperitoneally causes experimental diabetes (37).

Diabetes is induced in rats through the following:

- Diabetes was created by injecting freshly prepared STZ solution in 20 mM sodium citrate buffer (pH: 4.5) to 45 mg/kg (single dose) intraperitoneally in rats, in rats.
- In another study, rats that were fasted overnight before were administered STZ dissolved in 0.1 M citrate buffer (pH: 4.5) at a dose of 60 mg/kg, and after 72 h, rats with a blood glucose level of 350 mg/dL and above had diabetes mellitus.
- It was reported that it was accepted and included in the study and feed and water intake was released after STZ application (37).

Streptozotocin-induced diabetes is of two types: the adult type and neonatal type. In case of the adult type STZ-induced diabetes, rats weighing 140 to 300 g received a single STZ intraperitoneal injection (45– 70 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5) after an overnight fast. Control rats of the same age received only an injection of citrate buffer.

In the case of STZ-induced diabetes in mice, metformin does not directly affect insulin levels (33). However, the experiment performed by Han et al. (34) showed that metformin reduced insulitis in STZ-induced diabetes in mice through its anti-inflammatory action. The authors explained that STZ cause the destruction of β-cells in the pancreas leading to the secretion of TNF-α and IL-1β cytokines, which were evident through their infiltration levels. The use of metformin reduced the secretion of those proinlammatroy cytokines. The study presented metformin as a protective medication against diabetes development rather than a direction medication to cause hypoglycemia.

2.8. Anxiety Tests Used in this Research

2.8.1 Open Maze

Open field maze is the most commonly used test to test the behavior of animal models through several attributes. The ambulatory distance traveled within the maze is a indicator, where animal that travel intensively at the outer parameters are deemed to have higher anxiety levels. An increase in defecation is another indicator of increased anxiety, which was proportionally correlated with thigmotaxis (38). Freezing and self-grooming are other indicators that were associated with increased anxiety-like behavior in mice/rat in open field maze, in addition to crossing and rearing (39). The maze consists of a square field with closed parameters. The side length of the maze is 50 cm and can be increased depending on the animal size. The area of the maze is typically divided into sub-areas: centre and perimeter, or equal sub-areas, depending on the study type. The behaviour of the animal is entered from the top of the maze at the centre. After that, the specific behaviour of the animal is monitored, as well as the location of the behaviour to test the ambulatory or emotional traits of the animal.

2.8.2. Elevated Plus Maze

While the same attributes that are used for the open field maze are also used in the elevated plus maze, the main attributed targeted by the latter is the frequency and duration spent by the mouse in the closed arm and open arm. Increased fear and anxiety-like behavior is demonstrated by the avoidance of open arm due to fear of height and open spaces (40). The anxiety level is correlated reversely with the amount of activity demonstrated by the rate. Thus, the lower the activity and the tendency to stay in the closed arm part of the maze is correlated to higher anxiety levels (41). The maze consists of two perpendicularly crossed arms at their centres, where the perimeter in one arm is closed and the other is open. The width of each arm ranges between 5 to 10 cm, the length of the arms are typically 100 cm from tip to tip, and the height of the maze above the floor is 50 cm. The maze is used to observe anxiety-like behaviour in rodents. Anxiolytic or anxiogenic compounds are administrated to the animals.

3. MATERIALS AND METHODS

3.1. Experimental Animal

A total of 50 male, 8 weeks old age Wistar rats were used for this experiment (Figure 3.1). Animals was obtained from Başkent University, Experimental Animal Breeding Center. They were housed in standard cages; water and feed were given *ad libitum*. All animal experiments conducted in Başkent University, Experimental Animal Research Center. The experiments were conducted after the approval of the Başkent University Ethical Committee for Experimental Research on Animals (DA20/22).

Figure 3. 1. Wistar rats used in the experiment

3.2. Experimental Equipment

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

The equipment used for the experiment were as follows:

- Body weight scale equipment: In purpose to decide each animal's dose of drugs according to their body weight.

- Gavages: To give the drugs to the animals orally.

Figure 3. 2. Gavage for oral dose

- Streptozotocin (Sigma 50130): To induce diabetes.

Figure 3. 3. Streptozotocin

- GlucoDr: Glucose measurement equipment to measure & check the glucose blood levels.

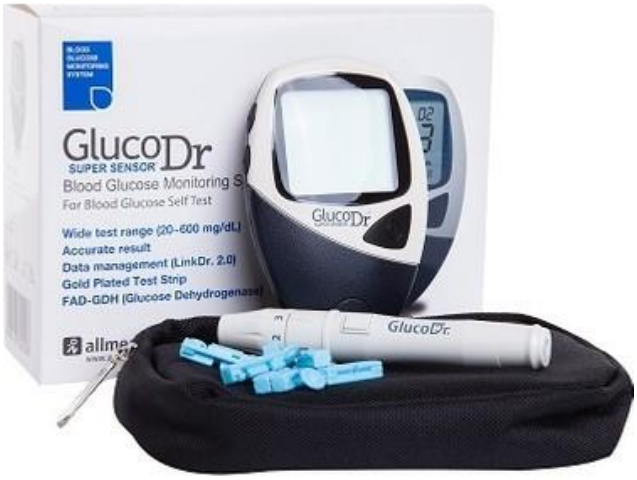

Figure 3. 4. Blood glucose monitoring equipment

- Sensitive scale: To get the precise dose of the animal depending on its body weight.
- Metformin (Glucophage 1000 mg, Brand?).
- Vildagliptin (Galvus 50 mg, brand?)

Figure 3. 5. Metformin and vildagliptin tablets used in experiment

3. 3. Maze experiments

Two maze types were used in the experiment:

- Open field maze: as shown in Figure 3.6, the maze consists of a wooden box with the center marked. The behavior of the rat is monitored through a video recording to measure anxiety levels.

Figure 3. 6. Open field maze

- Elevated plus maze: as shown in Figure 3.7, the maze is shaped into a plus sign configuration with two open and two closed sides. The maze is elevated from the floor and it indicates the behavioral anxiety of the rat. The behavior of the rat is monitored through a video recording for analysis.

Figure 3. 7. Elvated plus maze

3.4. Experimental groups and treatments

The animals were divided into five groups with 10 subjects in each one of them:

- The control group (C): Drank tap water until the treatment groups got diabetes. Then, took just water by gavage during the experimental period.

1. Body weights of treatment groups were measured to calculate the dosages of each applied drug. Drug dosages were calculated according to their weight. They were dissolved in normal saline (Figure 3.8).

Figure 3. 8. Diabetes treatment doses for each animal

- 45 mg/kg Streptozotocin (Sigma 50130) was injected intraperitoneally (Figure 3.9) after dissolving in normal saline and homogenized with a shaker to induce diabetes to rest of 40 of 50 animals (Figure 3.9).

Figure 3. 9. İntraperitoneal streptozotocin injection

- After 3 days, blood glucose levels were measured by glucometer with tail blood. After it was seen that their glucose levels were more than 250 mg/dl, they were divided into 4 treatment groups:
- Group with diabetes (D): Only water was administered by gavage for 15 days.
- Metformin group (DM): 500 mg/kg metformin (Glucophage 1000 mg, Merck Serono) was given by gavage for 15 days.
- Vildagliptin group (DV): 50 mg/kg vildagliptin (Galvus 50 mg, Novartis) was given by gavage for 15 days.
- Metformin+vildagliptin group (DMV): 500 mg/kg metformin+50 mg/kg vildagliptin was given by gavage for 15 days.
- As shown in Table 3.1, to perform the anxiety tests in all groups at the same time interval, the applications were made in 3 consecutive days and the anxiety tests were performed in the same order.
- Anxiety levels of animals were analyzed using an open field maze for 5 min and an elevated plus maze for 10 minutes, respectively. Waited 2 h between two tests. Behavior of animals were recorded using video footage. The rat was left at

the center of each maze and records of their rearing, location of movement, urination, and defecation were recorded.

Figure 3. 10. Diabetes treatment given to animals using savages

Table 3. 1. Drugs administration and analysis schedule for study groups

Days	Experimental step
1	Streptozocin injection to all animals except the control group
3	Blood glucose level was checked
4	Metformin was given by gavage to the DM group during 15 days
5	Vildagliptin was given by gavage to the DV group during 15 days
6	Metformin+vildagliptin was given by gavage to the DMV group during 15 days
19–21	Checked blood glucose again and conducted the anxiety tests in 3 consecutive days according to the treatment order.

3.5. Statistical Analysis

All data were analyzed by GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Comparisons were made by One-way analysis of variance (ANOVA) and followed by Tukey's multiple ranges to reveal the specific mean differences and statistical significance ($p < 0.05$).

All statistical analyses were considered statistically significant when the p value was 0.05, and highly significant when the p value was 0.001.

4. RESULTS

4.1. Blood glucose levels of rats on the 15th day of treatment

Blood glucose levels were measured 3 days after STZ injection and those above 250 mg/dl were considered diabetic. After the 15th day of treatment, blood glucose levels of the rats were remeasured and maze tests were started. As shown in Table 4.1, all the blood glucose values of the groups with diabetes (D, DM, DV, DMV) were still higher than the control group on 15th days.

Table 4. 1. Blood glucose values of the streptozocin-induced diabetic rats on 15th days (mg/dl)

Groups	**C** Mean±SE	**D** Mean±SE	**DM** Mean±SE	**DV** Mean±SE	**DMV** Mean±SE
Blood glucose levels	84.40±2.12	534,9±52.08	581,2±54.16	496,6±55.95	545,4±45.63

4.2. The Results of Open Field Test

4.2.1. The number of rearing

As shown in Figure 4.1, while the number of rearing of the D group was higher than the C group p<0.001), the number of rearing of the DM, DV and DMV group were lower than the diabetic control group (p<0.001, p<0.05 and p<0.001 respectively).

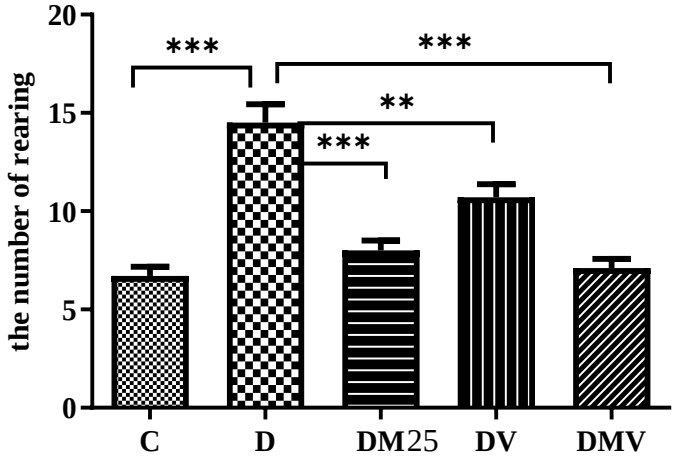

Figure 4. 1. The number of rearing of the control and treatment groups. **p<0.01 and ***p<0.001

4.2.2. Movement location

As shown in Figure 4.2, while there was a statistically significant between control and D group and DM groups in terms of time spent in the center, the time spent in the center of the DMV group was the highest compared to all treatment groups (D, DM, and DV) (p<0.01), time spent in periphery was lower in DMV group than the D, DM and, DV group (all were p<0.05) (Figure 4.3).

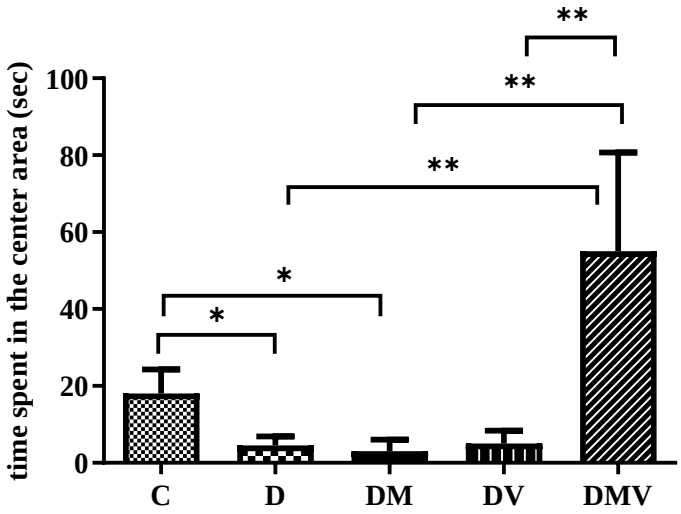

Figure 4. 2. Time spent in the center area of the control and treatment groups *p<0.05, **p<0.01

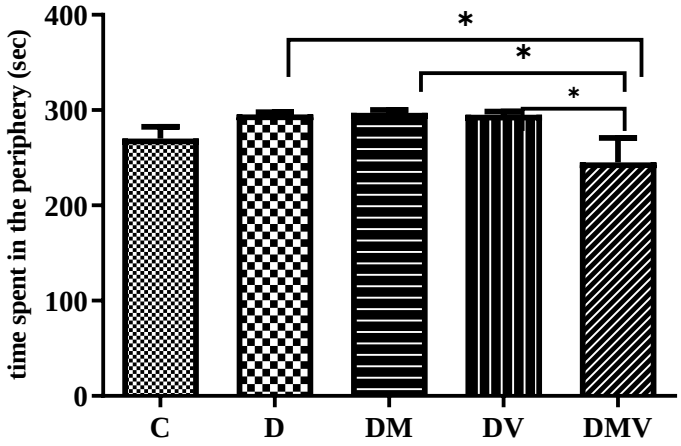

Figure 4. 3. Time spent in the periphery of the control and treatment groups. *p<0.05

4.2.3. Urination

The anxiety of the animals was also measured through the occurrence of urination during the period spent in the open field maze. As shown in Table 4.2, there were no significant differences in terms of urination number of the groups. However, when the table is examined, an increase in urination in the D, M, and, V groups compared to the control groups; and a decrease in the DMV group compared to the group with diabetes paid attention.

Table 4. 2. The number of urinations of the control and treatment groups

Groups	C Mean±SE	D Mean±SE	DM Mean±SE	DV Mean±SE	DMV Mean±SE
The number of urinations	0.60±0.22	1.20±0.13	1.20±0.13	1.20±0.13	0.80±0.20

4.2.4. Number of defecation

While the number of defecation was higher in the D group than the C group (p<0.05), the number of defecation of the DMV group was lower than the D group (p<0.1) (Figure 4.4)

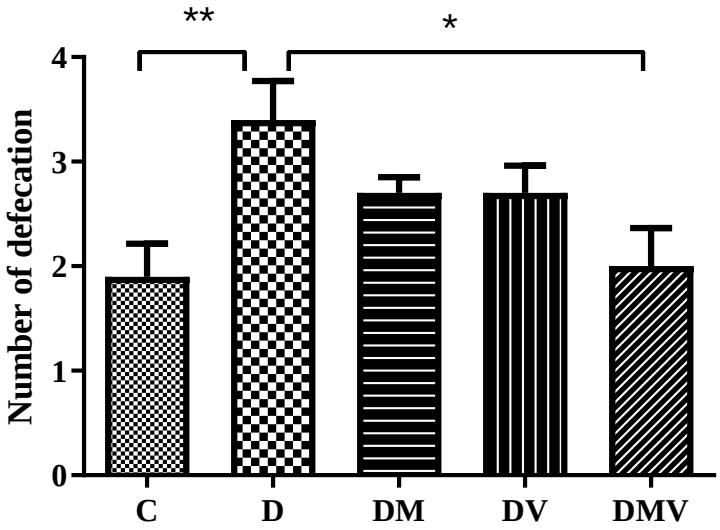

Figure 4. 4. The number of defecation of the control and treatment groups *p<0.05, **p<0.01

4.3. The Results of Elevated Plus Maze

Animals were left in the elevated plus maze for ten minutes and the timing according to the location of their movement was recorded. Timing was recorded for the time spent in the open arm and closed arm segments.

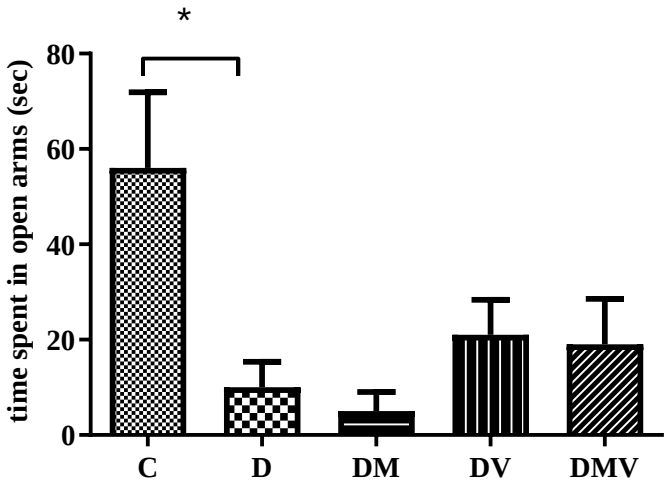

Figure 4. 5. Time spent in open arms of the control and treatment groups. *p<0.05

When evaluating the time spent in open arms, it was seen that the diabetic group spent lower time than the C in open arms (*p<0.05) (Figure 4.5).

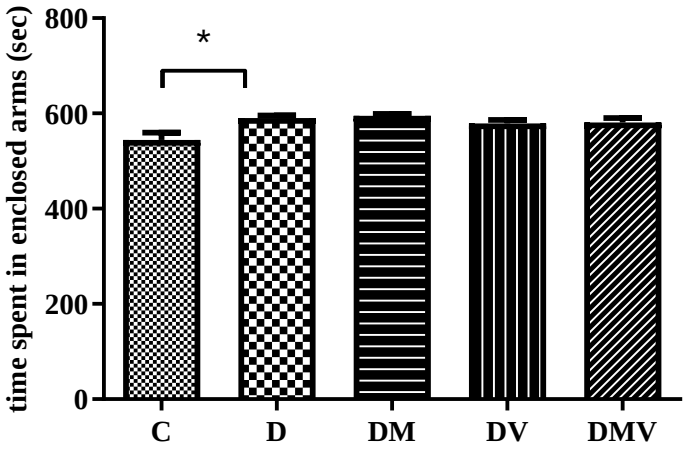

Figure 4. 6. Time spent in enclosed arms of the control and treatment groups.*p<0.05

As shown in fig 4.6, while the time spent in enclosed area of the groups with diabetes were not significant, diabetic control group was more spent time in enclosed arms compared to control group (*p<0.05).

5. DISCUSSION

The present study investigated the effects of metformin and vildagliptin and their combination on anxiety in streptozotocin-induced diabetic rats. We obtained STZ-induced diabetes, but it did not cause hypoglycemia. STZ-induced diabetes with metformin treatment did not lower blood glucose with the same mechanism of insulin as shown by Zhou et al. (33) and Han et al. (34). Zhou et al. (33) showed that metformin effect in STZ-induced diabetic mice is comparable to RGP through anti-oxidative effects on the islets. Similarly, Han et al. (34) attributed the effect of metformin in STZ-induced diabetic mice to its anti-inflammatory effects through the reduction in TNF-α and IL-1β cytokines' secretion.

In the open field test, anxiety behavior is triggered by the rats being taken from their accustomed environment and placed alone in a new environment by leaving their social group. Also, taking the animal from a familiar environment and leaving it alone in an unfamiliar environment is an important factor that causes anxiety. Because these spaces are larger than their natural environment (agoraphobia). Also, rodents show exploration behavior by changing their locomotion when they have anxiety. These are the behavioral features of rodents (42). Rearing behavior is a standing position on both hind paws of the rat. It is known as exploratory behavior. However, the views that rearing behavior is an indicator of decreased or increased anxiety are unclear. Some studies show that increased rearing is consistent with increased anxiety levels, while others postulate that decreased rearing behavior indicates increased anxiety (43). Our results obtained from the open field maze showed that while the number of rearing increased in the group with diabetes compared to control; the number of rearing decreased in the treatment groups compared to the D group. When this behavior is evaluated together with other behaviors in the open field test, it may consider that it shows the anxiogenic effect in diabetics and anxiolytic effect on the treatment groups.

Since rats are accustomed to being fed ad libitum, they try to search for food and water in the open field test and their travel increases due to this search (42). The desire of rats to spend time away from the center and close to the platform wall is called "thigmotaxis" and is considered an anxiety-like behavior. In other words, increased anxiety results in a preference to stay close to the wall of the field (44). In open field, while the time spent in the center was

decreased in a group with diabetes, DM and, DV groups (in DV groups, the decrease was no statistically significant), time spent in the center of the DMV group increased compared to other treatment groups (D, DM, and DV groups). However, although there was no statistical significance between control and D group in terms of time spent in the periphery, it was decreased in the DMV group compared to other diabetic groups. When all the ambulation results evaluate together, results may show while diabetes induces anxiety, combined treatment of metformin and vildagliptin may have an anxiolytic effect.

In 1934, Hall described the open field test for evaluating emotionality in rats. When he compared to non-emotional rats, emotional rats spent less time in the central part compared to the periphery and their defecation number was higher than the non-emotional ones. And, he reported that urination and defecation are the measures of individual differences in emotionality. The emotional animal moves less, avoids going to the center of the field, and the number of defecation increases. In our study, although there were no statistical differences in the number of urinations between groups, it could be seen that the number of urinations was higher than the control group than groups with diabetes, and it was reduced in the DMV group. Also, defecation numbers can be acceptable as a function of an autonomous function (45). In our study, while the number of defecation was higher than control in diabetics, it reduced in treatment groups, especially there was a statistically significant in the DMV group. Considering the abovementioned knowledge, we may say that while autonomic functions increased in the group with diabetes, this function was reduced by the treatment of metformin and vildagliptin combination compared to non-emotional rats, emotional ones had fewer entries in the central part of the arena and higher levels of defecation.

The overall results of the study show that diabetic animals have higher anxiety levels than healthy animals consisting of the control group. Additionally, the combined treatment of metformin and vildagliptin resulted in reduced anxiety levels compared to the diabetic control group, reducing the number of rearings, time spent in the periphery, and the number of defections in the open field maze.

The elevated plus maze is used to assess rats' behavior such as anxiety, exploration, learning, and memory. In rats, the tendency to stay close to vertical surfaces is considered exploratory behavior in this test, and the time spent in closed arms increases when it has

anxiety. In our study, while the time spent in the open arm decreased in the group with diabetes compared to the C group, there was no statistical significance among the group with diabetic and the treatment groups. However, parallel to the results of the Leo & Pamplona (40), our results showed that group with diabetes showed significantly more tendency to stay in the closed arm of the maze and showed lower movement than the control group, which is an indication of higher anxiety levels (41). Additionally, the time spent in the enclosed area was not statistically significant in the treatment groups. As a result, while the decrease in the time spent in the open arm in the group with diabetes compared with the control group showed that anxiety increased in the D group. The fact that there was no difference other than the increase in the time spent in the enclosed arm in the group with diabetes compared to the control group showed the same effect.

Research in the literature shows that there are strong connections between diabetes and anxiety. Rajashree et al. (1) declared that streptozotocin-induced diabetic rats had cognitive deficits, as well as increased levels of anxiety in the elevated plus maze test. Moreover, they found that the effects of these issues increase over time. Huerta-Cervantes et al. (2) reported that gestational diabetes cause increased anxiety in the second generation of diabetic rats, which indicates long-term effects and involvement of genetic factors. Also, Bilu et al. (3) declared that diabetic animals showed higher anxiety levels, which is confirmed through our results from the open field maze and the elevated plus maze. However, our results showed that metformin and vildagliptin combination therapy reduced rearing number, time spent in the periphery, and a range of defecation, which are indicators of anxiety reduction.

Gamma aminobutyric acid (GABA) is an inhibitory neurotransmitter, and decrease the concentration or expression of its receptor (called $GABA_A$ receptor) causes anxiety diseases (46). Fan et al. (32) reported that metformin has an anxiolytic-like effects by changing the function or expression of $GABA_A$ receptors. Additionally, Zemdegs et al. (4) showed that metformin decreases anxiety by reducing branched-chain amino acids that regulate tryptophan uptake in the brain. Also, it causes augmentation of hippocampal serotonergic neurotransmission which is a possible reason for anxiety. We think that the reason why we did not see an anxiolytic effect of metformin in the group administered only metformin, contrary

to the results of Fan and Zemdeg, may be due to the chronic administration of metformin in the first study and the in vitro study of the second.

Swain et al. (5) demonstrated improved memory and learning ability in mice with diabetes treated via a DPP-IV inhibitor, vildagliptin (10 and 20 mg/kg/day for 60 days). Also, it was reported that DPP-IV inhibitors reduced blood and brain oxidative damage in 3 and 30 mg/kg/day vildagliptin-treated of rats for 21 days (47). Additionally, it was declared that long term inhibitors of DPP-IV inhibitors cause to memory problems in Alzheimer's models by depositing amyloid-beta in the brain (48). Suh et al. (6), reported that the combination of metformin and vildagliptin was clinically more effective for decreasing blood glucose in type 2 diabetes. However, there was no effect of vildagliptin on STZ-induced anxiety in our study except for the number of rearing. The reason why we found no difference in the vildagliptin groups in our study may be that our treatment period was not as long as they did. Nevertheless, it's combination with metformin was more effective for anxiety compared to the use alone in our study.

The results indicated that diabetic animals had higher anxiety levels compared to nondiabetic, and combined treatment of metformin and vildagliptin may lessen the anxiety in streptozocin-induced diabetic rats.

6. CONCLUSION

The main aim of this research was to understand the effects of metformin and vildagliptin on anxiety. Fifty male Wistar rats were used for the study and divided equally on five groups. Diabetes was induced in all rats using streptozotocin, except for the control group. Apart from the control and groups with diabetes, the remaining three groups were treated with 500 mg/kg metformin, 50 mg/kg vildagliptin, and their combined for 15 days. After that, each rat was tested with an open field for 5 min elevated plus maze for 10 min.

Since streptozocin-induced diabetes cause β cell destruction, the islets cannot produce insulin and any antidiabetic drugs affect the blood glucose and, because of the β cell death, insulin-dependent diabetes develops. Diabetes can cause anxiety. The results indicate that vildagliptin and metformin treatments did not reduce anxiety solely when compared to diabetic subjects. However, usage of combined treatment of both drugs is possible to hinder this effect. It means these oral antidiabetics might be used to reduce anxiety in patients with diabetes.

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APPENDIX 1: PROJECT APPROVAL

Evrak Tarih ve Sayısı: 09.12.2020-35646

1993

BAŞKENT ÜNİVERSİTESİ

Tıp ve Sağlık Bilimleri Araştırma Kurulu

TS-EN-ISO 9001
KALİTE SİSTEM BELGESİ

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 Mohammed Abdulsalam Ammara Alshareefİn sorumluluğunda yürütülecek olan DA20/22 nolu "Effect of vildagliptin & metformin on anxiety in streptozotocin induced diabetic rats" başlıklı araştırma projesi Kurulumuz ve Hayvan Deneyleri Yerel Etik Kurulu Kurulu'nun 23/11/2020 tarih ve 20/20 sayılı kararı ile uygun görülmüştür. Projenin başlama tarihi ile çalışmanın sunulduğu kongre ve yayımlandığı dergi konusunda Kurulumuza bilgi verilmesini rica ederim.

e-imzalıdır

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 Resarch on Animals (Project no:…) and supported by Baskent University Research Fund.

DAĞITIM
Sağlık Bilimleri Enstitüsü Müdürlüğüne
Fizyoloji Anabilim Dalına

APPENDIX 2: ETHICS COMMITTEE APPROVAL

1993
BAŞKENT UNIVERSITY

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

Project DA20/22 no entitled "Effect of vildagliptin & metformin on anxiety in streptozotocin induced diabetic rats" 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.