

T.C.

YEDİTEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF PHARMACEUTICAL TOXICOLOGY

**INVESTIGATION OF THE EFFECTS OF
METFORMIN AND VILDAGLIPTIN ACTIVE
INGREDIENT PREPARATIONS ON RAT
TELOMERASE ENZYME AND OXIDATIVE
STRESS PARAMETERS IN COMPARISON WITH
HEALTHY CELLS**

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.



09.02.2023

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BEYAN

Bu tezin kendi çalışmam olduğunu, planlanmasından yazımına kadar hiçbir aşamasında etik dışı davranışımın olmadığını, tezdeki bütün bilgileri akademik ve etik kurallar içinde elde ettiğimi, tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara kaynak gösterdiğimi ve bu kaynakları kaynaklar listesine aldığımı, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını beyan ederim.

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LIST OF SYMBOLS AND ABBREVIATIONS

AGE: Advanced glycation end-product

AMPK: AMP-activated kinase

ATP: Adenosin triphosphate

BB: Bio-breeding

BGL: Blood glucose level

BMI: Body mass index

CAT: Catalase

DM: Diabetes mellitus

DPP4: Dipeptidyl peptidase-4

ER: Endoplasmic reticulum

FFA: Free fatty acid

FPG: Fasting plasma glucose

GAD: Glutamic acid decarboxylase

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

GDM: Gestational diabetes mellitus

GIP: Gastric inhibitory polypeptide

GLP-1: Glucagon-like peptide-1

GLUT-4: Glucose transporter type-4

GMO: Genetically modified organism

GPx: Glutathione peroxidase

GR: Glutathione reductase

GSH: Glutathione

GST: Glutathione S-transferase

H₂O₂: Hydrogen peroxide

H&E: Hematoxylin-eosin

HADYEK: Yeditepe University animal experiments local ethics committee

HbA1c: Glycosylated hemoglobin

HLA: Human leucocyte antigen

HNF: Hepatocyte nuclear factor

HOCl: Hypochlorous

IA-2: Islet antigen-2

IDDM: Insulin-dependent diabetes mellitus

IFCC: International Federation of Clinical Chemistry

IGF: Insulin-like growth factor

IGT: Impaired glucose tolerance

IPF: Insulin promoter factor

IRS: Insulin receptor substrate

L: Lipid radical

LHP: Lipid hyperperoxide

LOO: Lipid peroxy radical

LOOH: Lipid hydroperoxide

LP: Lipid peroxidation

MDA: Malondialdehyde

MELAS: Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like syndrome

MG: Methylglyoxal

MODY: Maturity onset diabetes of the young

MPO: Myeloperoxidase

mTOR: mammalian target of rapamycin

NADPH: Nicotinamide adenine dinucleotide phosphate

NIDDM: Non-insulin-dependent diabetes mellitus

NO: Nitric oxide

NOD: Non-obese diabetic

NOX: NADPH oxidase

OGTT: Oral glucose tolerance test

PB: Peripheral blood

PI3K: Phosphoinositide 3-kinase

PIP-3: Phosphatidylinositol (3,4,5)-triphosphate

PUFA: Poly-unsaturated fatty acid

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

SelP: Selenoprotein P

SGLT-2: Sodium-glucose co-transporter-2

SOD: Superoxide dismutase

STZ: Streptozotocin

T1DM: Type 1 diabetes mellitus

T2DM: Type 2 diabetes mellitus

TBA: Thiobarbituric acid

TBH: t-butyl hydroperoxide

TCA: Tricarboxylic acid

TEL: Telomere length

TERT: Telomerase reverse reductase

TNF: Tumor necrosis factor

TZD: Thiazolidinediones

ZnT8: Zinc transporter-8

ABSTRACT

Ezgi, T. (2023). Investigation of The Effects of Metformin and Vildagliptin Active Ingredient Preparations on Rat Telomerase Enzyme and Oxidative Stress Parameters in Comparison with Healthy Cells. Yeditepe University, Institute of Health Science, Department of Pharmaceutical Toxicology PhD thesis, İstanbul.

Aim: Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder which characterised by hyperglycemia and oxidative stress. First-choice treatment procedure is application of oral anti-diabetics such as metformin (MET) and vildagliptin (VILDA). Recently, MET published as a possible anti-catalyst for the aging process related with telomerase enzyme (TE) and telomere length. According to the experimental results, MET increased TE activity by decreasing the oxidative stress level caused by T2DM. Therefore, the main purpose of this study is to find out anti-diabetics' effects in both monotherapy (MET and VILDA) and combination (MET/VILDA) therapy on aging process associated with oxidative stress and enzymatic defense system.

Material & Method: Adult female Spraque-Dawley rats were used. T2DM was induced by single dose i.p. administration of 80 mg/kg streptozotocin (STZ). Treatment group was treated by 100 mg/kg/day MET and 6 mg/kg/day VILDA for 8 days. Erythrocyte and tissue samples were used for determination of malondialdehyde (MDA) formation, glutathione peroxidase (GPx) and catalase (CAT) activities by spectrophotometric method. Serum samples were used for determination of TE activity by Rat-Telomerase ELISA Kit.

Results: Erythrocyte MDA formation in MET/VILDA group was significantly higher than control group, MET, and VILDA however, in VILDA; were significantly lower than MET/VILDA and MET. Also, GPx and CAT activities in VILDA group were significantly higher than control group, MET, and MET/VILDA; GPx activity in MET was significantly lower than VILDA and MET/VILDA. TE activity is significantly higher in MET and VILDA than control. The effects of MET on TE activity are significantly higher than VILDA. In MET/VILDA, TE activity is significantly lower than control, MET, and VILDA.

Conclusion: Vildagliptin alert the GPx and CAT activities to reduce oxidative stress but metformin was found insufficient to activate GPx. Also, Vildagliptin increased TE activity as Metformin however, binary combination reduced TE activity.

Key words: Type 2 Diabetes Mellitus; Oxidative Stress; Telomerase Enzyme Activity



ÖZET

Ezgi, T. (2023). Metformin ve Vildagliptin Etken Maddeli Preparatların Sıçan Telomeraz Enzimi ve Oksidatif Stres Parametreleri Üzerine Etkilerinin Sağlıklı Hücrelerle Karşılaştırmalı Olarak Araştırılması. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Farmasötik Toksikoloji ABD., Doktora Tezi. İstanbul.

Amaç: Tip 2 Diabetes Mellitus (T2DM), hiperglisemi ve oksidatif stres ile karakterize metabolik bir hastalıktır. Tedavisinde ise ilk tercih olarak metformin ve vildagliptin gibi oral antidiyabetikler uygulanmaktadır. Son zamanlarda metformin, telomeraz (TE) enzim aktivitesi ve buna bağlı olarak telomer uzunluğu ile ilişkili yaşlanma süreci için olası bir anti-katalizör olarak yayınlanmıştır. Deneysel sonuçlara göre metformin, T2DM'nin neden olduğu oksidatif stres seviyesini azaltarak TE aktivitesini arttırmaktadır. Bu nedenle, çalışmanın temel amacı, hem monoterapide (MET ve VILDA) hem de ikili kombine tedavide (MET/VILDA) anti-diyabetiklerin oksidatif stres ve enzimatik savunma sistemi üzerine olan etkilerini yaşlanma süreci ile bağlantılı olarak araştırmaktır.

Materyal & Metot: Çalışmada, yetişkin dişi Spraque-Dawley ırkı sıçanlar kullanılmıştır. Deneysel gruplara tek doz i.p. 80 mg/kg streptozotocin (STZ) uygulanarak T2DM oluşturulmuştur. Tedavi grupları 100 mg/kg/gün metformin ve 6 mg/kg/gün vildagliptin ile 8 gün boyunca tedavi edilmiştir. Malondialdehit (MDA) oluşumu, glutatyon peroksidaz (GPx) ve katalaz (CAT) enzim aktivitelerinin spektrofotometrik yöntemle belirlenmesi için eritrosit ve doku örnekleri kullanılırken, Rat-Telomerase ELISA Kit ile TE aktivitesinin belirlenmesi için serum örnekleri kullanılmıştır.

Bulgular: MET/VILDA'da eritrosit MDA formasyonu; kontrol, MET ve VILDA'dan anlamlı derecede yüksek bulundu ancak, VILDA'da MDA formasyonu; MET/VILDA ve MET'den önemli ölçüde düşük olduğu saptanmıştır. Ayrıca, VILDA'daki GPx ve CAT enzim aktivitesi; kontrol, MET ve MET/VILDA'dan önemli ölçüde yüksek olduğu görülürken, MET'deki GPx enzim aktivitesi; VILDA ve MET/VILDA'dan önemli ölçüde düşük bulunmuştur. Bununla beraber, TE aktivitesi; MET ve VILDA'da kontrolden önemli ölçüde daha yüksek olduğu görülmüş olup, MET'in TE aktivitesi

zerindeki etkileri VILDA'dan daha yksek bulunmuřtur. Ancak, MET/VILDA'da TE aktivitesi; kontrol, MET ve VILDA'ya oranla daha dřk olarak saptanmıřtır.

Sonu: Vildagliptin, oksidatif stresi azaltmak iin GPx ve CAT enzim aktivitesini alarına geirmiřtir ancak metformin, GPx'i aktive etmek iin yetersiz kalmıřtır. Ayrıca Vildagliptin, Metformin gibi TE aktivitesini bir miktar arttırırken, ikili kombinasyon tedavinin TE aktivitesini azalttıęı grlmřtır.

Anahtar kelimeler: Tip 2 Diabetes Mellitus; Oksidatif Stres; Telomeraz Enzim Aktivitesi



1. INTRODUCTION

1.1. Diabetes Mellitus

Diabetes Mellitus (DM) is a metabolic disease associated with an elevated blood glucose level (BGL) and is categorized into type 1 (T1DM) and type 2 (T2DM). T1DM generally results from impaired insulin secretion and usually affects children and/or adolescents. T1DM has a lower incidence among middle-aged humans. T2DM results from insulin resistance or insufficient release of insulin and affects adults and pregnant women. T1DM and T2DM show different pathogenesis and etiologies; therefore, each type requires different treatments [1].

T1DM is characterized by the destruction of endocrine cells in the pancreas, and the most common outcome is impaired insulin secretion. Insufficient secretion of insulin or decreased cellular sensitivity to insulin may cause an imbalance in BGL. Obesity and aging are the most important factors in the development of insulin resistance. Moreover, genetic factors are assumed as risk factors for both types [1]. Accumulating evidence suggests that genetic factors are more strongly associated with the occurrence of T2DM is rather than T1DM [2].

T2DM development is usually related to defects in insulin secretion from the pancreas and the inability of tissues to utilize insulin [4]. The pancreas has two specific endocrine cells placed in Langerhans islets, namely the beta and alpha cells. Beta cells are responsible for insulin production, and alpha cells for glucagon secretion. Hormone secretion status from beta and alpha cells changes according to glucose levels. When the BGL increases, insulin is released by beta cells to lower the BGL, while a decrease in the BGL induces glucagon release from alpha cells to raise the BGL. Accordingly, insulin/glucagon imbalance is the most important reason underlying abnormal glucose levels [1].

Patients with DM have the potential to develop hyperglycemia. Consequentially, hyperglycemia leads to a disrupted metabolic state. A BGL higher than 180 mg/dL is usually recognized as of hyperglycemic grade. High BGL may cause saturation of the glucose transporters in the kidney; therefore, the most common symptoms are polyuria and polydipsia, especially in patients who show a BGL above 250 mg/dL. Insulin resistance might occur as a result of proinflammatory cytokine activity, which leads to

abnormal glucose and lipid metabolisms, such as non-enzymatic glycosylation of proteins and lipids. Therefore, glycosylated hemoglobin (HbA1c) measurement plays an important role in diagnosing DM [3].

1.2. Diabetes Diagnosis Criteria

Although DM causes several pathological conditions, the most widely accepted diagnostic criterion is the hyperglycemic state of patients. Diagnostic cut-off points for DM have been determined by the measurement of BGL, which is expected to increase diabetic episodes. Fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), and glycosylated hemoglobin (HbA1c) are used to identify patients at risk (Table 1). Patients with an FPG lower than 6.1 mmol/l and OGTT lower than 7.8 mmol/l are identified as non-diabetic, while an FPG higher than 7.0 mmol/l and OGTT higher than 11.1 mmol/l indicates persistent DM [7]. Furthermore, the severity of the hyperglycemic episode is higher in patients with an FPG threshold of 7.0 mmol/l than those with an OGTT threshold of 11.1 mmol/l [7,8].

Table 1. Diabetes mellitus and prediabetic states as defined by the OGTT (75 g anhydrous glucose).

FPG		OGTT	Interpretation
< 6.1 mmol/L	and	< 7.8 mmol/L	No diabetes
6.1-6.9 mmol/L	and	< 7.8 mmol/L	Impaired fasting glycaemia
< 7.0 mmol/L	and	7.8-11.0 mmol/L	Impaired glucose tolerance
			Consistent with diabetes
≥ 7.0 mmol/L	and/or	≥ 11.1 mmol/L	mellitus

*FPG: Fasting plasma glucose, *OGTT: Oral glucose tolerance test

In the following papers, new diagnostic criteria for DM have been reported by the American Diabetes Association (1997), the World Health Organization (1998), and the Japan Diabetes Society (1999) (Table 2) [7,8]. According to these criteria, the upper limit of FPG has been accepted as 7.0 mmol/l (126 mg/dl), HbA1c as 6.5%, and OGTT as 11.1 mmol/l (200 mg/dl) in the diagnosis of DM [13].

Table 2. Criteria for the diagnosis of DM

-
1. American Diabetes Association:
 - * FPG 5.3 mmol/L
 - * 1-hour OGTT 10.0 mmol/L
 - * 2-hour OGTT 8.6 mmol/L
 2. World Health Organization:
 - * FPG ≥ 7.0 mmol/L
 - * 2-hour OGTT ≥ 7.8 mmol/L
 3. Japan Diabetes Society:
 - * FPG ≥ 5.5 mmol/L
 - * 2-hour OGTT ≥ 8.0 mmol/L
-

*FPG: Fasting plasma glucose, *OGTT: Oral glucose tolerance test

1.3. Classification of Diabetes Mellitus

Since the etiology of DM depends on many factors, patients cannot be categorized into a single class. For example, a patient who receives steroids or thiazides for the treatment of certain diseases might develop DM years later, even after they stop the medication [14]. The etiologic classification of DM is shown in Table 3.

Table 3. The etiologic classification of diabetes mellitus.

-
- I. Type 1 diabetes
 - A. Immune-mediated
 - II. Type 2 diabetes
 - II Drug- or chemical-induced
 - I. 1. Vacor
 2. Nicotinic acid
 3. Glucocorticoids
 4. Others
 - I Infections
 - V. 1. Congenital rubella
 2. Cytomegalovirus
 3. Others
-

Etiologic implications of DM are as follows:

1. T1DM: depends on insulin deficiency caused by beta cell destruction [13].

2. Immune-mediated DM: antibodies respond to beta cells as antigens in the Langerhans islet [13,14].
3. T2DM: defined by insulin resistance or insulin deficiency due to the lack of insulin secretion. The most common type of DM. Insulin is not a key treatment protocol for patients with this type of DM [14].
4. Drug- or chemical-induced DM: many hormones and drugs, such as nicotinic acid and glucocorticoids, may impair insulin secretion. Rat poison Vacor can also destroy pancreatic beta cells [14].
5. Infections: caused by some viruses, such as congenital rubella, coxsackievirus B, cytomegalovirus, mumps, and adenovirus, that have a destructive effect on beta cells [14].

However, the current classification of DM has several limitations due to the similar phenotypic characteristics between different types of DM and the ability of the disease to change phenotype over time. For that reason, pathoetiology should be considered in the classification of DM [15,16,17].

1.3.1. T2DM

DM is an ancient disease reported in men. As aforementioned, the most common type of DM is T2DM, known as a metabolic syndrome that is not dependent on insulin [18,19,20]. T2DM is also known as insulin resistance and insulin deficiency, which may be caused by genetic or environmental factors [21, 22, 23]. Patients suffering from T2DM are more susceptible to short-term and long-term complications, even inopportune death. T2DM has especially become an important health threat in undeveloped countries due to late diagnosis and inadequate treatment opportunities [24].

T2DM can be defined by pancreatic beta cell malfunction and decreased insulin production [25,26]. This outcome reduces glucose transport into specific cells such as the liver, muscle, and fat cells. Recently, it has been reported that alpha cell function

may also be disrupted in T2DM, which may cause an inability to suppress glucagon during fasting [27].

In addition, incretins such as gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) play an important role as gut mediators of insulin release. In patients suffering from T2DM, GIP activity is disrupted, but the insulinotropic effect of GLP-1 remains. Therefore, GLP-1 may be considered a potential therapeutic option [27]. However, dipeptidyl peptidase-4 (DPP4) inactivates GIP and GLP-1. In this case, the most appropriate therapeutic approach is the prevention of endogenous GIP and GLP-1 degradation by inhibiting DPP4. Also, GLP-1 analogs with a longer half-life than GLP-1 may be considered as another therapeutic approach [27]. The main goal of these two approaches is to normalize the plasma glucose level and improve beta cell function [28].

Commonly, patients suffering from T2DM are obese. Therefore, adipose tissue plays an important role in T2DM pathogenesis. Ectopic fat storage syndrome, which involves the accumulation of triglycerides in the muscles, liver, and pancreas, can be associated with T2DM-induced obesity [27].

1.3.1.1. Insulin Resistance

Insulin resistance is commonly defined as the decreased ability of target tissues such as the liver, adipose tissue, and muscles to respond to normal circulating insulin concentrations and is characterized by uncontrolled hepatic glucose production and decreased glucose uptake by the muscles and adipose tissue.

Insulin resistance may also be defined as the inadequacy of the biological response against an increased insulin level and decreased sensitivity to glucose excretion, which is mediated by insulin. Glucose is the primary stimulus for insulin secretion. Similar to glucose, amino acids (ketones) are also insulin-releasing nutrients. In addition to nutrients, hormonal (growth hormone, glucagon, GIP, GLP-1, etc.) and neural (β -adrenergic, parasympathetic) stimuli can also initiate insulin secretion. Insulin suppressor agents can also be classified into two types as hormonal

(adrenocorticosteroids, somatostatin, adrenalin, noradrenalin, etc.) and neural suppressors (α -adrenergic molecules) [29].

Insulin resistance is the hallmark of T2DM, and most patients with T2DM are obese. Although the exact mechanism of obesity causing insulin resistance is not known, the effect might be partially explained by free fatty acids (FFAs) released from adipose tissue reaching the liver via portal circulation, increasing gluconeogenesis, and changing the effect of insulin in the liver. In addition, obesity reduces the binding of insulin to target tissues and reduces blood flow in the muscles, contributing to insulin resistance [30].

External factors such as high-calorie foods, lack of exercise, and weight gain lead to the development of insulin resistance, increasing the need for insulin and causing glucotoxicity due to hyperglycemia and lipotoxicity by increasing free fatty acid levels in the circulation, which may contribute to beta cell damage [31].

In most cases, insulin resistance is believed to arise at the cellular level through post-receptor defects in insulin signaling. Despite the presence of a number of insulin signaling defects in experimental animals, their relevance to human insulin resistance remains unclear [31].

1.3.1.2. Beta Cell Dysfunction

The pathogenesis of T2DM involves a progressive loss of beta cell mass and function, as well as the acquisition of insulin resistance. The decrease in the number of islet beta cells over time is also an important factor affecting the course of the disease. In an established T2DM, the beta cell mass is reduced by 20-40%. It has been suggested that, in addition to chronic hyperglycemia, the accumulation of amylin in pancreatic islets is responsible for this decrease. It has been recently noticed that FFAs and, especially, postprandial hypertriglyceridemia may impair insulin secretion through a toxic effect on beta cells. Researchers state that the primary consequence of beta cell dysfunction may be hyperlipidemia, although relevant studies have been ongoing [32].

Expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) can protect cells and tissues against

possible harmful effects of free radicals. Antioxidant capacity in pancreatic islet cells is known to be at the lowest level when compared to other tissues such as the liver, kidney, skeletal muscle, and adipose tissue. High glucose concentration in pancreatic islet cells causes cellular stress by increasing the number of free oxygen radicals. Since the activities of antioxidant enzymes are not high enough in the pancreas, beta cells become more susceptible to oxidative stress. Therefore, it is stated that oxidative stress is also responsible for the emergence of beta cell dysfunction and damage [32].

Beta cell damages cause inflammation induced by cytokine, obesity and insulin resistance, and excessive consumption of saturated fat and FFAs. A progressive decline in the function of beta cells leads to beta cell depletion [33]. When there is a loss of beta cell mass and function, the development of both T1DM and T2DM is inevitable [34].

In a previous study, it was reported that the proinflammatory cytokines might initiate an inflammatory response dependent on insulin resistance, and induction of mitochondrial stress by cytokines may cause beta cell loss [35]. Cytokine secretion executed by immune cells that infiltrate the pancreas is an important mediator of beta cell destruction [36]. Chronic hyperglycemia may result in inflammation and oxidative stress. This outcome might be responsible for the modulation of gene expression, leading to impaired insulin secretion and increased apoptotic response [37].

Neogenesis and apoptosis are generally in balance in tissues. Disruption of this balance towards apoptosis or shifting to necrosis may cause tissue damage as well as beta cell loss in diabetic patients. Apoptosis is believed to be the main cause of beta cell loss, especially in T2DM patients. The best indicator of this is the increase in the activities of apoptotic mediators such as caspase-3 and caspase-8 in beta cells of patients with T2DM. One of the factors affecting the activation of apoptotic pathways is the shifting of the oxidant/antioxidant balance in beta cells to scavenge the oxidants. However, the predicted consequences of beta cell loss in response to changes in insulin secretion characterizing T2DM cannot be adequately explained [32,38].

Overstimulation of mitochondria increases the amount of reactive oxygen species (ROS), and due to excessive production of nitric oxide (NO) in beta cells, the amount of reactive nitrogen species (RNS) also increases. Excess ROS and RNS lead to electron transport chain inhibition, which may result in the reduction of energy

production, DNA damage, and advanced glycation end-products (AGEs). Lower adenosine triphosphate (ATP) synthesis and inadequate antioxidant balance lead to beta cell dysfunction [32].

1.3.2. Experimental Models of DM

Experimental animal models provide many advantages to the investigations of diseases, such as enlightening the diagnostic factors, pathogenesis, and potential treatment strategies. DM is a chronic metabolic disorder that requires continuous follow-up and therapy. With the decrease in the lifespan and quality of life of patients, DM has a high morbidity and mortality rate. Therefore, elucidating its pathogenesis has great clinical value [39].

Streptozocin (STZ) and alloxan are used to establish DM models. For diabetic rat modeling with alloxan, the required intravenous (i.v.) dose is 65 mg/kg. For mice, the required i.v. alloxane dose is 100-200 mg/kg. However, in intraperitoneal (i.p.) or subcutaneous (s.c.) applications, the dosage should be increased. Administration of less than 150 mg/kg i.p. may not be adequate for successful induction of DM.

The most commonly used dose for modeling DM is 60-80 mg/kg STZ i.p. in rats and 150 mg/kg in mice. Female mice might be more tolerant than male mice, and doses less than 40 mg/kg is not sufficient to form DM. STZ solution must be prepared at pH 4.5 using a citrate tampon. BGL is measured from the tail vein two days after STZ administration, and the rats are considered diabetic when BGL is higher than 200-300 mg/dL. Another method for inducing DM is the total resection of the pancreas tissue. The requirement of surgical intervention and malabsorption are the disadvantages of this technique [40].

1.3.2.1. T1DM Modeling

The three main T1DM models are:

1. Chemically induced T1DM models: Alloxan, STZ, zinc chelators (dithizone, 8-hydroxyquinoline), rodenticide-Vacor, dietary nitrosamines, etc.,

2. Spontaneous models of T1DM: BB (Bio-Breeding) rat, NOD (non-obese diabetic) mouse, and others (Macaca nigra monkey, Keeshond dog, Chinese hamster, guinea pig, New Zealand white rabbit, KDP (Comedo Diabetes Prone) rat, and
3. Models created with viruses; transgenic models of T1DM [39].

1.3.2.2. T2DM Modeling

For T2DM modeling, spontaneous models have been used to produce severe (db/db mouse, Rhesus monkey, desert rodents) and moderate hyperglycemia models (ob/ob mouse). Experimental models established by chemical induction (STZ and alloxan), surgical models (pancreatectomy, hypothalamic lesion), diet-induction (high-fat and sugar diet) nutrition, hormones, womb malnutrition, high doses of anti-insulin hormones, prolonged exposure to hyperinsulinemia, and GMO models have also been used [39].

STZ synthesized by *Streptomyces achromogenes* is used to induce insulin-dependent and non-insulin-dependent DM. Usually, a single i.v. dose of 40-60 mg/kg body weight is used for adult rats. STZ may also be used by i.p. administration; however, a single i.p. dose should be higher than 40 mg/kg body weight [58].

Blood insulin and glucose levels are altered by STZ in beta-cells. Pancreatic beta-cells take up STZ by the glucose transporter GLUT2. It was found that the reduction of the expression of GLUT2 might prevent the diabetic action of STZ. When applied in multiple doses, STZ might restrict GLUT2 expression both *in vivo* and *in vitro* [59].

STZ is a nitrosourea derivative molecule that may change the DNA in pancreatic beta cells by alkylation of the DNA, especially affecting the guanine base. STZ acts as a nitric oxide (NO) donor, and NO induces DNA damage by destroying the pancreatic islet cells [60].

STZ also generates ROS that may affect DNA fragmentation. Both STZ activity in mitochondria and the increase in xanthine oxidase activity are related to the formation of superoxide anions. It was indicated that the Crebs cycle and oxygen

consumption in mitochondria might be inhibited by STZ. Inhibition of the Crebs cycle leads to decreased mitochondrial ATP production and may cause ATP depletion in beta-cells [61].

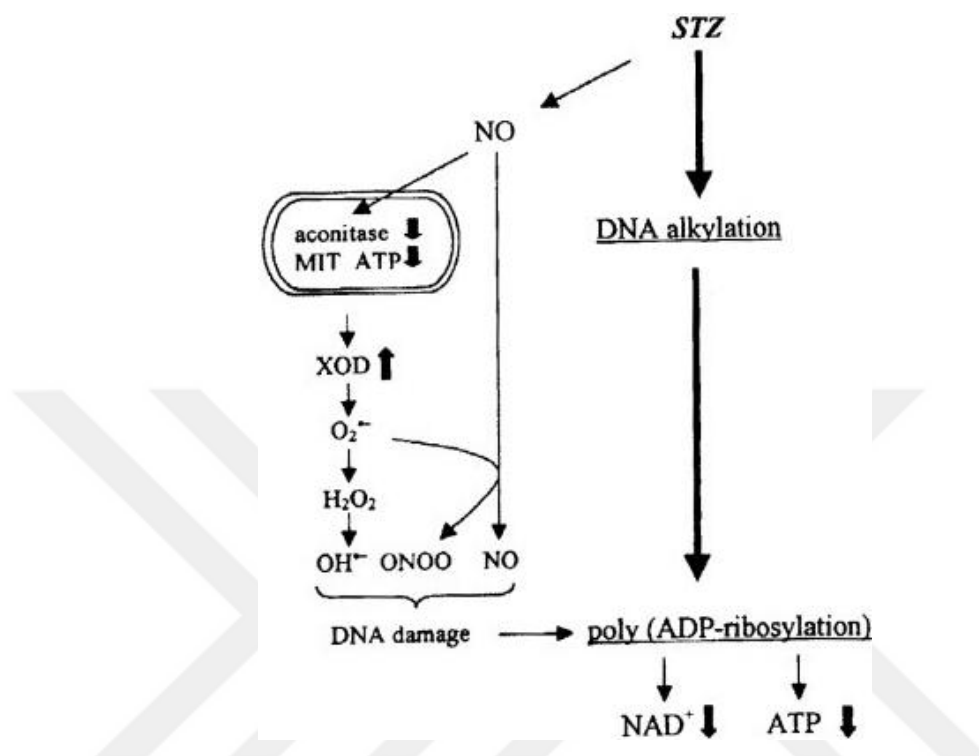


Figure 6. The mechanism of STZ-induced toxicity on beta-cells of rat pancreas. STZ: Streptozotocin; MIT: Mitochondria; XOD: xanthine oxidase [58].

1.4. Oxidant/Antioxidant Balance

Enzymatic and non-enzymatic antioxidants are responsible for the maintenance of the oxidant/antioxidant balance. There are many factors that may harm the tissues by interfering with the antioxidant activity. For that reason, antioxidants are important for the prevention and treatment of tissue injury that may be aggravated by oxidative stress. Studies reported that oxidant indicators (malondialdehyde [MDA] and myeloperoxidase [MPO]) are increased, and antioxidant indicators (glutathione [GSH] and glutathione S-transferase [GST]) are decreased during oxidative stress [41].

It has been suggested that patients with DM suffer from oxidative stress, and most diabetic complications seem to stem from oxidative stress. Also, oxygenated free

radicals may disrupt vascular function and homeostasis caused by the inhibition of NO synthesis in endothelial cells [104].

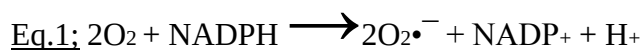
1.4.1. Enzymatic Antioxidants

Antioxidants are the most important inhibitors of cellular damage caused by free radicals. As mentioned in the previous section, there are two forms of antioxidants: enzymatic and non-enzymatic. Enzymatic antioxidants are responsible for converting oxidative products to hydrogen peroxide (H₂O₂) and eventually to water with the help of cofactors such as copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe). [42] Non-enzymatic antioxidants such as vitamin C, vitamin E, plant polyphenols, carotenoids, and glutathione mostly act by inhibiting the free radical chain reactions [42].

Free radicals are produced as a result of normal cellular metabolism. These molecules or atoms have at least one or more unpaired electrons. The odd number of electrons of a free radical makes it unstable, short-lived, and highly reactive. For that reason, they can abstract electrons from other compounds to become stabilized, and the molecule attacked by free radicals loses an electron, becoming a free radical itself, and initiating a chain reaction that consequently damages the living cell [43].

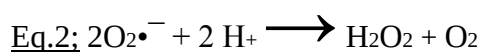
Oxygen uptake, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) activation, and superoxide anion (O₂•⁻) production lead to ROS generation (Eq.1) [42].

(oxidase)



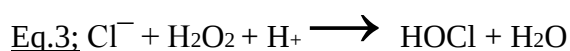
Then, the O₂•⁻ is converted to H₂O₂ (Eq. 2) by SOD [42].

(SOD)

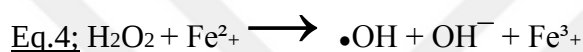


ROS usually act according to either Eq.1 or Eq.2, which are destructive for the organisms. ROS may also be produced by MPO located in neutrophils. MPO converts H_2O_2 and chloride ions to hypochlorous (HOCl), an oxidant agent, as shown in Eq. 3 [42].

(MPO)



ROS are also generated by Fenton (Eq.4) and Haber Weiss reactions (Eq.5) via respiration [42].



1.4.1.1. SOD

Increased oxidative stress plays an important role in the etiology and occurrence of complications in DM. Hyperglycemic status may stimulate ROS production, which leads to antioxidant defense system insufficiency. An elevated level of superoxide anion ($\text{O}_2^{\bullet-}$) is mostly caused by endothelial cells under hyperglycemic conditions and may inhibit important enzymes of the glycolytic pathway such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Accumulation of glucose and other intermediate metabolites of the glycolytic pathway is caused by the inhibition of GAPDH [44].

Superoxide is a potent oxidizing agent, which may also be generated through chain reactions. An excessive amount of superoxide is expected to damage vital biological macromolecules such as DNA, lipids, and proteins. Superoxide anion produces hydroxy radicals directly or indirectly from H_2O_2 , which results from its dismutation by SOD in the presence of Fe^{+2} (Fig. 1). SOD catalyzes the conversion of $\text{O}_2^{\bullet-}$ into H_2O_2 [44,47].

The major reason for ROS-mediated cell toxicity is hydroxy radicals. Besides superoxide, hydroxy radicals may affect membrane lipids and cause lipid radical ($\bullet\text{L}$) formation. Lipid radicals generate lipid peroxy radicals ($\bullet\text{LOO}$) in the presence of oxygen (O_2) molecules (Fig. 1) [47].

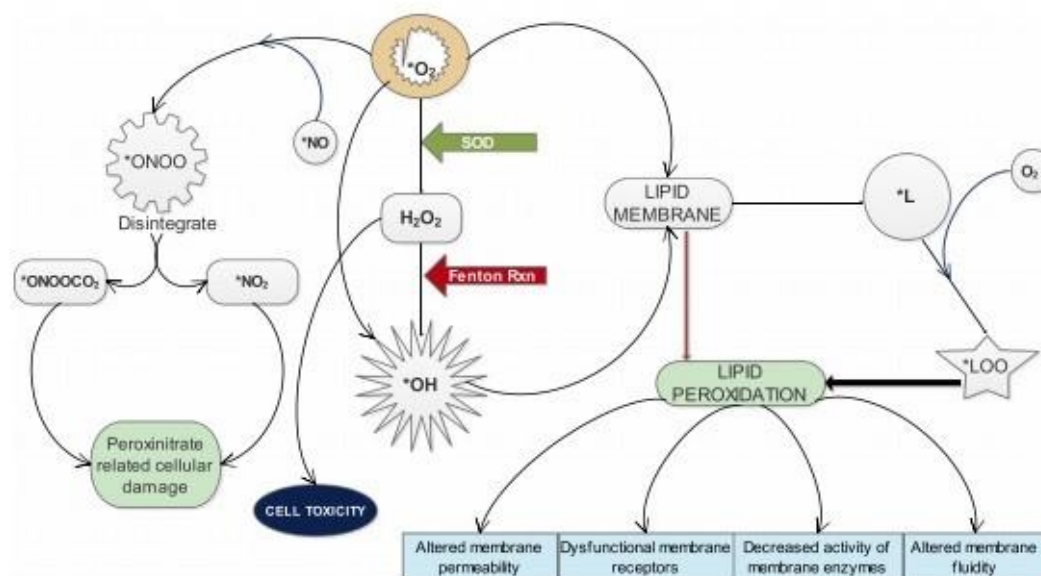
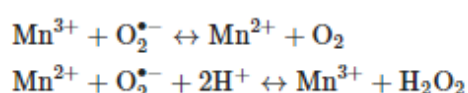


Figure 1. Role of superoxide anion in the generation of other reactive species [47].

The SOD enzyme converts two superoxide ions to oxygen and hydrogen peroxide using active site metals such as manganese (Mn). This redox shuffling is dependent on two protons per cycle [45].



Active site metals play a key role in the classification of SODs. Iron SOD (FeSOD) and Nickel SOD (NiSOD) are prokaryotic; FeSOD is also present in chloroplasts. Copper- zinc SOD (Cu/Zn- SODs) are located in the extracellular matrix and cytosol of eukaryotes. Manganese SOD (MnSOD) is present in bacteria and all eukaryotes. In eukaryotes, MnSOD is located in the mitochondrial matrix. The mitochondrial matrix is usually known as the region where superoxide formation is the most abundant. Electrons leak from the electron-transport chain and perform a one-electron reduction of diatomic oxygen to form superoxide [45]. Cu/Zn-SOD is one of

the most important enzymatic antioxidants, having the ability to neutralize superoxide [46].

1.4.1.2. CAT

CAT is one of the most common antioxidant enzymes found in almost all living aerobic tissues. CAT uses either Fe or Mn as a cofactor and catalyzes the degradation and/or reduction of H_2O_2 to H_2O and O_2 . The enzyme is located primarily in peroxisomes but absent in the mitochondria of mammalian cells, except in rats' hearts [47].

CAT is composed of four hemoprotein groups and has a molecular weight of 250 kDa. Parallel with the other antioxidant enzymes (Fig.2), CAT exists in erythrocytes, renal cells, and hepatic cells. Some of the prokaryotic and eukaryotic organisms may also produce CAT as an intracellular enzyme. CAT has been found in aerobic bacteria but not in anaerobes. It is the second most efficient enzymatic antioxidant (after SOD), able to prevent excessive ROS levels that could cause specific pathological disorders [48].

The pathogenesis of age-associated diseases, such as DM, hypertension (HT), anemia, and cancer, is related to the abnormal function or absence of the CAT enzyme [49]. However, according to data obtained from an experimental study conducted in recent years, enzymatic antioxidant activity cannot be altered in these diseases [105].

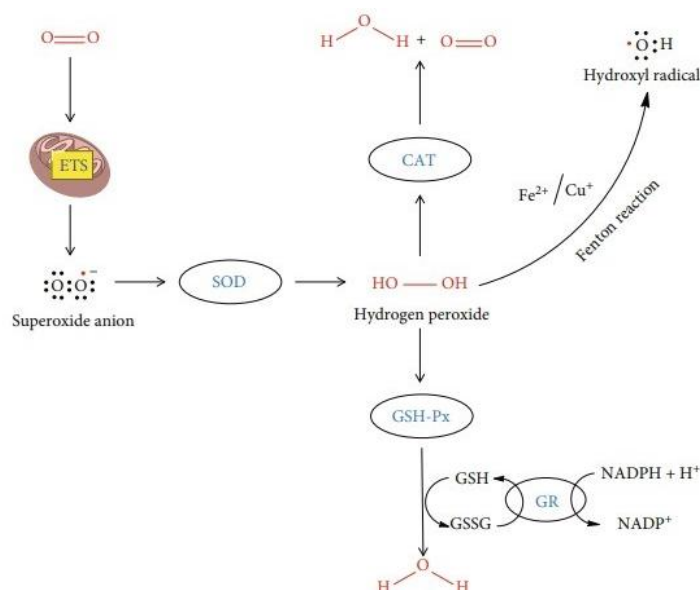
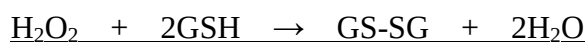


Figure 2. The interplay between catalase and other antioxidant enzymes. SOD: superoxide dismutase; CAT: catalase; GSH-Px [GPx]: glutathione peroxidase; GSH: glutathione; GSSG: oxidized glutathione; GR: glutathione reductase [49].

1.4.1.3. Glutathione Peroxidase

Reduction of H₂O₂ to water or alcohol using reduced GSH as an electron donor is carried out by GSH peroxidase (GPx) as follows [50]:



GPx has multiple isozymes. In mammalian tissues, there are four major selenium-dependent GPx isozymes:

1. classical GPx (GPx1)—normally found in red blood cells (RBCs), the liver, lung, and kidney;
2. gastrointestinal GPx (GPx2);
3. plasma GPx (GPx3)—mostly found in the kidney, lung, epididymus, placenta, seminal vesicle, heart, and muscle;
4. phospholipid GPx (PHGPx4 or GPx4)—broadly distributed in different tissues [50].

Hydroperoxides of polyunsaturated fatty acids (PUFAs) are reduced by GPx for the prevention of lipid peroxidation-induced toxic effects [51].

Non-enzymatic endogenous antioxidants may include metal-binding proteins; however, some of the metal-binding proteins cannot bind Fe and Cu. Ceruloplasmin (Cp), an important metal-binding protein, binds Cu ions to protect membrane lipids from lipid peroxidation caused by iron due to its ferroxidase-type activity. Exogenous factors such as selenium (Se) may support the endogenous antioxidant system. Se normally acts via specific proteins known as selenoproteins. Most of these proteins are involved in redox activities similar to the GPx enzymes. Due to the properties of the active site of these enzymes, Se plays a key role in GPx activity. Physiologically important selenoproteins include selenoprotein P (SelP), which is responsible for Se transport [51].

1.5. Oxidative Damage Biomarkers

The cellular antioxidant defense system might be insufficient against oxidative or nitrosative stress caused by excessive ROS/RNS production. The primary damage points of oxidative/nitrosative stress are lipids, proteins, and nucleic acid bases, which are responsible for cell viability and health. Additionally, oxidative stress may induce necrosis and/or apoptosis through secondary reactive species. Accumulating evidence suggests that oxidative stress might be related to acute and/or chronic human disorders (Table 4) [53].

Table 4. Human diseases may be associated with an increase in the biomarkers of oxidative damage.

<i>Biomarker</i>	<i>Diseases</i>	<i>Biomarker</i>
MDA	Alzheimer Disease	Spinal cord injury
	Asthma	Systemic sclerosis (scleroderma)
	Diabetes Mellitus	Decrease in GSH concentration
		Preeclampsia (41) and/or GSH:GSSG ratio

GSH: reduced glutathione; GSSG: oxidized glutathione.

The most important factor making aerobic life finite is oxygen. Occasionally, the oxygen produced as a result of aerobic metabolism can kill the cells through the generation of reactive species, which may cause necrosis and cell death. Similarly, reactive carbonyl species (RCS) may cause oxidative stress by interfering with the normal physiological processes in cells. Oxidative stress can be described as any disturbance in the antioxidant/pro-oxidant balance caused by various factors, including aging, drug actions and toxicity, inflammation, and/or addiction [53].

Biomarkers of oxidative stress in DM;

Proteins: ROS react with amino acids, which may cause oxidative degeneration and protein glycation. The degree of protein glycation is estimated by glycated hemoglobin and fructosamine biomarker levels. Due to glycation enhanced by oxidative stress in DM, the structure and function of antioxidant enzymes may be altered. According to *in vitro* studies, MPO catalyzes oxidation reactions, such as the conversion of tyrosine to the highly reactive crosslinked dityrosine, which makes it an important biomarker for protein oxidation [53].

Lipids: DM renders cells vulnerable to lipid peroxidation by producing some defects in lipid monomers. The cell membrane consists of PUFAs, which consist of multiple bonds. These bonds make the cell membrane susceptible to the attacks of free radicals. A critical biomarker of oxidative stress is lipid peroxidation, and lipid peroxides are usually measured by MDA in the presence of thiobarbituric acid (TBA) [53].

Vitamins: Some of the free radicals may be detoxified by antioxidant vitamins such as vitamins A, C, and E. Small alterations in antioxidant vitamin levels are another important biomarker of oxidative stress. Since these vitamins may also increase toxicity by producing pro-oxidants under specific conditions, vitamin E levels have been reported to be either increased or decreased in DM [53].

Glutathione: Alterations in GPx and glutathione reductase enzyme activity is a specific outcome of T2DM. These enzymes are responsible for metabolizing peroxide to water and converting glutathione disulfide back into glutathione in cells. Interruption of this metabolic pathway will make the cells sensitive to oxidative stress and cell injury [53].

Metals: Iron (ferric) and copper, which are known as heavy metal derivatives, have free radical properties. Due to their unpaired electrons in the outer layer, they may bind to other biomolecules to modify them. It is also known that these ions may oxidize proteins, lipids, and nucleic acids, causing the dysfunction of the tissues or even breaking them [53].

Apoptotic cells: The beta-cell function may be impaired by oxidative stress via several molecular actions, such as the reduction of insulin production and exocytosis of proinsulin vesicles into circulation in response to glucose. Apoptotic processes may also be induced in pancreatic cells, leading to the loss of beta cells and death. A series of proapoptotic agents are highly sensitive to oxidative stress and can activate the apoptotic process in the pancreatic cells. Moreover, an overload of free radical species negatively affects metabolic pathways in beta cells and impairs ATP-sensitive potassium (K_{ATP}) channels, leading to lower insulin secretion (Figure 3). [54] For that reason, tracking the proteins, lipids, vitamins, glutathione, metals (iron and copper), and apoptotic cells in the bloodstream may be helpful to detect T2DM-induced oxidative stress.

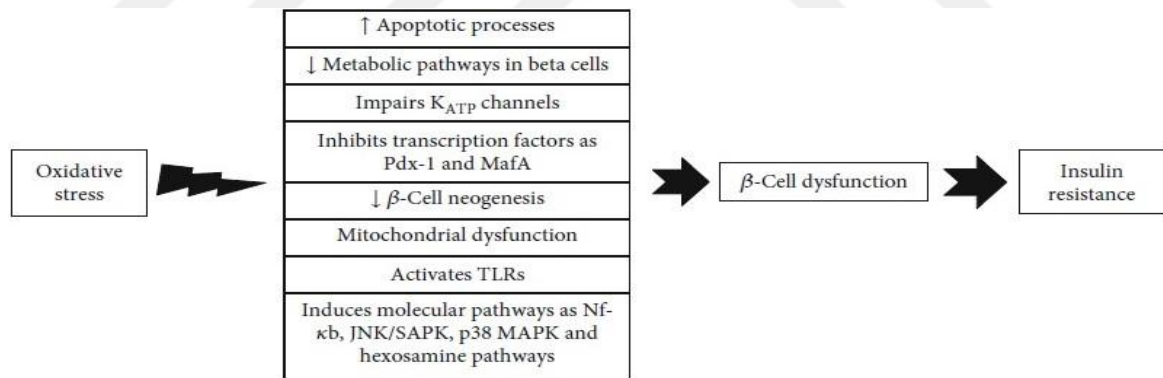


Figure 3. Proposed molecular mechanisms relating oxidative stress and beta-cell dysfunction, leading to DM. Nf-κB: nuclear factor-kappa B; JNK/SAPK: c-jun NH2-terminal kinase/stress-activated protein kinase; Pdx-1: pancreatic duodenal homeobox 1; p38 MAPK: p38 mitogen-activated protein kinase [54].

1.5.1. Lipid Peroxidation

Oxidative disruption of carbon-carbon double bond-containing lipids can be defined as lipid peroxidation (LP). Due to the presence of PUFAs in the phospholipids, the membrane becomes susceptible to LP, which causes a decrease in membrane fluidity, changes the phase properties of membranes, and decreases electrical resistance. The steps involved in iron-induced LP are described in Figure 4 [55]. The oxidative stress-induced LP end-product is MDA, which can be used as a specific biomarker of T2DM.

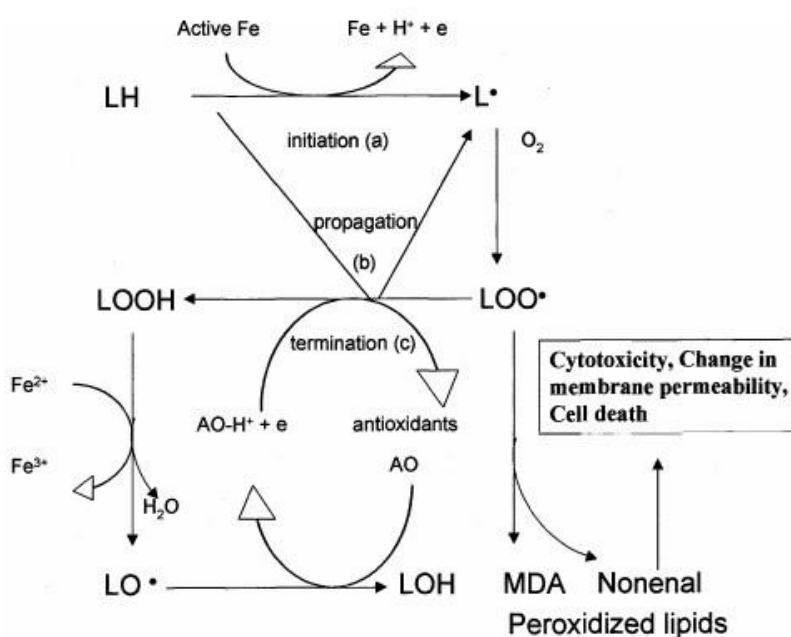


Figure 4. Mechanisms involved in iron-induced LP. LH: lipid membrane; L•: lipid radical; LOOH: lipid hydroperoxide; LOO•: lipid peroxy radical; LOH: stable lipid alcohol; LO•: lipid alkoxyl radical; MDA: malondialdehyde [55].

1.5.1.1. MDA

The LP process in organisms is mostly generated by free radicals. MDA is one of the end products of PUFA peroxidation in cells. The increase in free radicals may cause MDA overproduction. As mentioned before, MDA level is commonly known as a biomarker of oxidative stress and antioxidant status. PUFAs are also essential molecules in cellular membranes as a component of phospholipids, and peroxidation may occur

under physiological conditions, which causes oxidation of these fatty acid chains. MDA forms as a result of this mechanism. The structure of MDA is shown in Figure 5 [56]. Among many different aldehydes, LOOH, propanal, hexanal, and 4-hydroxynonenal (4-HNE) may be formed as secondary products during LP [62].

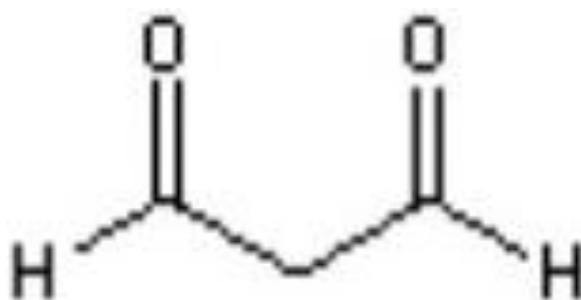


Figure 5. The structure of MDA.

As an indicator of oxidative stress; MDA, the end product of lipid peroxidation, 8-hydroxy-2'-deoxyguanosine, which is an indicator of oxidative DNA damage, protein oxidation, antioxidant enzymes such as SOD, GPx, CAT, glutathione-S-transferase, glutathione reductase, and the antioxidants such as alpha tocopherol, ascorbic acid, glutathione ubiquinone and cysteine are studied. The main methods used to assess oxidative stress are summarized in Table 5 [57].

Table 5. Oxidative stress biomarkers.

	<i>Parameters</i>
1 <u>Measurement of Radicals</u>	<i>Electron Paramagnetic Resonance Spectroscopy (EPR)</i>
	<i>a) Determination of lipid peroxidation products</i>
	Malondialdehyde (MDA)
	Aldehyde
2 <u>Measurement of Oxidative Stress Biomarkers</u>	<i>b) Determination of protein damage</i>
	<i>c) Determination of DNA damage</i>
	8-hydroxy-2'-guanosine (8-OHdG)

3 <u>Measurement of Antioxidant Defense Systems</u>	<i>a) Evaluation of antioxidant enzyme</i> Superoxide Dismutase (SOD) Glutathione Peroxidase (GPx) Catalase (CAT) Glutathione-S-Transferase (GST) Glutathione reductase (GR) <i>b) Determination of total antioxidant activity</i> <i>c) Measurement of low molecular weight antioxidants</i> Alpha-tocopherol Ascorbic Acid Glutathione Melatonin
4 <u>Measurement of Enzyme Cofactors</u>	Cu, Zn, Mn, Se, Fe elements

The determinant factor for identifying MDA as a biomarker for LP is the peroxidation of omega-3 and omega-6 fatty acids by the TBA reaction [63]. The TBA test was initially used by food chemists to evaluate the autoxidative degradation of fats and oils, making use of the reactivity of TBA toward MDA [64].

MDA is also known as a potent biomolecular danger for cellular proteins or DNA. It may form DNA adducts, inducing cellular damage. Early studies showed that the biochemical pathway for the metabolism of MDA involves its oxidation via mitochondrial aldehyde dehydrogenase, followed by decarboxylation for acetaldehyde production, which is oxidized by aldehyde dehydrogenase to form acetate and, eventually, carbon dioxide and water (Figure 7) [62].

On the other hand, phosphoglucose isomerase is a possible metabolizer of cytoplasmic MDA to methylglyoxal (MG), eventually leading to the formation of D-lactate via the action of glyoxalase system enzymes, using GSH as a cofactor. A fair amount of MDA is excreted by urine as N-epsilon-(2-propenal)lysine and N-2-(propenal) serine [62].

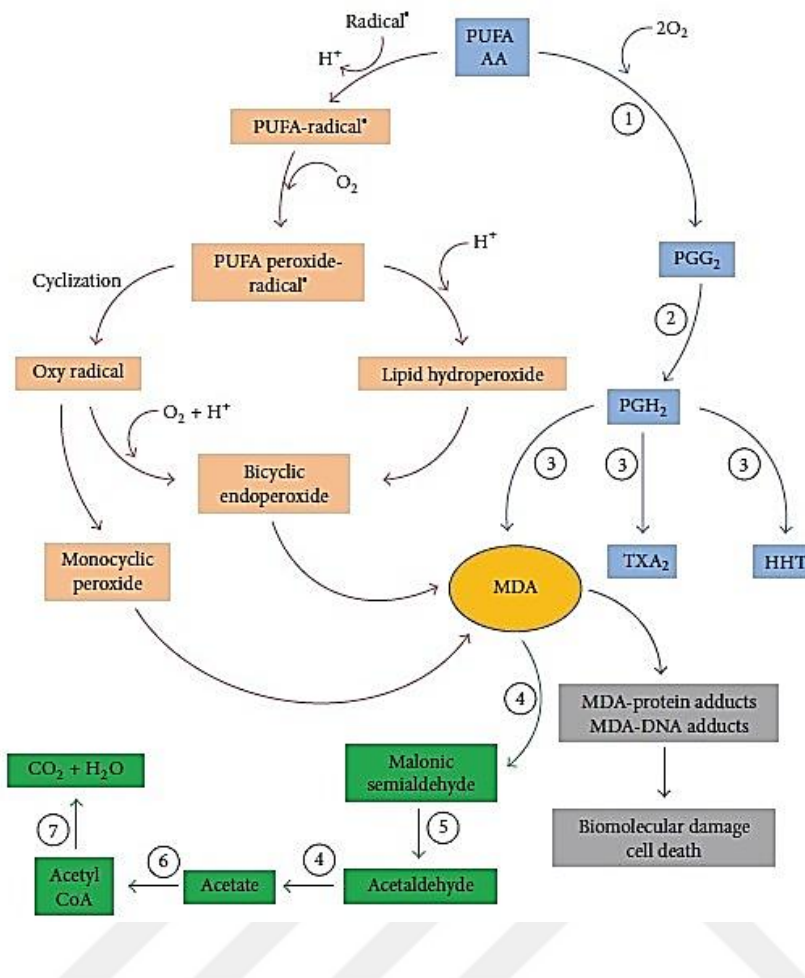


Figure 7. MDA formation and metabolism [62].

1.6. T2DM and Oxidative Stress

Many studies showed that oxidative stress plays a key role in various T2DM complications [65]. ROS levels are elevated as a result of inadequate destruction of free radicals by the enzymatic and non-enzymatic antioxidant systems. Variations in the levels of antioxidant system molecules render the tissues susceptible to oxidative stress, leading to diabetic complications [66].

Enzymes damage and increased insulin resistance are mostly caused by free radical formation due to non-enzymatic glycation of proteins, glucose oxidation, and increased lipid peroxidation [65].

According to the latest research, lipids forming the apolipoprotein component of LDL may form insoluble aggregates that are responsible for oxidative damage in diabetic complications [66].

In DM, mitochondria are the main source of oxidative stress. During oxidative metabolism in mitochondria, utilized oxygen is first reduced to water, and then the remaining oxygen molecules are transformed into oxygen free radicals (O), known as an important ROS [67]. On the other hand, the ROS/RNS ratio negatively affects insulin signaling, which may cause the development of insulin resistance, a known risk factor for T2DM [68].

Increasing evidence from experimental studies has elucidated the link between T2DM and oxidative stress. Free radicals' ability to damage biological materials, such as lipids, proteins, and DNA, is very important for the onset and progression of T2DM complications. Many diseases related to endothelial dysfunction in the blood vessels of patients with T2DM are caused by free radicals and oxidative stress (Figure 8) [65]. In addition, with persistent hyperglycemia, inappropriate amounts of glucose are delivered to the cells. This results in an enhanced glucose flux through glycolysis and the tricarboxylic acid (TCA) cycle. This leads to an overdrive of the mitochondrial electron transport chain, which generates greater amounts of superoxide radicals than mitochondrial SOD [65,68].

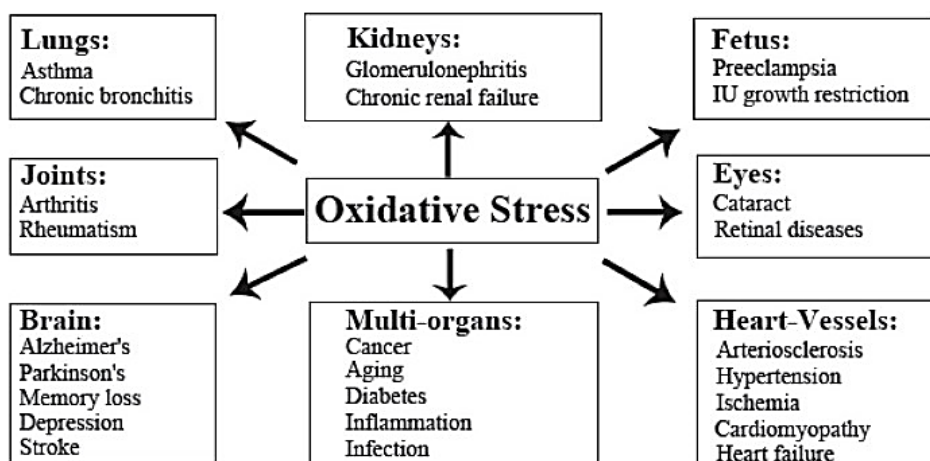


Figure 8. Oxidative stress-induced diseases in humans [66].

Evidence has indicated that hyperglycemia-induced excess mitochondrial superoxide radical production plays a key role in the generation of other ROS. Glucose may react with proteins to produce several intermediate products before generating AGEs by the non-enzymatic glycation reaction. ROS generation may be observed in each step of these reactions. Moreover, the excess glucose in T2DM is expected to undergo other pathways, such as sorbitol and hexosamine pathways, through which glucose is metabolized, and ROS are generated [68].

1.6.1. Free Radical Formation in T2DM

According to experimental and clinical studies, patients are affected by DM through the destruction of beta cells, which may be the outcome of oxidative stress. Increased level of oxidative stress in the tissue and bloodstream plays a critical role in DM [69].

Generally, it is believed that approximately 10,000-20,000 free radicals invade most of the cells. Some of them are beneficial, helping our body to fight against acute and chronic inflammation, destroying bacteria, and controlling smooth muscle contraction and relaxation. On the contrary, free radicals are responsible for the induction of several types of diseases, such as Parkinson's disease, cancer, heart diseases, arthritis, Alzheimer's disease, and DM, when produced in excess amounts [70, 71].

T2DM occurs when insulin secretion by pancreatic beta cells is impaired. Further, ROS triggers the pathogenesis of inflammatory response and ischemic reperfusion, promoting DM. Hyperglycemia is an important concern that may enhance the production of free radicals via several mechanisms. Diabetic patients are more occupied with acute and chronic oxidative stress, which increases the possibility of diabetes-related macrovascular complications [66, 69, 70, 72].

In addition, NOX activation is a major intracellular source of free radicals, leading to a variety of signaling events that may cause endothelial dysfunction, vascular smooth muscle cell proliferation, proinflammatory cytokine expression, and extracellular matrix disruption. In DM, free radical production is increased by

hyperglycemia through glycoxidation and sorbitol system activation and by functional limitation of the hexose monophosphate shunt, leading to decreased glutathione synthesis. Furthermore, there is evidence that hyperinsulinemia, the hallmark of insulin resistance, may be involved in the generation of oxidative stress. Insulin stimulates NADPH-dependent H_2O_2 generation in human adipocyte plasma membranes and increases superoxide anion (O_2^-) production through NADPH oxidase in aortic segments from hyperinsulinemic rats [73].

In animal models of insulin resistance, insulin signaling via the phosphatidylinositol 3'-kinase (PI3K) is impaired, whereas the extracellular signal-regulated kinase (ERK)-1 and -2 pathways appear to be unaffected [73]. Insulin generates free radicals in isolated human fibroblasts; the activation of NOX, achieved through a PI3K-dependent mechanism, was necessary to elicit this effect. The inhibition of this oxidase prevented not only free radical generation but also insulin-mediated ERK-1 and -2 activation [73].

1.7. Cellular Life Cycle

All biological cells are referred as membrane-bound compartments. The cell membrane creates an internal environment where genetic materials located and metabolic activities can take place. It is also known that modern cell membranes are consisted of amphiphilic molecules such as phospholipids, sterols, other lipids and various proteins. Phospholipid membranes are stable under a wide range of temperature, pH, and salt concentration conditions. Such membranes are extremely good permeability barriers, so that modern cells have complete control over the uptake of nutrients and the export of wastes through the specialized channel, pump and pore proteins embedded in their membranes. A great deal of complex biochemical machinery is also required to mediate the growth and division of the cell membrane during the cell cycle [74]. The cell cycle can be thought of as the life cycle of a cell. In other words, it is the series of growth and development steps a cell undergoes.

In cell division, four important processes may take place such as cell growth, DNA replication, distribution of duplicated chromosomes to daughter cells and division. In bacteria, cell growth and DNA replication take place throughout most of the cell

cycle, and duplicated chromosomes are distributed to daughter cells in association with the plasma membrane. In eukaryotes, however, the cell cycle is more complex and consists of four discrete phases [111].

1.7.1. Cell Division

Cell division is the reproduction of unicellular organisms and the means of tissue growth/maintenance for multicellular organisms. Survival of the eukaryotes depends on the interactions and balance between different cell types. The growth and division of different cell populations are regulated in various ways, but the basic mechanisms are similar among all multicellular organisms [75].

Most tissues of the body grow by increasing their cell number, but this growth is to maintain a balance between different tissues. In adults, cell division is usually required for tissue renewal rather than growth; many types of cells undergo a continuous replacement process. For example, skin cells are constantly being sloughed off and replaced; in this case, the mature differentiated cells do not divide, but their population is renewed by the division and differentiation of immature stem cells. In certain other cells, such as those of the liver, mature cells remain capable of division to allow growth or regeneration after injury [75].

In contrast to these patterns, several other types of cells are either differentiated to a certain level that they are no longer capable of cell division, or their division is inhibited by certain signaling molecules produced by nearby cells. As a result, in adult organisms, some tissues have a greatly reduced capacity to replace damaged or diseased cells. Examples of such tissues include the heart muscle, nerve cells of the central nervous system, and lens cells in mammals. Maintenance and repair of these cells are limited to replacing intracellular components rather than replacing entire cells [75].

Before a cell can divide, it must duplicate the genetic information coded in the DNA in order for its daughter cells to function properly and survive. This is a complex process because of the length of the DNA molecules. Each human chromosome consists of a long double spiral, also called a helix, and each strand consists of more than 100 million nucleotides [75].

The duplication of DNA is called DNA replication, and it is regulated by DNA polymerases, complex machines consisting of several subunits. These enzymes read the adjacent nucleotides linked together to make up the DNA chains. During the process, each strand of the DNA double helix acts as a template specifying the nucleotide structure of the new growing chain. After replication, each of the two daughter DNA double helices consists of one parental DNA strand wound around one newly synthesized DNA strand [75].

In order for DNA to replicate, the two strands must be unwound from each other. Enzymes called helicases unwind the two DNA strands, and additional proteins bind to the separated strands to stabilize and prevent them from re-binding during replication. In addition, a remarkable class of enzymes called DNA topoisomerase removes the helical twists by cutting either one or both strands and then resealing the cut. These enzymes can also untangle and unknot the DNA when it is tightly coiled into a chromatin fiber [75].

In the circular DNA of prokaryotes, replication starts at a unique site called the origin of replication and then proceeds in both directions around the molecule until the two ends meet, producing two daughter molecules. In rapidly growing prokaryotes, a second round of replication can start before the first has been completed. The situation in eukaryotes is more complicated, as replication proceeds more slowly than in prokaryotes. At 500 to 5,000 nucleotides per minute (versus 100,000 nucleotides per minute in prokaryotes), it would take a human chromosome about a month to replicate if the process was initiated at a single site. In fact, replication begins at many sites on the long chromosomes of animals, plants, and fungi. Distances between adjacent initiation sites are not always the same; for example, they are closer in the rapidly dividing embryonic cells of frogs or flies than in adult cells of the same species [75].

Accurate DNA replication is crucial to ensure that daughter cells have exact copies of the genetic information to synthesize proteins. Accuracy is achieved by the “proofreading” ability of the DNA polymerase itself. It can check for and erase its own errors and then re-synthesize the mismatched portions. Other repair mechanisms also exist, which correct the wrong bases in the DNA. For example, the incorporation of an incorrect nucleotide during replication or damage caused by mutagenic agents can be

corrected by cutting out the particular section of the daughter strand and recopying the parental strand [75].

In eukaryotes, the processes of DNA replication and cell division occur at different points of the cell division cycle. During cell division, DNA condenses to form short, tightly coiled, rod-like chromosomes. Each chromosome then splits longitudinally, forming two identical chromatids. Each pair of chromatids is distributed to the two daughter cells during mitosis—division of the nucleus—a process during which the chromosomes are propelled by attachment to a bundle of microtubules called the mitotic spindle [75]. Mitosis can be divided into five phases (Figure 9). In prophase, the mitotic spindle forms, and the chromosomes condense. In prometaphase, the nuclear envelope breaks down (in many but not all eukaryotes), and the chromosomes attach to the mitotic spindle. Both chromatids of each chromosome attach to the spindle at a specialized chromosomal region called the kinetochore. In metaphase, the condensed chromosomes align in a plane across the equator of the mitotic spindle. Anaphase follows as the separated chromatids move abruptly toward opposite spindle poles. Finally, in telophase, a new nuclear envelope forms around each set of unraveling chromatids [75].

Mitosis, or somatic cell division

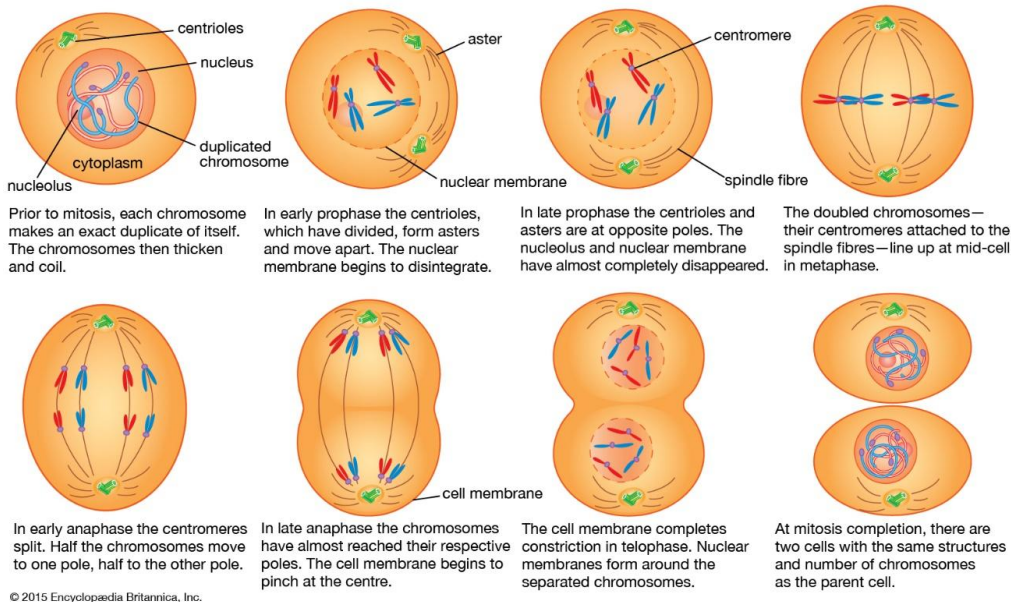


Figure 9. Mitosis, or somatic cell division [75].

An essential feature of mitosis is the attachment of the chromatids to the opposite poles of the mitotic spindle. This ensures that each of the daughter cells will receive a complete set of chromosomes. The microtubules of the mitotic spindle form a tubular assembly of molecules of the protein tubulin. Some microtubules extend from one spindle pole to the other, while a second class extends from one spindle pole to a chromatid. Microtubules can grow or shrink by the addition or removal of tubulin molecules. The shortening of spindle microtubules at anaphase propels attached chromatids to the spindle poles, where they unravel to form new daughter cell nuclei [75].

The two poles of the mitotic spindle are occupied by centrosomes, which organize the microtubule arrays. In animal cells, each centrosome contains a pair of cylindrical centrioles, which are composed of complex arrays of microtubules themselves. Centrioles duplicate at a precise time point in the cell division cycle, usually close to the start of DNA replication [75].

Mitosis is followed by cytokinesis, the division of the cytoplasm. This is another process that differs in animal and plant cells. In animal cells, cytokinesis is achieved through the constriction of the cell by a ring of contractile microfilaments consisting of actin and myosin, the proteins involved in muscle contraction and other forms of cell movement. In plant cells, the cytoplasm is divided by the formation of a new cell wall, namely the cell plate, between the two daughter cells. The cell plate arises from small Golgi-derived vesicles that coalesce in a plane across the equator of the late telophase spindle to form a disk-shaped structure. In this process, each vesicle contributes its membrane to the forming cell membranes and its matrix contents to the forming cell wall. A second set of vesicles extends the edge of the cell plate until it reaches and fuses with the sides of the parent cell, thereby completely separating the two new daughter cells. At this point, cellulose synthesis commences, and the cell plate becomes a primary cell wall [75].

Meiosis, a specialized type of cell division involving a different chromosomal event occurs during the formation of the reproductive cells, or gametes, of sexually reproducing organisms (Figure 10). Gametes such as ova, sperm, and pollen begin as germ cells, which, like other types of cells, contain two copies of each gene in their nuclei. The chromosomes composed of these matching genes are called homologs.

During DNA replication, each chromosome duplicates into two attached chromatids. The homologous chromosomes are then separated to opposite poles of the meiotic spindle by microtubules similar to those of the mitotic spindle. A crucial process takes place at this stage in the meiosis of germ cells: the two chromatids making up each chromosome remain together so that the entire chromosomes are separated from their homologous partners. Cell division then occurs, followed by a second division that resembles mitosis more closely in that it separates the two chromatids of each remaining chromosome. In this way, when meiosis is complete, each mature gamete receives only one copy of each gene instead of the two copies present in other cells [75].

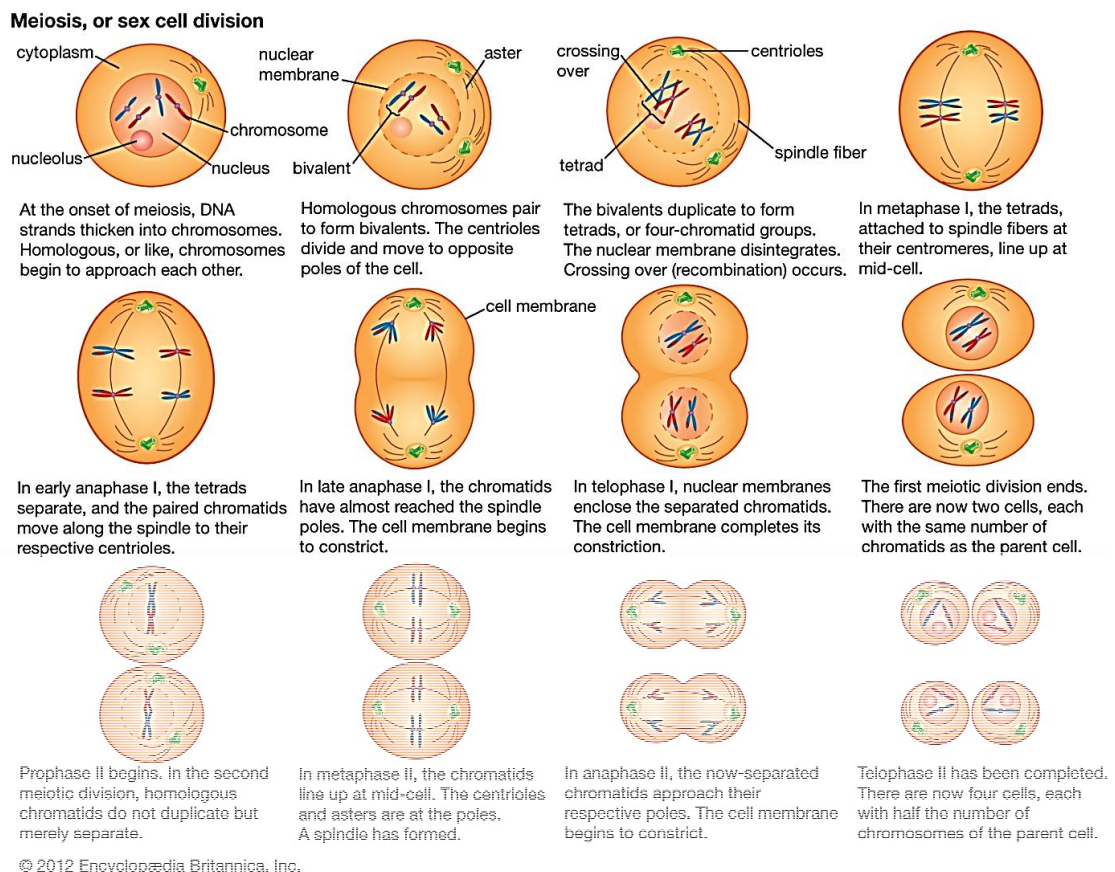


Figure 10. Meiosis, or sex cell division [75].

In prokaryotes, DNA synthesis can take place uninterrupted between cell divisions, and new cycles of DNA synthesis can begin before previous cycles have been completed. In contrast, eukaryotes duplicate their DNA exactly once during a discrete

period between cell divisions. This period is called the S phase (for “synthetic”). It is preceded by a period called G1 (meaning “the first gap”) and followed by a period called G2, during which nuclear DNA synthesis does not occur [75].

The four periods G1, S, G2, and M (for “mitosis”) make up the cell division cycle. The cell cycle characteristically lasts between 10 to 20 hours in rapidly proliferating adult cells, but it can be arrested for weeks or months in quiescent cells or a lifetime in neurons of the brain. A prolonged arrest of this type usually occurs during the G1 phase and is sometimes referred to as G0. In contrast, some embryonic cells, such as those of fruit flies (vinegar flies), can complete entire cycles and divide in only 11 minutes. In these exceptional cases, G1 and G2 are undetectable, and mitosis alternates with DNA synthesis. In addition, the duration of the S phase varies dramatically. The fruit fly embryo takes only four minutes to replicate its DNA, compared with several hours in adult cells of the same species [75].

Several studies have identified the transition from the G1 to the S phase as a crucial control point of the cell cycle. Stimuli are known to cause resting cells to proliferate by inducing them to leave G1 and begin DNA synthesis. These stimuli, called growth factors, are naturally occurring proteins specific to certain groups of cells in the body. Examples include nerve growth factor, epidermal growth factor, and platelet-derived growth factor. Such factors may have important roles in the healing of wounds as well as the maintenance and growth of normal tissues. Many growth factors are known to act on the external membrane of the cell by interacting with specialized protein receptor molecules. These respond by triggering further cellular changes, including an increase in calcium levels that makes the cell interior more alkaline and the addition of phosphate groups to the amino acid tyrosine in proteins. The complex response of cells to growth factors is of fundamental importance to the control of cell proliferation [75].

1.7.1.1. Telomeres and the Telomerase Enzyme System

Genetic information is stored in linear DNA molecules—chromosomes—in eukaryotic cells. It was found that complete chromosomes and their fragments behave differently in the cells. The different properties of chromosomes could be due to the

presence of special nucleotide sequences at the chromosomal ends called telomeres. Telomeres consist of repeating nucleotide sequences and a set of special proteins that interact with DNA to form a nucleoprotein complex [76].

Unlike bacterial chromosomes, the chromosomes of eukaryotes are linear (rod-shaped), with clear ends (Figure 11). These ends pose a problem for DNA replication. The DNA at the very end of the chromosome cannot be fully copied in each round of replication, resulting in a slow, gradual shortening of the chromosome. In human cells, the last RNA primer of the lagging strand may be positioned as much as 70 to 100 nucleotides away from the chromosome end. Thus, the single-stranded overhangs produced by incomplete end replication in humans are fairly long, and the chromosome shortens significantly with each round of cell division due to the deletion of terminal RNA primer [79].

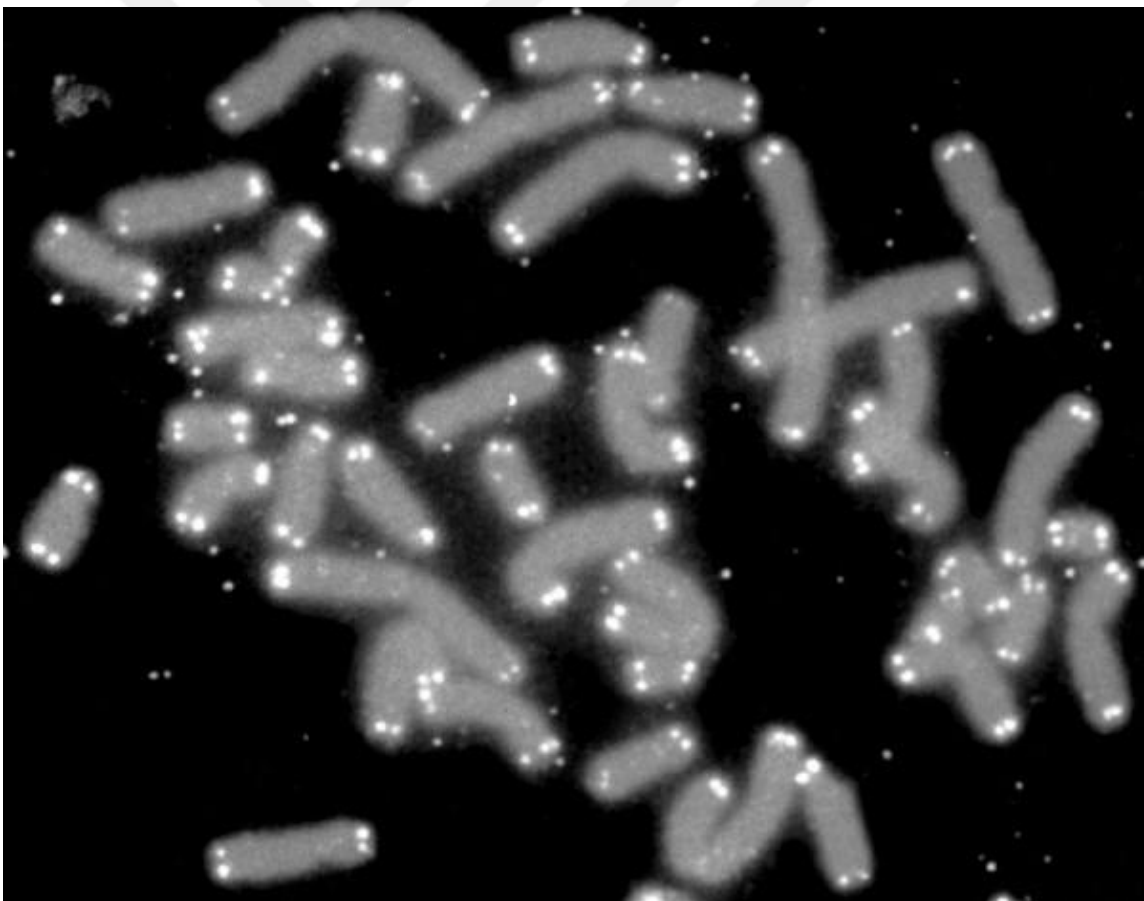


Figure 11. Telomeres appear as bright spots at the ends of each chromosome.

Aging is caused by degenerative diseases, which generate an irreversible accumulation of mutations, inability of cell division, increased susceptibility to morbidity, and ultimately death. Human cells can undergo a limited number of cell divisions and eventually reach an indivisible state called replicative senescence, as telomeres get shorter in each cell division. [76] Progressive telomere shortening also occurs in all dividing normal cells due to the incomplete-end-replication issue, oxidative damage, and other end-processing events [77].

In living organisms, there are repetitive DNA sequences to cope with the loss of DNA during replication. Accordingly, telomeric RNA primers can be found in different living groups containing different series (Table 6). For instance, the telomere repeat sequence is TTAGGG in humans and TTGGGG in Tetrahymena, belonging to protozoans [78]. Telomeres are found in small amounts in organisms, and telomeric DNAs make up approximately 0.003% of the total DNA [78].

Table 6. Sequences of telomeric RNA primers in different living groups.

<i>Organism</i>	<i>Telomeric Sequences</i>	<i>RNA Primer Sequences</i>	<i>RNA Sizes (kb)</i>
Tetrahymena	TTGGGG	CAACCCCAA	160
Euplotes	TTTTGGGG	CAAAACCCCAAAACC	190
Oxytricha	TTTTGGGG	CAAAACCCCAAAACC	190
Human	TTAGGG	CUAACCCUAAC	450
Mouse	TTAGGG	CCUAACCCU	450
S. Cerevisiae	TG(1-3)	CACCACACCCACACAC	1300
	TTTGATTAGGTAT	UCAAUUUCCGUACACC-	
K. Lactis	GT-GGTGTCGGA	AAUACCUAUAUCAA	1300

Telomerase, also called telomere terminal transferase, is an enzyme made up of protein and RNA subunits that elongates chromosomes by adding TTAGGG sequences to the ends of existing chromosomes. Telomerase is found in fetal tissues, adult germ cells, as well as tumor cells. Telomerase activity is regulated during development and has a very low, almost undetectable activity in somatic (body) cells. Somatic cells age

because they do not use telomerase regularly. The result of aging cells is an aging body. If telomerase is activated in a cell, the cell will continue to grow and divide [79].

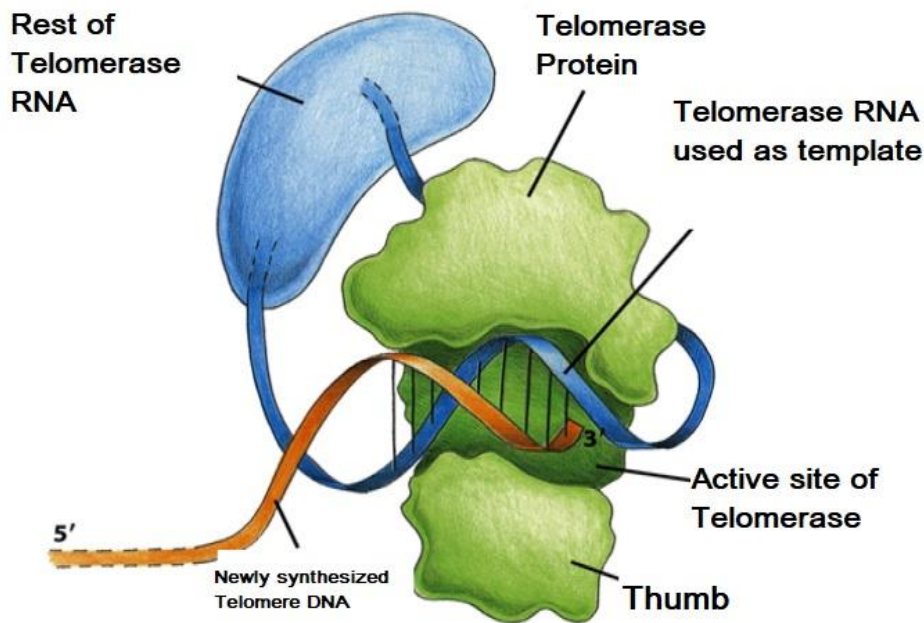


Figure 12. The structure of the telomerase enzyme [78].

It is known that shortening of telomeric regions of DNA to critically short length can serve as a signal for the replicative senescence of somatic cells and destabilization of their chromosomes. Telomerase activity combined with the telomere length (TEL) reflects the cell's proliferative potential. Telomerase activity is mainly present in stem, sex, and tumor cells but there is also evidence of detectable activity in intestinal mucous cells, lymphocytes of peripheral blood (PB), and thymus. During life, telomeres of PB lymphocytes gradually shorten due to cell division, accumulating with age *in vivo* [76].

Telomerase acts in lymphocytes to reduce telomere shortening during cell proliferation. A decrease in telomerase activity may explain why telomeres shorten faster under pathological conditions [76]. Harsh environmental conditions or demanding biological processes are expected to trigger the production of ROS, and may also raise the cell division rate. Stress-induced ROS production would accelerate the telomere abrasion rate by increasing damage to the telomeric sequence, decreasing its ability to

replicate, ultimately translating into a faster mitotic clock and a shorter cell lifespan [77].

1.8. The Relationship Between Diabetes and Telomerase Enzyme Activity

Accumulating evidence indicates that diabetes may trigger changes in telomere length. Studies have shown that the leukocyte telomere length in patients with diabetes was shorter than that in healthy individuals. The authors suggested that, with the development of diabetes, islet beta cells undergo senescence or apoptosis, because the telomere length gradually becomes shorter, leading in turn to a cascade of damaging events characteristic of diabetes complications [80].

Telomere length in patients with diabetes is reduced compared with that of healthy individuals, albeit with variances based on the geographical region. Specifically, European patients with diabetes displayed a reduced telomere length compared with that of Asian and US patients. Additionally, it was demonstrated that telomere length was affected by the type of diabetes, body mass index (BMI), age, and sex. As such, patients with T1DM, obese patients, patients over 60 years of age, and female patients exhibited a shorter telomere length compared with that of patients with T2DM, lean patients, patients below 60 years of age, and male patients, respectively [80].

Telomere length is often used as a biological marker for cell aging due to its shortening with increased age. However, the exact mechanisms underlying the influence of diabetes on telomere length as a function of age remain unclear. Moreover, there is no consensus on the relationship between diabetes and telomere length since some studies have identified no age-related decline in telomere length between patients with T1DM and non-diabetic patients, while others have demonstrated an age effect [80].

Previous studies have demonstrated that oxidative stress is a major contributor to T2DM development. The telomere sequence is rich in guanine residues, and guanine is particularly inclined to oxidative stress, which is markedly increased in uncontrolled diabetes. Therefore, it is plausible to assume that, at least in the case of T2DM, telomere length is affected by oxidative stress. Once the telomere undergoes shortening, it increases the risk of beta cell injury and apoptosis, leading to a decline in islet cell functioning and diabetes development and progression [80].

There is a growing body of evidence in support of an association between short telomeres and T2DM. However, most studies have been cross-sectional in nature, precluding causal inferences: it is not clear whether the metabolic perturbances of T2DM cause telomere attrition or shorter telomeres lead to T2DM. Possible biological hypotheses support both scenarios (Figure 13). Short telomeres may lead to premature beta cell senescence, resulting in reduced beta cell mass and subsequent impaired insulin secretion and glucose tolerance. Indeed, experimental evidence suggests that telomerase is important in maintaining glucose homeostasis in mice. Conversely, elevated BGL increases oxidative stress, potentially interfering with telomerase function and resulting in shortened telomeres [81].

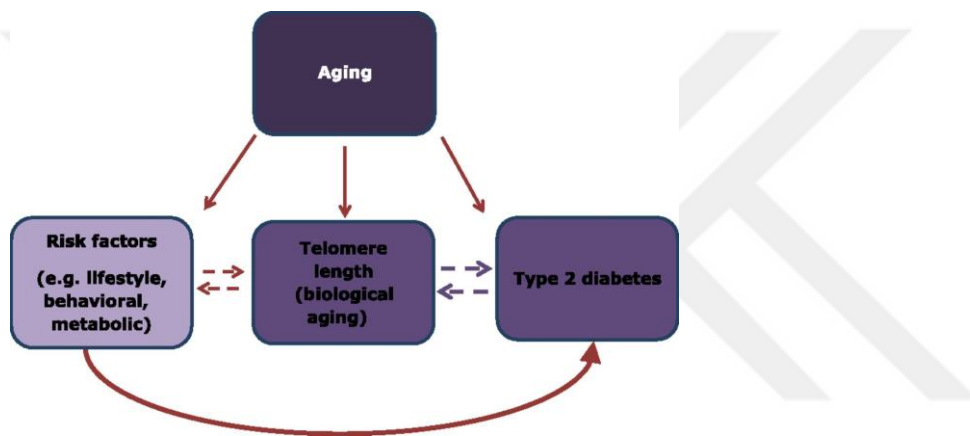


Figure 13. Conceptual framework relating telomere length and T2DM risk [81].

T2DM is a disease characterized by the dysfunction of various organs. Recent studies have shown a close relationship between DM and telomere attrition in leukocytes. In patients with DM or impaired glucose tolerance, excessive oxidative stress induces damage to telomeres and shortens their length. Furthermore, it is suggested that telomere length is a good surrogate marker for mortality and diabetic complications in DM patients. It was found that telomere length in pancreatic beta cells is also shortened in patients with DM, potentially leading to an impaired capacity for proliferation and insulin secretion and accelerated cell death. In contrast, leukocyte telomere length has also been reported in patients with obesity or insulin resistance, both of which are frequently associated with T2DM. In an animal model, it has been shown that telomere attrition in adipose tissue induces insulin resistance. Taken

together, the available data suggest that hyperglycemia, oxidative stress, and telomere attrition in pancreatic beta cells and adipocytes create a vicious cycle that underlies the pathophysiology of T2DM. Inhibition of telomere attrition in various organs, including pancreatic beta cells, could be a new approach to prevent the progression of DM and its complications [82].

Pancreatic beta cells differentiate from precursor cells during the prenatal period, but after birth, they are supplied mainly by autoreplication. However, it has been shown that the replication capacity of beta cells decreases with aging, possibly as a result of telomere shortening, and, in fact, a previous report has shown that telomerase activity is associated with the proliferative capacity of beta cells. Some studies reported that both telomerase reverse reductase (TERT) expression and telomerase activity were upregulated in liver-specific insulin receptor-knockout mice, which show an accelerated proliferative capacity of beta cells. In contrast, TERT expression and telomerase activity were reduced in cyclin D2-knockout and beta cell-specific insulin receptor-knockout mice, which show a low beta cell proliferative capacity [82].

Hyperglycemia induces systemic oxidative stress, including beta cells and adipocytes, whereas obesity induces oxidative stress in adipocytes. In both cell types, oxidative stress induces telomere attrition. Telomere attrition in beta cells further induces senescence and loss of beta cells and impairment of insulin secretion, which leads to hyperglycemia. Thus, two vicious cycles are formed between hyperglycemia and telomere attrition, and p53 plays a crucial role in both processes [82].

1.9. T2DM Treatment Procedures

Inadequate control of BGL in T2DM patients poses a significant risk for the formation of many pathological conditions such as coronary heart diseases, hypertension, endothelial inflammation, myocardial infarction, nephropathy, and retinopathy [83]; therefore, it is necessary to determine blood glucose and HbA1c levels every 3-6 months and establish a treatment scheme suitable for the course of the disease [84].

Like many other proteins, hemoglobin undergoes non-enzymatic glycation. Glycosylated hemoglobin, glycated hemoglobin, HbA1c, and HbA1 are all terms used

to identify the non-enzymatic addition of glucose residues to hemoglobin [85]. N-terminal (valine) amino of the β -chain of HbA1 durable structure formed by the bonding of glucose to the group [-(1-deoxyfructosyl) hemoglobin] has been defined as HbA1c by the International Clinic Society for Biochemistry (IFCC) [85]. In recent years, following the diagnosis of T2DM, either one of the five types of oral antidiabetic drugs are used: sulfonylurea derivatives, non-sulfonylurea secretagogues, biguanides (metformin), thiazolidinediones (TZDs or glitazones), or α -glucosidase inhibitors. These agents act by different mechanisms to increase insulin production, decrease glucose production, or minimize insulin resistance of cell receptors in relevant tissues and help to keep the impaired glucose homeostasis in balance (Figure 14) [83].

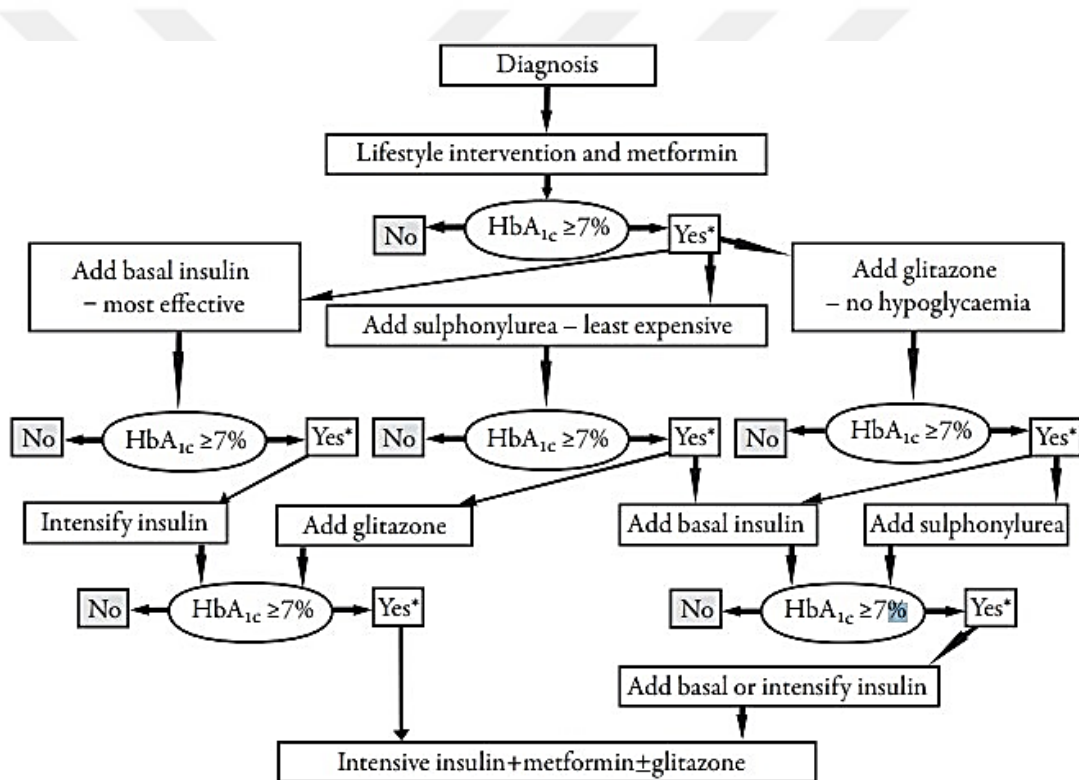


Figure 14. Algorithm for the management of hyperglycemia in type 2 diabetes [83].

Today, despite the application of medical nutrition therapy, exercise, and many antidiabetic drug groups in the treatment of diabetes, the results of treatment may deteriorate over time due to the nature of diabetes. In general, problems regarding inconsistent efficacy, short duration of efficacy, inconsistent dosage, and tolerability are

encountered with the preferred antidiabetic agent. According to current reports, sulfonylurea, meglitinide, and insulin can cause hypoglycemia and weight gain, while thiazolidinediones cause weight gain, and metformin and alpha-glucosidase inhibitors cause complications such as gastrointestinal problems. Although achieving glucose control with metformin reduces the risk of the aforementioned side effects, a decrease in the effectiveness of metformin can be observed throughout the treatment process [85].

Impairment of the biphasic insulin response, which is characteristic of diabetes, slow insulin response to food, and hyperglucagonemia are several conditions that arise in T2DM due to reduced insulin secretion by pancreatic beta cells even under nutrient-rich conditions or abnormal apoptosis. Considering the need for drugs that act by different mechanisms, in recent years, new drug groups have been found for the treatment of diabetes. These include analogs of incretin hormones (glucagon-like peptide-1 “GLP-1” agonists), DPP4 enzyme inhibitors that degrade incretins, dapagliflozin that selectively inhibits the kidney sodium-glucose co-transporter 2 enzyme (DGZ), and endogenous amylin molecule agonist (pramlintide) [86].

Oral glucose intake is much more efficient in stimulating insulin secretion than intravenous glucose infusion when administered in similar concentrations. This so-called “incretin effect” is estimated to be responsible for 50 to 70% of the insulin response to glucose and is caused mainly by the two intestinal insulin-stimulating hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP). GIP is a 42 amino acid polypeptide released from K cells in the duodenum. Digestion of carbs and fats induces GIP to enter the circulation. Protein-heavy diets are less efficient in inducing this process than carbohydrates and lipids. On the other hand, GLP-1 is secreted by L cells in the small intestines. GLP circulates within minutes when food is ingested. Both GIP and GLP-1 are inactivated immediately after secretion. GLP-1 inactivation is carried out by an enzyme called DPP4. In GLP-1 activation and DPP4 inhibition, presuming that insulin secretion will increase and this incretin may interfere with the mechanism, a new generation of anti-diabetic agents has been developed [87]. The contributions of these hormones have been confirmed in mimicry experiments, as well as experiments with antagonists of their actions and those where the genes encoding their receptors have been deleted. In patients with T2DM, the incretin effect is either greatly impaired or absent, and it is assumed that this could contribute to the inability of these patients to adjust their insulin secretion to their needs.

Through the studies addressing the mechanism of impaired incretin effect in patients with T2DM, it was found that the secretion of GIP is usually normal, whereas the secretion of GLP-1 is reduced, presumably as a consequence of the diabetic state. It might be of even greater importance that the effect of GLP-1 is preserved, whereas the effect of GIP is severely impaired. The impaired GIP effect seems to have a genetic background but could be aggravated by the diabetic state. The preserved effect of GLP-1 has inspired attempts to treat T2DM with GLP-1 or analogs thereof, and intravenous GLP-1 administration has been shown to be able to near-normalize both fasting and postprandial glycemic concentrations in patients, probably since the treatment compensates for both the impaired secretion of GLP-1 and the impaired action of GIP [87]. The incretin effect seems to play a major role in the regulation of glucose metabolism in healthy subjects [87].

1.9.1. Usage of Biguanide Derivatives in T2DM

Various biguanides were used in different countries for the treatment of DM; however, all biguanides except metformin were removed from the market due to the associated high risk of lactic acidosis [88]. The most widely used biguanides for T2DM treatment are 1,1-dimethylbiguanide (metformin) and phenylethylbiguanide (phenformin) (Figure 15) [89].

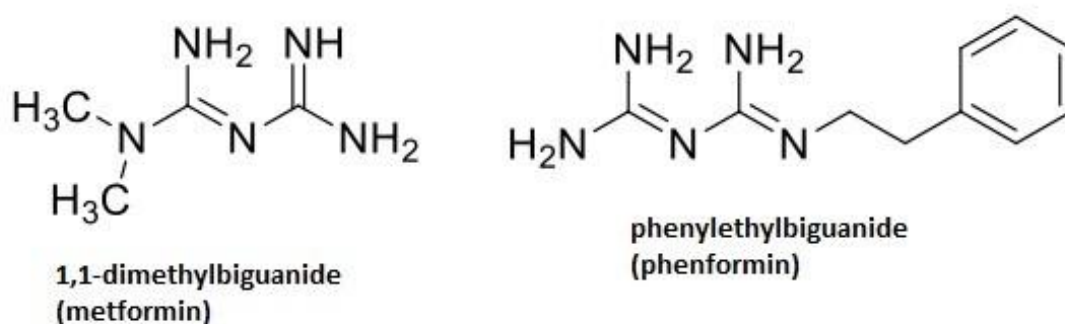


Figure 15. chemical structures of 1,1-dimethylbiguanide (metformin) and phenylethylbiguanide (phenformin).

The mechanisms by which metformin exerts its antihyperglycemic effects are still not entirely clear. Its major action in patients with diabetes is decreasing the hepatic glucose output primarily by decreasing gluconeogenesis; to a lesser extent, it may also increase glucose uptake by skeletal muscles. Metformin activates hepatic and muscle adenosine monophosphate-activated protein kinase (AMPK), which is an enzyme activated by adenosine monophosphate, the breakdown product of adenosine triphosphate and a cellular signal for increased energy requirements. Activation of hepatic AMPK decreases the expression of sterol-regulatory-element-binding-protein-1 (SREBP-1), a transcription factor implicated in the pathogenesis of insulin resistance, dyslipidemia, and diabetes. AMPK activation appears to be an important step in the metformin-mediated reduction of hepatic glucose production and increase in glucose uptake by skeletal muscles. AMPK is a major regulator of lipid and glucose metabolism and may be the key mediator underlying all effects of metformin (Figure 16) [88].

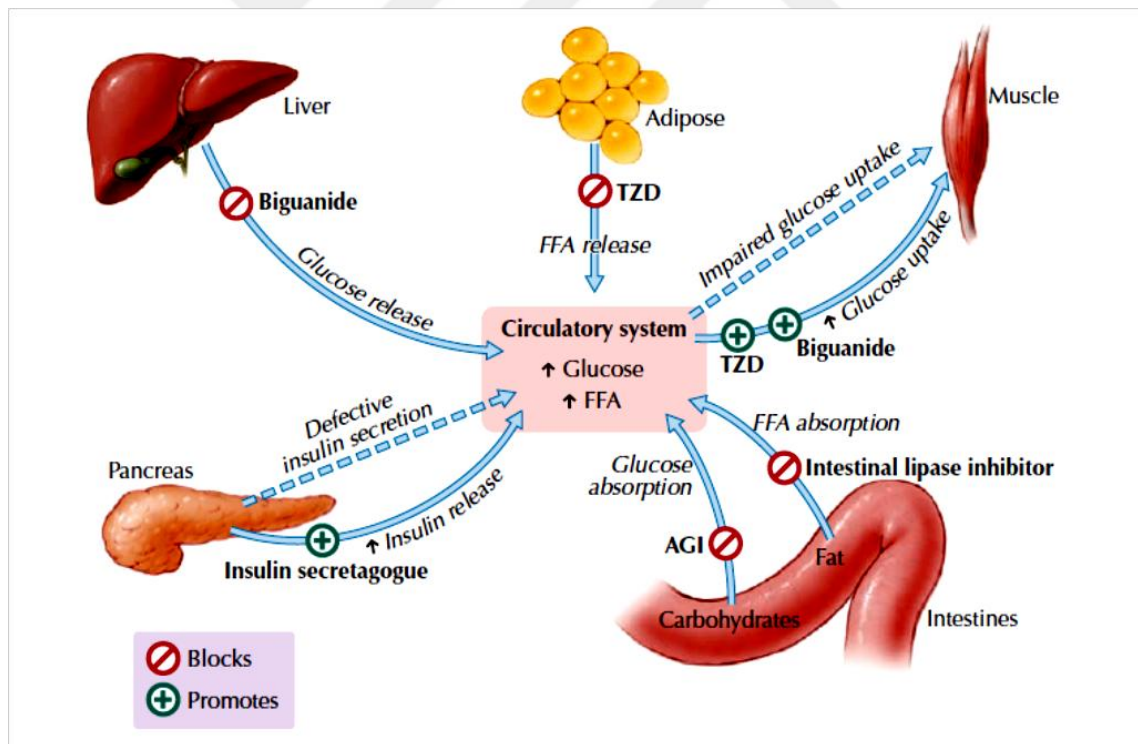


Figure 16. Major target organs and actions of orally administered antihyperglycemic agents in type 2 diabetes mellitus. TZD: thiazolidinedione; FFA: free fatty acid; AGI: α -glucosidase inhibitor [88].

Around 50% of phenformin is absorbed from the gastrointestinal tract. It has a poor protein binding ability, which is about 20%. Phenformin is distributed throughout the major organs, and it is mainly metabolized in the liver by hydroxylation, producing N-P-hydroxy-B-phenyl-ethyl biguanide as metabolite. About 66% of the biguanide is excreted unchanged and the remaining 33% as a metabolite. Phenformin's half-life in the plasma is 7-15 h, whereas metformin's is 1.5 h and buformin's 4-6 h. Metformin and buformin are excreted largely in an unchanged form. The renal clearances of buformin, metformin, and phenformin are 393, 440, and 42-262 ml min⁻¹, respectively. Biguanides are known to show interaction with furosemide, nifedipine, and cationic drugs [90].

The drug has a two-fold mechanism of action: it enhances the peripheral muscle glucose uptake and utilization and inhibits glucose release from the liver. Biguanides induce an increase in peripheral gluconeogenesis and a decrease in intestinal absorption of glucose, vitamin B12, and bile acids. Biguanides do not usually decrease blood sugar in a normal individual unless ethanol or another hypoglycemic agent is simultaneously administered or there is severe hepatic insufficiency. Biguanides are also known to cause decreased absorption of folic acid. In the medical registry, it has been advised to avoid biguanide treatment in patients with hepatic insufficiency, renal insufficiency, peripheral vascular disease, and coronary diseases [90].

Phenformin generally lowers the blood sugar level in patients with diabetes and nutritional starvation. Phenformin appears initially to produce gastric mucosal irritability, which may predispose a person to a number of gastrointestinal symptoms, including gastric hemorrhage. Phenformin may act on the cell membrane to decrease oxidative phosphorylation, produce tissue anoxia, increase peripheral glucose uptake (Pasteur effect), and lead to lactic acidosis (accumulation of lactic acid) by inhibition of lactic acid metabolism [90].

1.9.1.1. Usage of Metformin in T2DM

Metformin hydrochloride, a biguanide, is the most popular oral glucose-decreasing medication for newly diagnosed T2DM, known as 'foundation therapy' [91]. A derivative of guanidine, which was initially obtained from the plant *Galega officinalis*

or French lilac, metformin was first synthesized in 1922 and marketed as a medication in humans in 1957 [93]. The efficacy of the drug has been demonstrated in monotherapy as well as in combination with other glucose-lowering medications for T2DM [92].

Most combination therapy studies including metformin with members of new glucose-lowering drug classes, such as DPP4 inhibitors, GLP-1 receptor agonists, and SGLT2 inhibitors, have added to metformin therapy. In general, the changes in HbA1c repeated the approximate glucose-lowering effect of these drugs as monotherapy. Such studies, therefore, provide little insight into metformin itself, other than the observation that these respective combinations are effective and well tolerated without adding any substantial risk of hypoglycemia [92]. Other investigations have examined initial combination therapy, mainly in treatment-naïve individuals; these have further reinforced the concept that combining two drugs with distinct mechanisms of action leads to greater glucose-lowering potential than the constituents alone, allowing patients to better achieve HbA1c targets [94].

In 1998, the United Kingdom prospective diabetes study revealed data from newly diagnosed patients with T2DM receiving glucose-lowering treatment for more than a decade. This epic study, which redefined the therapeutic strategy for the management of T2DM noted that, in addition to glucose-lowering effects, weight neutrality, and low hypoglycemia risk, long-term metformin therapy might reduce cardiovascular events and improve survival. The reduced cardiovascular risk appeared to be largely independent of glucose-lowering efficacy, and attention was drawn to substantial literature noting potentially advantageous effects of the drug on the macro- and microvasculature (Table 7) [93].

Table 7. Pharmacodynamic effects of metformin in the treatment of type 2 diabetes.

Clinical feature	Effect of metformin
Hyperglycemia	Improves glycemic control in T2D; reduces progression of IGT and IFG to T2D
Insulin resistance	Counters insulin resistance by several insulin-dependent and -independent actions that reduce hepatic glucose output, improve peripheral glucose disposal, increase intestinal anaerobic glucose metabolism, and assist endothelial function

Hyperinsulinemia	Reduces fasting hyperinsulinemia
Abdominal obesity	Usually stabilizes body weight; can facilitate reduction of excess adiposity
Dyslipidemia	May modestly improve blood lipid profile in some hypertriacylglycerolemic and hypercholesterolemic individuals
Blood pressure	No significant effect on blood pressure in most studies but blood pressure control may be improved in overweight individuals achieving weight loss
Proinflammatory state	May reduce CRP and some adipocytokines
Procoagulant state	Some antithrombotic activity, e.g., decrease in PAI-1, fibrinogen and platelet aggregation; improved capillary perfusion
Atherosclerosis	Reduced myocardial infarction and increased survival in T2D: reduced carotid intima-media thickness and reduced levels of adhesion molecules; other evidence for antiatherogenic activity, mostly from animal studies

CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor-1; T2D, type 2 diabetes

1.9.2. Usage of DPP4 Inhibitors in T2DM

DPP4, also known as CD26, is a type II transmembrane glycoprotein of 110 kDa, which can be cleaved off the membrane and released into circulation by a process called shedding [96]. DPP4 is a multifunctional enzyme that serves as a binding partner for numerous peptides, including adenosine deaminase and extracellular matrix proteins. Moreover, as a serine protease, DPP4 cleaves numerous substrates, which further amplifies its complexity of action: the enzyme is involved in several signaling processes and immune cell activation, and its dysregulated expression and release are associated with numerous diseases [96].

As mentioned in Section 1.9, incretin-based treatments are also used for patients suffering from T2DM. The increase in plasma insulin levels following oral glucose administration and the increase in insulin levels after intravenous glucose administration is similar. This effect created by intestinal hormones is called the incretin effect. The two most important incretin hormones are GIP and GLP-1; in patients with T2DM, GIP and GLP-1 activities are found to be significantly reduced. It has been stated in many studies that there is also a decrease in the amount of GLP-1. GIP is a 42 amino acid polypeptide released from K cells in the duodenum. By the digestion of foods containing carbs and fats, GIP enters the circulation. On the other hand, GLP-1 is secreted by L cells located in the small intestines. Both GIP and GLP-1 are inactivated

immediately after secretion. This inactivation is carried out by DPP4. DPP4 inhibitors have been developed due to the involvement of DPP4 in GIP and GLP-1 inactivation. Inhibiting the DPP4 enzyme may cause activation of GIP and GLP-1, helping to increase the incretin effect [86].

1.9.2.1. Usage of Vildagliptin in T2DM

Vildagliptin as a DPP4 inhibitor (Galvus®) has been approved for use as monotherapy and combination therapy in T2DM. A combination of vildagliptin and metformin (Galvus-Met®) is also available in the market. Improvements in glycemic control were observed with vildagliptin in geriatric patients with T2DM and those with renal impairment. Vildagliptin was generally well tolerated in patients with T2DM because of the low risk of hypoglycemic response. Many studies demonstrated vildagliptin at doses of 50 or 100 mg daily, when added to metformin monotherapy, results in a significant and dose-related decrease in fasting plasma glucose and HbA1C levels. These effects are associated with an improvement of beta-cell function, with no weight gain and no increase in the incidence of hypoglycemia [97].

The incretin hormones and their effects are summarized in Table 8. Reduced incretin effect may be the main reason underlying the abnormalities in beta-cell function in T2DM. Vildagliptin provides a modern mechanism of action for treating T2DM and may be useful both as monotherapy and in combination with other antidiabetic agents such as metformin. Vildagliptin has been shown to be safe and effective in targeting incretin deficiencies through the inhibition of DPP4 [98].

Table 8. Incretin hormones and their effects.

Parameters	GLP-1	GIP
Production Site	L-cells in the distal small intestine	K-cells in the proximal small intestine
Effects in the body	1. Enhancement of glucose-dependent insulin secretion, stimulation of glycogen synthesis.	7. Glucose-dependent stimulation of insulin secretion.
	2. Glucose-dependent suppression of postprandial glucagon secretion.	8. Induction of beta-cell growth. 9. Induction of lipoprotein-lipase activity.

- | | | | |
|----|--|-----|--|
| 3. | Delay of gastric emptying. | 10. | Stimulation of fatty acid synthesis. |
| 4. | Reduction of appetite and food intake. | 11. | Enhancement of fatty acid incorporation into adipose tissue. |
| 5. | Induction of beta-cell growth and regeneration. | | |
| 6. | Transdifferentiation of ductal pancreatic tissue into insulin-secreting cells. | | |

GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1

The pharmacokinetic effects of vildagliptin have been described in many reports. It was found that vildagliptin was orally well-absorbed, excreted mainly through the kidney, and did not inhibit or induce hepatic CYP enzymes. Also, hypoglycemic outcomes and gastrointestinal side effects were significantly reduced compared to other anti-diabetic drug therapies [99].

1.10. Possible Effects of Metformin and Vildagliptin on Telomerase Enzyme Activity and Oxidative Stress Parameters

In recent years, metformin started to attract attention in anti-aging research. Aging is a process regulated by a variety of signaling mechanisms, including nutritional sensing pathways, ROS-regulated oxidative stress, protein homeostasis, telomeres, and epigenetics (Figure 17). It was reported that metformin organizes the aging-relating pathways and may delay the aging processes in human cells. Most studies focus on the anti-aging effects of metformin and the associated molecular mechanisms [100].

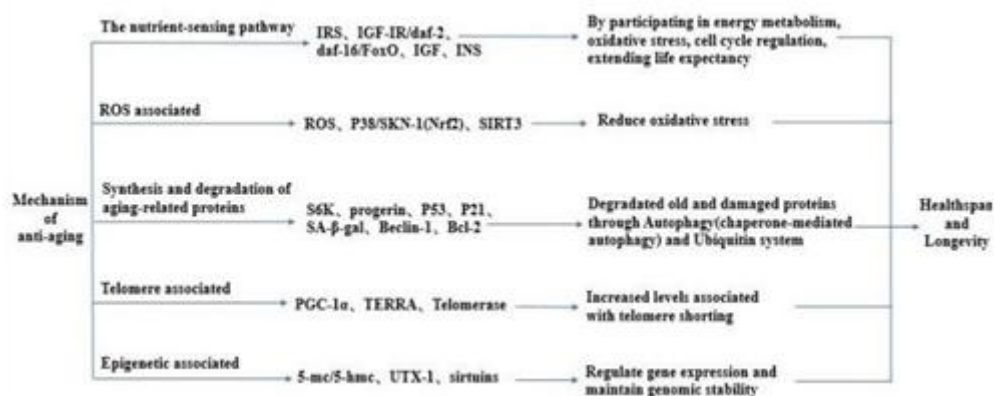
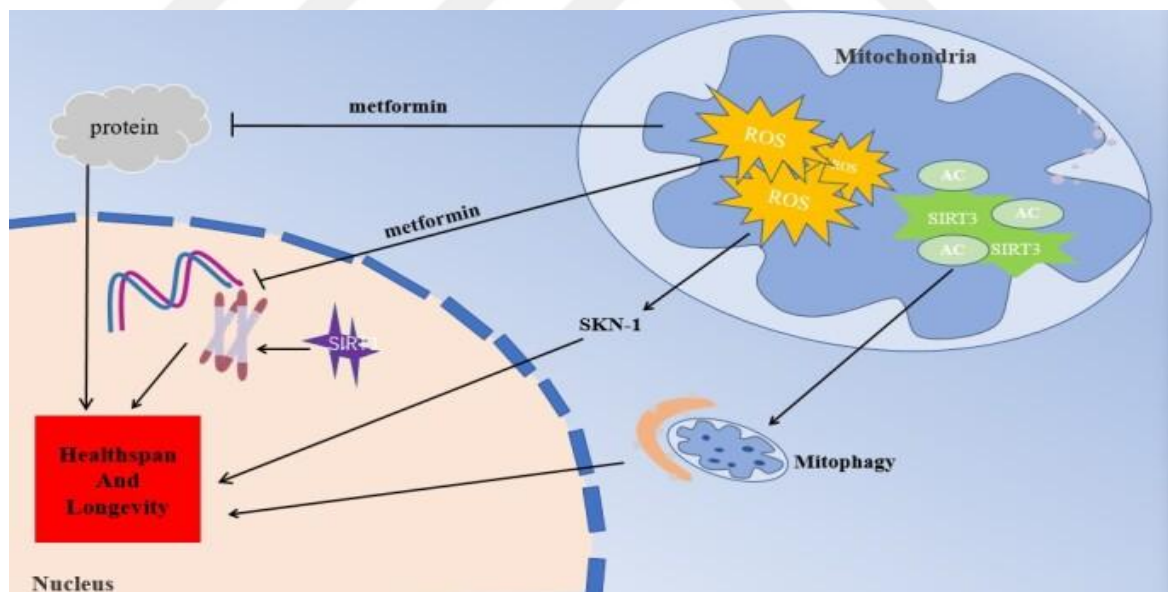


Figure 17. Possible mechanisms associated with lifespan [100].

Nutrient sensing is a major mechanism regulating nutrient uptake and signaling, affecting multiple signaling and metabolic processes. The IIS pathway (The nutrient-sensing pathway mediated by insulin/insulin-like growth factor-1 [INS/IGF-1] signaling), consisting of the INS/IGF-1 receptor, is key in this process. In the mouse brain, constant low IGF-I levels lead to an elongated lifespan and reduced age-related mortality. Also, the downregulation of IRS2, an adaptor protein of the IGF-1 receptor in the whole body or brain, could significantly extend the mouse lifespan. This extension in lifespan was related to the reduced secretion of IGF-1. Additionally, the impairment of IR/IRS2 signaling reduced β -amyloid accumulation in the brains of mice presenting Alzheimer's disease, thus demonstrating improvement in this aging-related disease. High level of insulin contributes to age-dependent insulin resistance, ultimately resulting in aging. Therefore, improving insulin sensitivity by decreasing insulin levels might extend the lifespan [100]. Epigenetic modifications are expected to become potential therapeutic strategies for anti-aging and aging-related diseases (Figure 18).



ROS, reactive oxygen species; SIRT1, sirtuin 1; SIRT3, sirtuin 3; SKN-1, transcription factor involved in anti-aging mechanisms.

Figure 18. Metformin delays aging by reducing oxidative stress [100].

Metformin protects biological macromolecules such as proteins and DNA by reducing excessive ROS production in the mitochondria. Furthermore, metformin can activate the SKN-1 pathway through ROS, which acts as a signaling molecule to delay aging. In addition, metformin activates SIRT3, participates in mitochondrial autophagy, and reduces oxidative stress, thereby delaying cell senescence [100].

Mechanisms underlying the anti-aging effect of metformin may be explained by the delay in aging through the IIS pathway, regulation of the ROS levels, and regulation of protein homeostasis. Also, metformin delays aging through telomeres and epigenetic mechanisms [100]. It was found that the mean average telomere length of patients not receiving metformin was lower than those of patients receiving metformin as well as healthy subjects [101].

In this study, in addition to the effects of metformin on the aging mechanism, we focused on the possible anti-aging effects related with ROS, and TE activity of vildagliptin, both as monotherapy and combination therapy with metformin.

2. MATERIALS AND METHODS

2.1. Materials

43 adult (12-18 weeks old) female Sprague–Dawley rats were obtained with the approval of Yeditepe University Animal Experiments Local Ethics Committee (HADYEK; decision number 2021/03-9). Also, used chemical agents and devices are presented below;

Chemical Agents	tri-Sodium citrate dehydrate / Sigma Aldrich
	Streptozocin (STZ) / Farmasina
	Metformin (MET) / Bilim İlaç
	Vildagliptin (VILDA) / Novartis
	Trisma Base / Sigma Aldrich
	Ethylenedinitrilotetraacetic acid disodium salt dihydrate (Na ₂ EDTA) / Sigma Aldrich
	Nicotinamide adenine dinucleotide phosphate (NADPH) / Sigma Aldrich
	Sodium Azide / Sigma Aldrich
	Glutathione (GSH) / Sigma Aldrich
	Glutathione Reductase (GR) / Sigma Aldrich
	t-butyl hydroperoxide (TBHP) / Sigma Aldrich
	Monosodium diphosphate (NaH ₂ PO ₄) / Sigma Aldrich
	Disodium phosphate (Na ₂ HPO ₄) / Sigma Aldrich
	Citric Acid / Sigma Aldrich
	Hydrogen peroxide (H ₂ O ₂) / Sigma Aldrich
	Potassium Chloride (KCl) / Sigma Aldrich
	Sodium Carbonate (Na ₂ CO ₃) / Sigma Aldrich
	Copper Sulfate (CuSO ₄) / Sigma Aldrich
	Sodium Potassium Tartrate (KNaC ₄ H ₄ O ₆) / Sigma Aldrich
	Bovine Serum Albumin (BSA) / Sigma Aldrich
	Formaldehyde / Sigma Aldrich
	Sodium dodecyl sulfate (NaC ₁₂ H ₂₅ SO ₄) / Sigma Aldrich
	Acetic Acid / Sigma Aldrich
	Thiobarbituric Acid (TBA) / Sigma Aldrich
	Tetramethoxy propane / Sigma Aldrich
	Trichloro acetic acid (TCA) / Sigma Aldrich
	Liquid paraffine / Sigma Aldrich
	Hematoxyline-Eosin / Sigma Aldrich
	Periodic Acid / Sigma Aldrich
	Basic Fucsin / Sigma Aldrich
	Potassium metabisulfide / Sigma Aldrich
	Hydrochloric Acid / Sigma Aldrich
	Activated Charcoal/ Sigma Aldrich
	Rat-Telomerase ELISA Kit (96 strip) / BT Lab

Devices	Glucometer / VivaCheck ®
	Spectrophotometer / Thermo Scientific, Evolution 300
	Centrifuge / Sigma 3-16 PK
	Microscope / Olympos
	Tissue Homogeniser / Heidolph, Silent Crusher S
	Microplate Reader / Thermo, Multiskan Ascent

2.1.1. Preparation of the Feed Used in the Study

Rats were fed *ad libitum* with baits (supplied from Optima Besin Maddeleri San. ve Tic. A.Ş.) composed of soy sauce, bonkalit, rice bran, corn, sugar beet molasses, calcium carbonate, dicalcium phosphate, vitamin-mineral mix, lignosulfanate (e 565), sodium chloride, bentonite (1m558), yeast (*Saccharomyces cerevisiae*), calcium propionate (e 282), and corn DDGS. The vitamin-mineral mix components are listed in Table 9.

Table 9. Components of the vitamin-mineral mix in rat feed.

VITAMINS	
Vitamin A	36000 IU/Kg
Vitamin D3	65000 IU/Kg
Calcium Iodate Anhydrous	1.3 mg/kg
ELEMENTS	
Cobalt II Carbonate	0.15 mg/kg
Copper Sulfate Pentahydrate	16 mg/kg
Manganese Oxide	155 mg/kg
Zinc Oxide	130 mg/kg
Sodium Selenite	0.35 mg/kg

2.1.2. Preparation of the Solutions Used

2.1.2.1. Sodium Citrate Buffer Preparation

Since a maximum of 1 mL intraperitoneal (i.p.) injection can be applied, 36 mL of 100 mg/mL sodium citrate solution (pH 4.5) was prepared for 36 animals that received STZ by dissolving 3600 mg of tri-Sodium citrate dihydrate (VWR) in 36 mL of distilled water.

2.1.2.2. Preparation of STZ

The STZ solution should be prepared freshly for each injection and injected within 5 min of being dissolved. For that reason, immediately prior to injection, STZ was dissolved in 50 mM sodium citrate buffer to a final volume of 36 mL. Since 80 mg/kg single dose STZ was applied to four different test groups (n=9 each), 490 mg of STZ was weighed and dissolved in the previously prepared sodium citrate buffer solution (pH 4.83). Using a 1-ml syringe and 23-G needle, STZ solution was injected i.p. for the study group. An equal volume of citrate buffer (pH 4.73) was injected i.p. for the control group. The solutions were applied in volumes appropriate for the rats' weight.

2.1.2.3. Preparation of the Antidiabetic Drug Metformin (Glifor)

Since a maximum of 1 mL oral gavage can be applied to each animal for metformin, and 18 animals were to receive the drug for 8 days, 2806 mg of Glifor (metformin) was dissolved in 144 mL of 0.9% sodium chloride solution for the test groups that received the drug. Dose calculations are as follows:

Metformin (MET) dose = 100 mg/kg/day

BWort = 170 g

100 mg MET is required for 1000 g animal

17 mg MET is required for a 170 g animal (the weight of 1 animal)

306 mg MET was required for a total of 18 animals (n=9 x 2 groups) for 1 day

2806 mg MET was required for a total of 18 animals (n=9 x 2 groups) for 8 days

2.1.2.4. Preparation of the Antidiabetic Drug Vildagliptin (Galvus)

Since a maximum of 0.5 mL oral gavage can be applied to each animal for vildagliptin, and 18 animals were to receive the drug for 8 days, 144 mg Galvus (vildagliptin) was dissolved in 72 mL of 0.9% sodium chloride solution for the test groups that received the drug. Dose calculations are as follows:

Vildagliptin dose= 6 mg/kg/day

BWort= 170 g

6 mg VILDA is required for 1000 g animal

1 mg VILDA is required for a 170 g animal (the weight of 1 animal)

18 mg VILDA was required for a total of 18 animals (n=9 x 2 groups) for 1 day

144 mg VILDA was required for a total of 18 animals (n=9 x 2 groups) for 8 days

2.2. Establishing the T2DM Model

BGL of the animals was measured 72 hours after the i.p. administration of the STZ solution. A VivaCheck Eco® Glucometer and VivaCheck Eco® Strips (50 pcs) were used to measure the BGL (mg/dL) in each animal group. Rats that showed BGL higher than 200 mg/dl were considered diabetic (Table 10). Rats that developed T2DM were divided into groups for the next treatment phase of the study.

Table 10. Blood glucose level (mg/dL) of rats 24 h after the i.p. injection of the STZ solution.

Groups	Blood Glucose Level (mg/dL)
A (n=7)	118.71 ± 11.19

B (n=9)	200.74 ± 41.44
C (n=9)	210.89 ± 12.81
D (n=9)	291.78 ± 35.04
E (n=9)	295.78 ± 40.91

2.3. Experimental Groups

Seven rats were included in the control group, and nine rats were included in each experimental group (four groups of rats with T2DM) as shown in Table 11.

Table 11. Experimental design.

GROUPS	METHOD	DOSE	DURATION
GROUP A (n=7)	Healthy, untreated rats	-	8 days
GROUP B (n=9)	T2DM rat group with pancreatic injury induced by STZ	80 mg/kg (single dose)	8 days
GROUP C (n=9)	T2DM treatment with metformin (Glifor®; 1000 mg, 100 tablets)	100 mg/kg/day	8 days
GROUP D (n=9)	T2DM treatment with vildagliptin (Galvus®; 50 mg, 56 tablets)	6 mg/kg/day	8 days
GROUP E (n=9)	T2DM treatment with metformin + vildagliptin	100 mg/kg/day +	8 days
		6 mg/kg/day	

2.4. Metformin and Vildagliptin Administrations

Drug applications to the treatment groups were initiated on the second day of the study and continued for 8 days. 1 mL of metformin solution was applied to Group C and Group E by oral gavage. Similarly, 0.5 mL of vildagliptin solution was applied to Group D and Group E by oral gavage. All gavage applications were performed at noon.

2.5. Body Weight and Fasting Blood Glucose Measurements

Rats were weighed before starting the experiment. The results of the measurements are shown in Table 12. Blood glucose measurements of the rats were repeated every day for a total of 8 days. The results of the measurements are shown in Table 14.

2.6. Termination of the Study

Blood samples taken from the cardia of animals anesthetized with high-purity carbon dioxide were collected in 2.5 mL K2-EDTA and gelly yellow-colored blood tubes mentioned previously. First, BGL measurements were performed with the collected blood. Then, the animals were dissected for tissue sampling. Liver, kidney, and pancreas tissues were stored in a 10% formaldehyde solution for histopathological analysis. For the determination of oxidative stress parameters, 2 grams of liver tissue samples were weighed and frozen on dry ice and stored at -80 degrees. Total liver weight and hemogram were measured for each animal (Table 13 and Table 15).

2.7. Biochemical Analysis

At the end of the study, erythrocyte and liver MDA level, antioxidant enzyme activity were measured by spectrophotometric methods. In addition, activities of enzymatic antioxidants such as CAT, and GPx were determined.

2.7.1. Preparation of the Plasma and Erythrocyte Hemolysate

Preparation of the Plasma: Blood samples transferred into heparinized tubes were centrifuged at 3000 $\times g$ at +4°C, and the plasma was transferred to a fresh tube after separation from shaped elements. The separated plasma samples were divided into portions and stored at -20°C for laboratory analysis.

Preparation of Erythrocyte Hemolysate: Blood samples (n=43) kept at room temperature (25°C) for 2 hours were centrifuged at 4000 $\times g$ for 10 minutes at 4 °C. After the supernatant was discarded, the remaining part was washed 3 times with 2 layers of

isotonic saline solution. With a 1:4 ratio of erythrocytes and cold distilled water, erythrocytes were lysed and incubated at 4°C for 15 minutes. Then, the sample was centrifuged at 2000 $\times g$ for 10 minutes at 4°C. Cell remnants were removed, and the obtained erythrocyte hemolysate was stored at -70°C.

2.7.1.1. Liver GPx Analysis

GPx activities were measured in liver homogenates. The reaction mixture was 50 ml, containing 0.303 g of Trizma base, 0.0186 g of Na₂EDTA, 0.00834 g of NADPH, 0.0130 g of sodium azide, 0.0307 g of GSH, and 50 U of GR. 10 μ l of homogenate and 990 μ l of the reaction mixture were mixed, and the reaction was initiated with 10 μ l of t-butyl hydroperoxide (TBH). The decrease in absorbance was followed at 340 nm for 3 min (U/g protein) [113].

2.7.1.2. Liver CAT Analysis

To prepare the phosphate buffer solution, 1.29 g of monosodium diphosphate and 2.57 g of disodium phosphate were dissolved in 500 mL of distilled water. pH was adjusted to 7.0 using a citric acid solution. 200 mcl of 30% H₂O₂ solution was added to 50 mL of phosphate buffer solution to prepare the hydrogen peroxide solution. Eight standard solutions of CAT were prepared by dilution in phosphate buffer. Spectrophotometric measurement of CAT enzyme activity depending on increasing H₂O₂ concentration was carried out at 240 nm (kU/g protein) [113].

2.7.1.3. Tissue Homogenization

1 g of the liver tissue sample obtained from each rat was homogenized by the addition of 1.15% KCl (potassium chloride) solution and homogenized at 75 x 1000 rpm using a tissue homogenizer. For each sample, 1 mL of liver homogenate was collected and placed in a deep freezer until used for protein assay.

2.7.1.4. Lowry Protein Assay

Lowry protein assay is a biochemical assay applied to determine the total protein concentration. The presence of proteins can be observed by the concentration-dependent color change in the sample solution, which can be measured by colorimetric techniques. Shortly, 250 mL of 2% 0.1 N Na_2CO_3 (sodium carbonate), 2.5 mL of 0.5% CuSO_4 (copper sulfate), and 5 mL of 1% NaKTar (sodium potassium tartarate) are mixed to prepare the Lowry Reagent. Folin solution is diluted by distilled water at a ratio of 1:2 (v/v). Six standard solutions of known protein concentrations were prepared from the 2% stock solution of bovine serum albumin (BSA). Spectrophotometric measurement of the absorbance values of proteins was carried out at 640 nm [113].

2.7.2. Measurement of Oxidative Damage Biomarkers—MDA Analysis

To prepare 0.2 mL of 10% samples and standard solutions, 8.1% sodium dodecyl sulfate (SDS) solution, 20% acetic acid solution, and 0.8% thiobarbituric acid (TBA) solution were used. The final volume of all samples was 4 mL. Six standard solutions were prepared from 0.05% tetramethoxy propane stock solution. Following the addition of distilled water and trichloroacetic acid (TCA) solution to the samples, spectrophotometric measurements were carried out by determining the absorbance values at 532 nm (nmol/g protein)[113].

2.7.3. Determination of the Cellular Life Cycle Status

2.7.3.1. Telomerase Enzyme Activity Analysis

The telomerase enzyme activity test was carried out using a Rat-Telomerase ELISA Kit (96-strip) obtained from BT Lab. Following the addition of the standards and serum samples to the wells, streptavidin-HRP and anti-TE antibody was added to the wells except for those containing the standards. The plate was covered with aluminum folio and incubated at 37°C for 60 minutes. The wells were washed, substrate solutions were added, and the plate was incubated about at 37°C for 10 minutes in the dark. After the addition of the stop solution, an immediate color change from blue to

yellow was observed. The microplate reader was set to 450 nm to determine the optical density (OD) values of the samples.

2.8. Histopathological Analysis

All tissue samples were placed in capped embedding cassettes for tissue tracking and placed into the tissue tracking device for paraffin embedding. Paraffinized tissue incisions were carried out using a microtome device. The incisions were placed on microscope slides and covered with 24x60 mm coverslips. Hematoxylin-eosin (H&E) staining was applied to the tissue slides to detect cell damage under microscopic examination. Detected cell damages were scored and statistically analyzed.

2.8.1. Tissue Sample Tapering

Kidney, and liver tissue samples were obtained from the animals, placed on a cassette, and soaked in 0.1 M 10% neutral formaldehyde solution. Afterward, the kidneys were separated into two sections to allow better absorption of the chemicals.

2.8.2. Organization of Devices

2.8.2.1. Tissue Tracking Device

The contents of the 11 BOXes in the tissue tracking device were checked, and the device was run in Program 3. Neutral formaldehyde solution containing 37% formaldehyde in 0.1 M phosphate buffer was used.

2.8.2.2. Tissue Embedding Machine

Solid paraffin was placed in the embedding device and heated up to 65°C to obtain a liquid form. Some of the liquid paraffin was transferred into sample cups. Then, the tissue cassettes were placed into sample cups, applying a slight pressure on top to fix the tissue. The dry ice section of the device was used to fix the tissues. The sample cups were filled with liquid paraffin, and the samples were allowed to freeze.

2.8.2.3. Microtome

Incisions of paraffin-embedded tissues were obtained using a microtome device and disposable microtome blades. Selected tissue incisions were placed into hot water and then placed immediately on microscope slides.

2.8.3. Tissue Staining

2.8.3.1. H&E Staining

In the H&E staining method, calcium precipitants and nucleic acids (cell nucleus) are stained by hematoxylin (blue-purple), and proteins, amyloids, and the cytoplasm are stained by eosin (pink).

Tissue samples were placed in xylol for about 10 minutes to keep the tissues deparaffinized. The samples were placed in 95% ethanol for 10 minutes, 80% ethanol for 10 minutes, and 70% ethanol for 10 minutes for dehydration. All samples were then incubated in hematoxylin for 5 to 6 minutes (the hematoxylin step). The sections were washed with distilled water for another 5-10 minutes (first wash) and treated for 1-2 seconds in acid alcohol until a pale blue color was observed (second wash). Tissue samples were then placed in eosin for 3-4 minutes (the eosin step) and washed with 70%, 80%, and 95% ethanol solutions sequentially. The samples were then placed in absolute ethanol and incubated for 15-20 minutes (exit alcohol step). Each tissue was passed through 3 xylols every 10 minutes (exit xylol step). The nucleus appeared in dark blue-violet color, the cytoplasm was colored pink-red, and bacteria were colored sky blue.

2.8.3.2. PAS Staining Method

PAS staining technique was also used for the second sections obtained from the kidneys. Two grams of periodic acid was weighed and dissolved in 400 mL of distilled water to prepare the periodic acid solution. Then, to prepare the Schiff Solution, 2 g of basic fuchsin was weighed and dissolved in 400 mL of distilled water in an Erlenmeyer flask and boiled using a hot plate. The solution was allowed to cool down to 50°C, and 4

g of potassium metabisulfide was added. The mixture was then cooled down to 25°C (room temperature). Finally, 4 mL of HCl and 4 g of activated charcoal were added, and the solution was left to rest overnight. The tissues were deparaffinized and incubated in the periodic acid solution for 5 minutes. They were washed with distilled water and left in Schiff solution for 15 minutes. The tissues were washed for 5-10 minutes (wash 1). The nucleus was stained by hematoxylin differentiated with acid alcohol. The samples were washed again with distilled water (wash 2), rinsed with absolute ethanol, and finally, cleaned with xylene. Glycogen and carbohydrates appeared in red-purple, and the nucleus appeared in blue color at the end of the process.

2.8.3.3. Organization of the Tissue Staining Device

As mentioned in previous sections, an automatic staining device was used for tissue staining. The device was run in program 7 for H&E staining and program 3 for PAS staining. Tissue samples were kept in the oven for 10 minutes at 37 °C before placement in the staining device. The samples were then stained using the automatic staining device for approximately 50 minutes and covered with 24x 60 mm coverslips using an automated coverslip closure device.

2.9. Statistical Analysis

The data were analyzed using the SPSS 25.0 (Statistical Packages of Social Sciences) software. The conformity of the data to the normal distribution was evaluated using the Shapiro–Wilk's test. Explanatory statistics are shown as mean $\pm$ standard deviation (SD), median, and minimum and maximum values for continuous variables. The Kruskal-Wallis test was used to compare the variables that did not fit the normal distribution between the groups. Mann–Whitney U test was used as the post-hoc test for the variables that were found to be statistically significant, and Bonferroni correction was applied to the results. Variables with normal distribution within the group were evaluated with the dependent sample t-test. If $p < 0.001$, the difference was considered significant.

3. RESULTS

3.1. Body and Liver Weights of Rats

After the rats were adapted to the laboratory conditions, their weights were measured on Days 1, 2, 3, 5, and 7. Changes in the body weights of rats during the experiment are shown in Table 12 and Figure 7.

Table 12. Body weights of the rats in each experimental group.

Groups	Body Weight (g)							Day 8
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Control (n=7)	162.14 ± 8.69	168.29 ± 7.59	171.00 ± 6.40	-	176.00 ± 5.89	-	176.57 ± 7.55	-
STZ (n=9)	168.44 ± 6.75	168.22 ± 11.38	166.33 ± 9.51	-	173.22 ± 11.43	-	172.89 ± 11.02	-
MET (n=9)	175.33 ± 7.91	167.56 ± 7.16	164.22 ± 8.14	-	170.00 ± 8.80	-	170.79 ± 9.04	-
VILDA (n=9)	171.22 ± 7.73	163.44 ± 8.52	158.78 ± 8.57	-	165.56 ± 10.89	-	163.67 ± 11.82	-
MET/VILDA (n=9)	175.89 ± 8.25	168.00 ± 10.30	162.00 ± 10.85	-	166.89 ± 13.27	-	169.44 ± 14.04	-

STZ: diabetic rats; MET: metformin applied rats; VILDA: vildagliptin applied rats; MET/VILDA: metformin plus vildagliptin applied rats. Data are presented as mean ± SD.

Body weight results obtained on the 7th day were used in the final data analysis. Based on the average weight of rats on days 1 and 7, increased body weight was observed in the control, and STZ groups. Meanwhile, body weight was reduced in the VILDA and MET/VILDA groups. The average body weight of the rats in the VILDA group was significantly lower than those in the control, STZ, MET, and MET/VILDA groups. The average body weight of the control group on day 7 was the highest among all experimental groups.

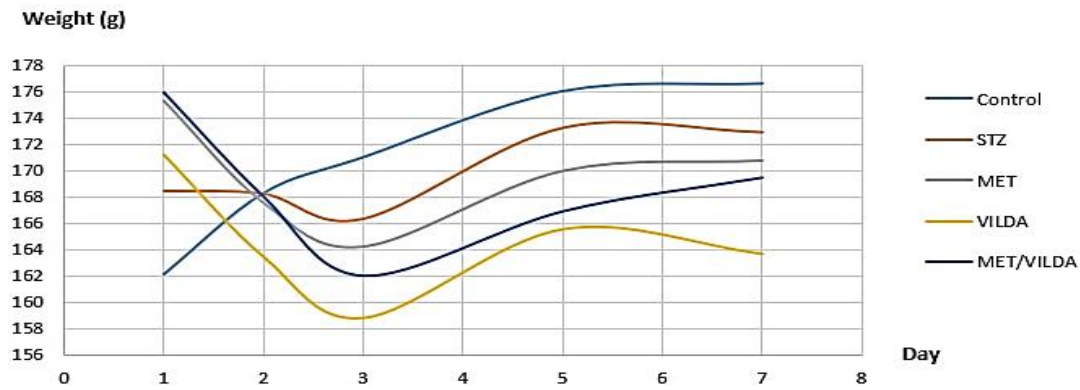


Figure 19. Weight change of rats over days 1 to 7.

At the end of the experiment, liver weight of rats were measured. The average liver weight data (mean $\pm$ SD) are given in Table 13.

Table 13. Liver weights of the rats in each experimental group

Groups	Liver Weight (g)
Control (n=7)	7.417 $\pm$ 0.981
STZ (n=9)	6.552 $\pm$ 0.695
MET (n=9)	7.404 $\pm$ 0.516
VILDA (n=9)	6.774 $\pm$ 0.833
MET/VILDA (n=9)	6.706 $\pm$ 0.444

STZ: diabetic rats; MET: metformin applied rats; VILDA: vildagliptin applied rats; MET/VILDA: metformin plus vildagliptin applied rats. Data are presented as mean $\pm$ SD.

No significant difference was observed in the average liver weights of rats between the control and experimental groups (STZ, MET, VILDA, and MET/VILDA). Also no significant difference was found between MET and VILDA, VILDA and MET/VILDA, MET and MET/VILDA.

3.2. BGL of Rats

After the adaptation process of the rats was completed, the BGL of the control and experimental groups were measured. The values were measured once after oral

gavage on each experimental day except for day 6. In order to observe drug efficacy, BGL was measured twice (before and after oral gavage) on Day 6. The data are shown in Table 14.

Table 14. Blood glucose levels of the experimental groups

Blood Glucose Level (mg/dL)									
Groups	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6		Day 7	Day 8
						Before Oral Gavag e	After Oral Gavag e		
Control (n=7)	126.71	-	-	126.86	-	-	-	-	135.86
± SD	6.99	-	-	9.48	-	-	-	-	23.23
STZ (n=9)	116.56	151.33	210.11	199.78	179.44	176.67	170.78	178.22	181.56
± SD	9.62	24.64	118.39	125.17	79.75	107.92	112.02	103.13	65.04
MET (n=9)	122.78	270.22	398.22	425.67	438.78	406.67	360.44	387.33	449.67
± SD	13.33	99.72	79.95	118.04	114.36	99.97	104.63	97.45	123.93
VILDA (n=9)	122.78	252.22	418.22	431.56	418.89	396.67	379.89	388.67	426.78
± SD	10.37	107.43	63.78	67.88	65.81	49.89	74.79	72.96	89.27
MET/VILDA (n=9)	112.44	272.78	381.00	387.56	380.33	360.67	330.44	364.78	443.44
± SD	4.48	68.28	93.88	106.43	100.62	89.95	124.61	94.08	123.27

STZ: diabetic rats; MET: metformin applied rats; VILDA: vildagliptin applied rats; MET/VILDA: metformin plus vildagliptin applied rats.

The lowest BGL was observed in the control group. Blood glucose lowering effect of the combination of drugs in the MET/VILDA group were significantly higher than those of MET and VILDA alone. However, no significant difference was observed in the effects of single drugs between the MET and VILDA groups. Figure 20 shows the changes in BGL over the experimental days.

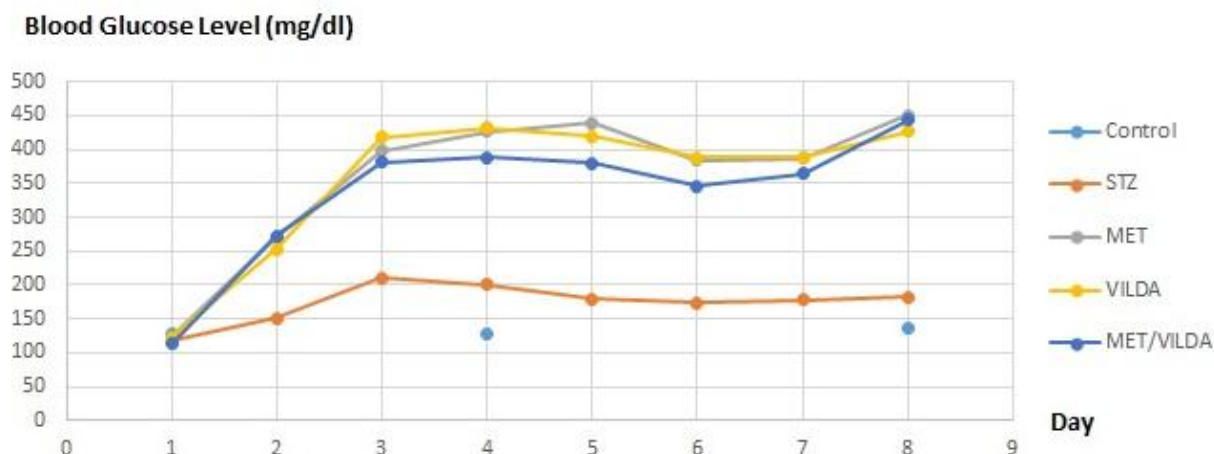


Figure 20. BGL changes in rats from day 1 to day 8.

3.3. Hemogram Values of Rats

In order to follow the health status of the rats, hemogram values were checked on the first and last days of the experimental process (days 1 and 8; Table 15).

Table 15. Hemogram values of the rats in the experimental groups on days 1 and 8.

Groups	Day 1				Day 8			
	WBC ($10^3/\mu$ L)	RBC ($10^6/\mu$ L)	HGB (g/dL)	PLT ($10^3/\mu$ L)	WBC ($10^3/\mu$ L)	RBC ($10^6/\mu$ L)	HGB (g/dL)	PLT ($10^3/\mu$ L)
Control (n=7)	10.600	7.916	19.414	433.857	6.086	3.199	7.186	242.000
± std dev.	1.526	0.517	1387	62.350	6.528	2.763	6.574	445.141
STZ (n=9)	10.589	8.067	20.389	366.556	20.411	5.621	12.800	260.000
± std dev.	2.901	0.492	1.215	68.464	20.295	1.476	4.691	219.196
MET (n=9)	10.122	8.226	20.422	370.111	12.963	7.016	15.625	729.125
± std dev.	1.781	0.666	1.965	121.236	7.771	0.271	0.537	382.000
VILDA (n=9)	12.122	8.658	21.544	425.222	11.144	7.172	16.100	761.000
± std dev.	2.631	0.448	1.532	83.600	5.789	0.136	0.536	268.869
MET/VILDA (n=9)	12.956	8.909	22.711	365.556	12.822	6.850	15.633	784.667
± std dev	2.416	0.673	1.790	62.466	2.102	0.336	0.718	275.421

STZ: diabetic rats; MET: metformin applied rats; VILDA: vildagliptin applied rats; MET/VILDA: metformin plus vildagliptin applied rats. WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; PLT, platelet.

Increased PLT values indicated that all experimental groups experienced anemia and thrombocytosis. RBC and HGB values might have decreased due to a lack of nutrition, leading to anemia.

3.4. GPx, CAT, and Telomerase Enzyme Activities and MDA Analysis

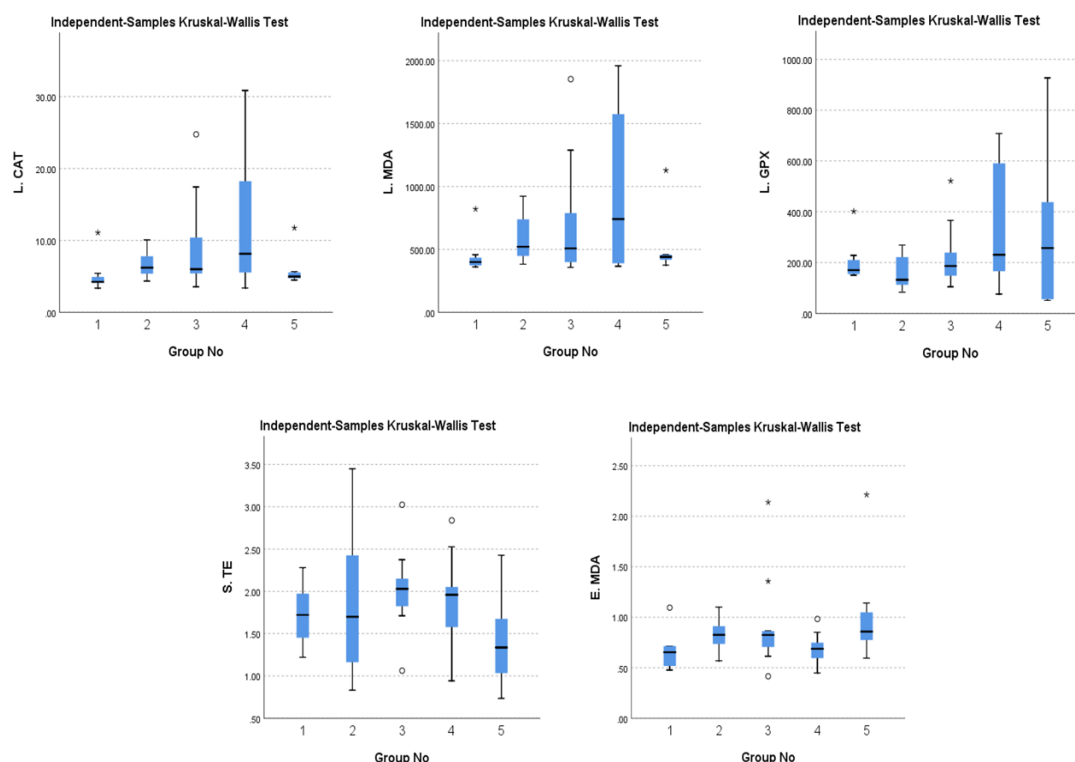
Erythrocyte MDA formation in MET/VILDA rats was higher than that in the control, MET, and VILDA groups and lower in VILDA rats compared with the MET/VILDA and MET groups. Opposite to that, liver MDA formation in MET/VILDA rats was significantly lower than that in the control, MET, and VILDA groups and higher in VILDA compared with the MET/VILDA and MET groups. Also, GPx and CAT activities in the VILDA group were higher than those in the control, MET, and MET/VILDA groups. GPx activity in the MET group was lower than VILDA and higher than MET/VILDA groups. TE activity was higher in the MET and VILDA groups compared to the control rats. The drug effect on TE activity in the MET group was higher than in the VILDA group. In MET/VILDA rats, TE activity was lower compared to those in the control and monotherapy groups (Table 16 a).

Table 16 a. Erythrocyte and liver MDA levels, hepatic antioxidant enzyme activities (GPx, CAT), and serum telomerase enzyme activity data.

Groups	E.MDA (nmol/g protein) (Mean $\pm$ S.D.)	L.MDA (nmol/g protein) (Mean $\pm$ S.D.)	L.CAT (kU/g protein) (Mean $\pm$ S.D.)	L.GPX (U/g protein) (Mean $\pm$ S.D.)	S.TE (ng/ml) (Mean $\pm$ S.D.)
Control (n=7)	0.69 $\pm$ 0.20	456.02 $\pm$ 164.00	207.36 $\pm$ 90.07	5.23 $\pm$ 2.65	1.72 $\pm$ 0.38
STZ (n=9)	0.83 $\pm$ 0.18	589.79 $\pm$ 195.01	160.45 $\pm$ 67.16	6.64 $\pm$ 1.89	1.89 $\pm$ 0.87
MET (n=9)	0.95 $\pm$ 0.51	743.06 $\pm$ 509.28	229.86 $\pm$ 133.12	9.48 $\pm$ 7.15	2.02 $\pm$ 0.53
VILDA (n=9)	0.69 $\pm$ 0.16	993.40 $\pm$ 664.18	344.24 $\pm$ 253.40	11.82 $\pm$ 9.29	1.88 $\pm$ 0.58
MET/VILDA (n=9)	1.00 $\pm$ 0.48	516.27 $\pm$ 248.95	309.98 $\pm$ 306.61	5.84 $\pm$ 2.41	1.26 $\pm$ 0.70

STZ: diabetic rats; MET: metformin applied rats; VILDA: vildagliptin applied rats; MET/VILDA: metformin plus vildagliptin applied rats. E, erythrocyte; L, liver; S, serum; MDA, malondialdehyde; GPX, glutathione peroxidase; CAT, catalase; TE, telomerase. Data are presented as mean $\pm$ SD.

The data were statistically analyzed as described in Section 2.9. According to the Kruskal–Wallis test results, there was no statistically significant difference in the median values of the measurements between the groups ($p>0.05$). Box plots of the measurements for the groups are given in Figure 22.



Group No:1, Control; Group No: 2, STZ; Group No: 3, MET; Group No: 4, VILDA; Group No: 5, MET/VILDA

Figure 22. Box plots of the erythrocyte MDA formation, liver MDA formation, liver GPx activity, liver CAT activity, and serum telomerase activity measurements.

In order to increase the interpretability of the results; statistically analyzed data (E.MDA, L.MDA, L. CAT, L.GPX, S.TE) corrected by neglecting the mismatched scores. Revised data obtained from corrected scores of each experimental groups were presented in Table 16 b.

Table 16 b. Corrected data of erythrocyte and liver MDA formation, liver enzymatic defense system (GPx, CAT) and serum telomerase enzyme activity.

Groups	E.MDA (nmol/g protein) (Mean $\pm$ S.D.)	L.MDA (nmol/g protein) (Mean $\pm$ S.D.)	L.CAT (kU/g protein) (Mean $\pm$ S.D.)	L.GPX (U/g protein) (Mean $\pm$ S.D.)	S.TE (ng/ml) (Mean $\pm$ S.D.)
Control (n=7)	0.60 ^g $\pm$ 0.10	395.31 $\pm$ 33.04	4.20 $\pm$ 0.14	164.26 $\pm$ 15.17	1.72 ^c $\pm$ 0.35
STZ (n=9)	0.82 $\pm$ 0.06	494.54 $\pm$ 85.80	6.05 $\pm$ 0.74	182.15 $\pm$ 57.71	1.69 $\pm$ 0.65
MET (n=9)	0.72 ^b $\pm$ 0.15	502.46 $\pm$ 147.49	5.46 $\pm$ 1.59	68.74 ^a $\pm$ 25.26	2.02 ^a $\pm$ 0.50
VILDA (n=9)	0.65 ^e $\pm$ 0.12	506.24 ^d $\pm$ 144.29	6.77 ^f $\pm$ 2.03	168.76 ^{e, f} $\pm$ 41.58	1.88 ^d $\pm$ 0.55
MET/VILDA (n=9)	0.77 $\pm$ 0.10	428.81 ^a $\pm$ 28.16	5.00 $\pm$ 0.39	155.29 ^d $\pm$ 50.12	1.41 $\pm$ 0.51

STZ: diabetic rats; MET: metformin applied rats; VILDA: vildagliptin applied rats; MET/VILDA: metformin plus vildagliptin applied rats. E, erythrocyte; L, liver; S, serum; MDA, malondialdehyde; GPX, glutathione peroxidase; CAT, catalase; TE, telomerase. Data are presented as mean $\pm$ SD. a; MET&MET/VILDA $p < 0.001$, b; MET&MET/VILDA $p > 0.05$, c; Control&STZ $p > 0.05$, d; VILDA&MET/VILDA $p < 0.001$, e; MET&VILDA $p < 0.001$, f; MET&VILDA $p < 0.001$, g; Control&VILDA $p > 0.05$.

Erythrocyte MDA formation in MET/VILDA rats was higher than those in the control, MET, and VILDA groups and lower in VILDA rats compared with the MET/VILDA and MET groups. Also, CAT activities in the VILDA group were higher than those in the control, MET, and MET/VILDA groups and lower in MET/VILDA rats compared with the MET and VILDA. GPx activities in the MET group were higher than those in control, VILDA and MET/VILDA groups and lower in the MET/VILDA rats were lower than those in the control, STZ, MET and VILDA groups. TE activity was higher in the MET and VILDA groups compared to the control rats. The drug effect on TE activity in the MET group was higher than in the VILDA group. In MET/VILDA rats, TE activity was lower compared to those in the control, diabetic rat and monotherapy groups (Table 16 b).

3.5. Cellular Damage Scores of Tissue Samples

Microscopic examinations of the H&E-stained tissues are shown in Figure 21. Interstitial edema and hemorrhagic response were observed in STZ-induced T2DM rats, which were diminished in the MET/VILDA group. Also, Histopathological scores' mean and SD values are shown in Table 17 and Table 18.

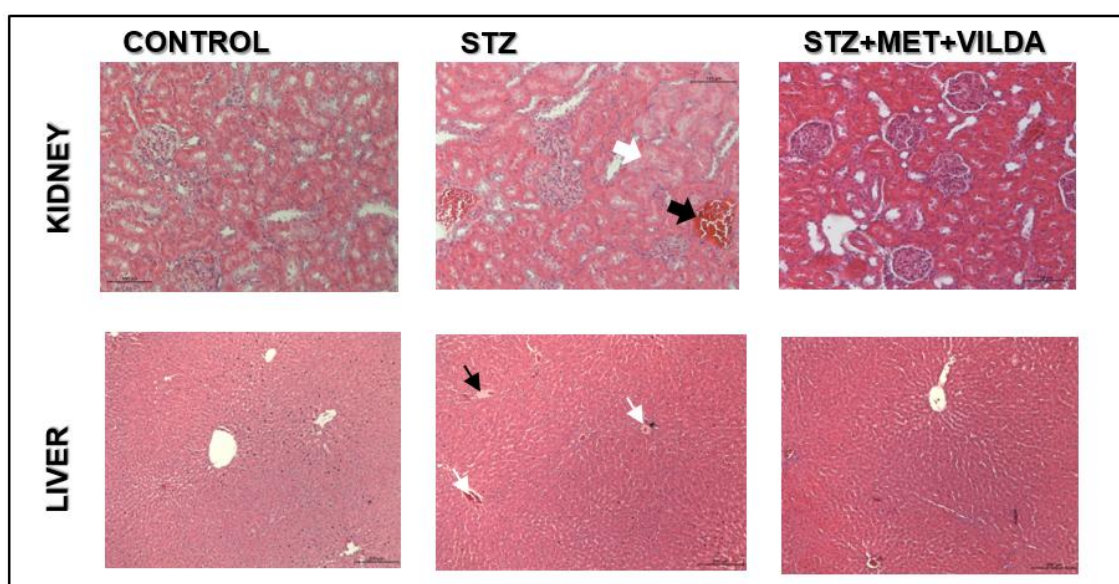


Figure 21. Cellular damage analysis of tissue samples stained with H&E. White arrows indicate interstitial edema, and black arrows indicate hemorrhagic response.

Table 17. Histopathological scores of liver damage.

Types of Tissue Damage		Intertisial Edema	Sinuzoid Capillary Congestion	Hepatocellular Necrosis	Hepatocyte Growing (Vacuol)	Leukocyte Infiltration	Total Score
Experimental Groups							
Control	mean	0.429	0.429	0.429	0.286	0.286	1.857 ^{c,f}
	±SD	0.535	0.535	0.535	0.488	0.488	0.900
STZ	mean	2.222	2.333	2.444	1.222	1.111	9.333
	±SD	0.667	0.500	0.527	0.441	0.333	1.225
MET	mean	1.333	1.444	1.778	1.000	0.444	6.000 ^a
	±SD	0.500	0.527	0.441	0.500	0.527	1.000
VILDA	mean	1.333	1.222	1.333	1.222	1.333	6.444 ^d
	±SD	0.500	0.833	0.500	0.441	0.707	1.424
MET/VILDA	mean	0.667	1.222	1.444	1.333	0.333	5.000
	±SD	0.500	0.441	0.527	0.500	0.500	1.000

Table 18. Histopathological scores of kidney damage.

Types of Tissue Damage		Intertistial Edema	Sinusoid Capillary Congestion	Hepatocellular Necrosis	Hepatocyte Growing (Vacuolation)	Leukocyte Infiltration	Total Score
Experimental Groups							
Control	mean	0,857	0,714	0,857	1,714	0,429	4,571 c,f
	std. Dev.	1,069	0,488	0,690	1,113	0,535	2,507
STZ	mean	2,111	2,556	2,444	2,889	1,625	11,444 b
	std. Dev.	0,782	0,527	0,726	0,333	0,744	0,726
MET	mean	2,444	2,889	2,667	1,444	1,222	10,667 a,e
	std. Dev.	0,882	0,333	0,500	0,726	0,972	2,179
VILDA	mean	2,778	2,667	2,889	2,000	1,111	11,444 d
	std. Dev.	0,441	0,500	0,333	1,000	0,928	1,333
MET/VILDA	mean	2,333	2,333	2,444	1,778	0,889	9,778
	std. Dev.	0,707	1,000	0,726	1,302	0,928	1,922

STZ: diabetic rats; MET: metformin applied rats; VILDA: vildagliptin applied rats; MET/VILDA: metformin plus vildagliptin applied rats. Data are presented as mean $\pm$ SD. **a**; MET&MET/VILDA $p < 0.001$, **b**; STZ&VILDA $p > 0.05$, **c**; Control&MET/VILDA $p > 0.05$, **d**; VILDA&MET/VILDA $p < 0.001$, **e**; MET&VILDA $p < 0.001$, **f**; Control&MET/VILDA $p < 0.001$

According to the median value of total scores, the highest cellular damage was found in the STZ group compared to the control, MET, VILDA, and MET/VILDA groups. This suggestion supported by Figure 21. Among the treatment groups, the lowest damage was found in MET/VILDA.

On the other hand; liver damage in MET and VILDA was almost equal to each other however, kidney damage was higher in VILDA compare to MET. Also kidney damage in VILDA and STZ was almost equal to each other.

3.6. Statistical Analysis of the Results

The data which was presented Table 16 b. were statistically analyzed as described in Section 2.9. According to the one way ANOVA, there was statistically

significant difference in the median values of the measurements between the groups (p<0.001). Average Total Score $\pm$ Standart Deviation data are presented in Table 20.

Table 20. Statistical Analysis of corrected datas of erythrocyte and liver MDA formation, liver enzymatic defense system (GPx, CAT) and serum telomerase enzyme activity.

Name of Experimental Group	E.MD A (Average Total Score $\pm$ Standart Deviation)	L.MD A (Average Total Score $\pm$ Standart Deviation)	L.CAT (Average Total Score $\pm$ Standart Deviation)	L.GPX (Average Total Score $\pm$ Standart Deviation)	S.TE (Average Total Score $\pm$ Standart Deviation)	p E.M DA	p L.M DA	p L.C AT	p L.G PX	p S.T E
CONTR	0,60 $\pm$ 0,10 & 0,82 $\pm$ 0,06	395,31 $\pm$ 33,04	4,20 $\pm$ 0,14 & 6,05 $\pm$ 0,74	164,26 $\pm$ 15,17	1,72 $\pm$ 0,35 & 1,69 $\pm$ 0,65	0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,08 1
OL & STZ	0,60 $\pm$ 0,10 & 0,72 $\pm$ 0,15	395,31 $\pm$ 33,04	4,20 $\pm$ 0,14 & 5,46 $\pm$ 1,59	164,26 $\pm$ 15,17	1,72 $\pm$ 0,35 & 2,02 $\pm$ 0,50	0,00 0*	0,00 0*	0,00 0*	0,13 4	0,00 0*
CONTR	0,60 $\pm$ 0,10 & 0,65 $\pm$ 0,12	506,46 $\pm$ 502,24	0,14 & 6,77 $\pm$ 2,03	168,76 $\pm$ 155,29	0,35 & 1,88 $\pm$ 0,55	0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*
OL & MET	0,60 $\pm$ 0,10 & 0,77 $\pm$ 0,10	395,31 $\pm$ 33,04	4,20 $\pm$ 0,14 & 5,00 $\pm$ 0,39	164,26 $\pm$ 15,17	1,72 $\pm$ 0,35 & 1,41 $\pm$ 0,51	0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*
CONTR	0,60 $\pm$ 0,10 & 0,82 $\pm$ 0,06	506,46 $\pm$ 502,24	0,14 & 6,77 $\pm$ 2,03	168,76 $\pm$ 155,29	0,35 & 1,88 $\pm$ 0,55	0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*
OL & VILDA	0,60 $\pm$ 0,10 & 0,77 $\pm$ 0,10	395,31 $\pm$ 33,04	4,20 $\pm$ 0,14 & 5,00 $\pm$ 0,39	164,26 $\pm$ 15,17	1,72 $\pm$ 0,35 & 1,41 $\pm$ 0,51	0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*
CONTR	0,60 $\pm$ 0,10 & 0,82 $\pm$ 0,06	506,46 $\pm$ 502,24	0,14 & 6,77 $\pm$ 2,03	168,76 $\pm$ 155,29	0,35 & 1,88 $\pm$ 0,55	0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*
OL & MET/VI	0,60 $\pm$ 0,10 & 0,77 $\pm$ 0,10	395,31 $\pm$ 33,04	4,20 $\pm$ 0,14 & 5,00 $\pm$ 0,39	164,26 $\pm$ 15,17	1,72 $\pm$ 0,35 & 1,41 $\pm$ 0,51	0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*
CONTR	0,60 $\pm$ 0,10 & 0,82 $\pm$ 0,06	506,46 $\pm$ 502,24	0,14 & 6,77 $\pm$ 2,03	168,76 $\pm$ 155,29	0,35 & 1,88 $\pm$ 0,55	0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*
OL & STZ & MET	0,60 $\pm$ 0,10 & 0,82 $\pm$ 0,06	506,46 $\pm$ 502,24	0,14 & 6,77 $\pm$ 2,03	168,76 $\pm$ 155,29	0,35 & 1,88 $\pm$ 0,55	0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*

		0,72 ± 0,15	85,80 & 506,46 ± 147,49 494,54 ± 85,80	5,46 ± 1,59	57,71 & 168,76 ± 41,58 182,15 ± 57,71	2,02 ± 0,50					
STZ & VILDA		0,82 ± 0,06 & 0,65 ± 0,12	& 502,24 ± 144,29 494,54 ± 85,80	6,05 ± 0,74 & 6,77 ± 2,03	& 155,29 ± 50,12 182,15 ± 57,71	1,69 ± 0,65 & 1,88 ± 0,55	0,00 0*	0,45	0,34	0,00 0*	0,00 0*
STZ & MET/VI LDA		0,82 ± 0,06 & 0,77 ± 0,10	& 428,81 ± 28,16 506,46 ± 147,49	6,05 ± 0,74 & 5,00 ± 0,39	& 68,74 ± 25,26 168,76 ± 41,58	1,69 ± 0,65 & 1,41 ± 0,51	0,00 0,00 0*	0,00 0*	0,07 5	0,00 0*	0,00 0*
MET & VILDA		0,72 ± 0,15 & 0,65 ± 0,12	& 502,24 ± 144,29 506,46 ± 147,49	5,46 ± 1,59 & 6,77 ± 2,03	& 155,29 ± 50,12 168,76 ± 41,58	2,02 ± 0,50 & 1,88 ± 0,55	0,00 0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*
MET & MET/VI LDA		0,72 ± 0,15 & 0,77 ± 0,10	& 428,81 ± 28,16 502,24 ± 144,29	5,46 ± 1,59 & 5,00 ± 0,39	& 68,74 ± 25,26 155,29 ± 50,12	2,02 ± 0,50 & 1,41 ± 0,51	0,00 0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*
VILDA & MET/VI LDA		0,65 ± 0,12 & 0,77 ± 0,10	& 428,81 ± 28,16	6,77 ± 2,03 & 5,00 ± 0,39	& 68,74 ± 25,26	1,88 ± 0,55 & 1,41 ± 0,51	0,00 0,00 0*	0,00 0*	0,00 0*	0,00 0*	0,00 0*

STZ: Streptozotocin, MET: Metformin, VILDA: Vildagliptin, MDA: Malondialdehyde. (*p< 0.001)

According to the results, a significant difference was found between Control and STZ, Control and MET, Control and VILDA, Control and MET/VILDA for erythrocyte MDA formation (p<0.001). Also, a significant difference was found between each experimental groups except STZ and VILDA for liver MDA formation.

For liver CAT activities, a significant difference was found between control and each experimental groups also between MET and VILDA, MET and MET/VILDA, VILDA and MET/VILDA rats however; a significant difference was found between each experimental groups except between Control and MET for liver GPx activities.

Serum TE activity was found significantly different in the each experimental groups compared to the control rats. However no significant difference was found between control and STZ groups.

Histopathological differences in liver and kidney cells are presented in Table 19.

Table 19. Statistical analysis of the histopathological parameters.

Name of Experimental Group	Liver (Average Total Score $\pm$ Standart Deviation)	Kidney (Average Total Score $\pm$ Standart Deviation)	p Liver	p Kidney
Control & STZ	1,85 $\pm$ 0,340 & 9,33 $\pm$ 1,224	2,14 $\pm$ 0,670 & 13,11 $\pm$ 0,309	0,000*	0,000*
Control & STZ MET	1,85 $\pm$ 0,340 & 6,00 $\pm$ 0,408	2,14 $\pm$ 0,670 & 9,00 $\pm$ 0,408	0,000*	0,000*
Control & STZ VILDA	1,85 $\pm$ 0,340 & 6,44 $\pm$ 0,474	2,14 $\pm$ 0,670 & 11,66 $\pm$ 0,408	0,000*	0,000*
Control & STZ MET VILDA	1,85 $\pm$ 0,340 & 5,00 $\pm$ 0,333	2,14 $\pm$ 0,670 & 6,00 $\pm$ 0,288	0,000*	0,000*
STZ & STZ MET	9,33 $\pm$ 1,224 & 6,00 $\pm$ 0,408	13,11 $\pm$ 0,309 & 9,00 $\pm$ 0,408	0,000*	0,000*
STZ & STZ VILDA	9,33 $\pm$ 1,224 & 6,44 $\pm$ 0,474	13,11 $\pm$ 0,309 & 11,66 $\pm$ 0,408	0,000*	0,105
STZ & STZ MET VILDA	9,33 $\pm$ 1,224 & 5,00 $\pm$ 0,333	13,11 $\pm$ 0,309 & 6,00 $\pm$ 0,288	0,000*	0,000*
STZ VILDA & STZ MET	6,44 $\pm$ 0,474 & 6,00 $\pm$ 0,408	11,66 $\pm$ 0,408 & 9,00 $\pm$ 0,408	0,92	0,000*
STZ VILDA & STZ MET VILDA	6,44 $\pm$ 0,474 & 5,00 $\pm$ 0,333	11,66 $\pm$ 0,408 & 6,00 $\pm$ 0,288	0,073	0,000*
STZ MET & STZ MET VILDA	6,00 $\pm$ 0,408 & 5,00 $\pm$ 0,333	9,00 $\pm$ 0,408 & 6,00 $\pm$ 0,288	0,352	0,000*

STZ: Streptozotocin, MET: Metformin, VILDA: Vildagliptin. (*p< 0.001)

According to the results, a significant difference was found between Control and STZ, Control and MET, Control and VILDA, Control and MET/VILDA both in liver and kidney cells. Also; a significant difference was found between STZ and MET, STZ and VILDA, STZ and MET/VILDA in liver cells. In kidney cells; STZ and MET, STZ

and MET/VILDA showed a significant difference but no significant difference was found between STZ and VILDA.

In kidney cells, all experimental groups were showed significant differences except STZ and VILDA. In liver cells, no significant difference was found between VILDA and MET, VILDA and MET/VILDA, MET and MET/VILDA. ($p < 0.001$)



4. DISCUSSION & COCLUSION

In our study, the effects of two anti-diabetic drugs, metformin and vildagliptin, on telomere length and oxidative stress parameters were investigated in STZ-induced diabetic rat models. Vildagliptin was found the most effective in decreasing lipid peroxidation by enhancing the activities of the GPx and CAT enzymes. Moreover, it was observed that metformin is more effective in increasing CAT activity rather than GPx but less efficiently than vildagliptin. Ávila Dde L et.al. (2013) studied the antioxidant status of vildagliptin in rats, and in parallel with our study they concluded that 5 mg/ kg/day vildagliptin exerts antioxidant effects in rats after 4 weeks, potentially increasing the elements of the antioxidant defense system and, consequently, decreasing ROS formation in the pancreatic tissue [105].

The highest MDA formation due to lipid peroxidation was observed in the combination group (metformin plus vildagliptin), which might be caused by the ldeficient activity of CAT and GPx enzymes. Therefore, it can be concluded that vildagliptin induces GPx and CAT to reduce oxidative stress, but metformin is insufficient in activating GPx. Interestingly, the combination was found insufficient for both enzymes. In agreement with this finding, Tomášová et al. (2019) suggested that drug combinations might not mimic the effects of monotherapies in mice [110]. Combining vildagliptin with another anti-diabetic drug that can act on GPx more effectively than metformin might be suitable to reduce oxidative stress.

In contrast to our results, a previous study in patients with diabetes treated with the metformin/gliclazide and metformin/vildagliptin combinations showed that the addition of vildagliptin more efficiently reduced the levels of thiobarbituric acid reactive substances, including MDA [106]. This difference in the results might have arisen from the differences between the experimental subjects (rats vs. humans) used in the studies. Also, in our study, the effects of metformin and vildagliptin were examined both as monotherapy and combination therapy. However, Hissa et al. studied the differences between two different combinations (vildagliptin vs. gliclazide) of metformin.

According to a study published in 2021, metformin plays an important role in telomere stability in patients with diabetes. In the metformin monotherapy group,

patients with T2DM demonstrated significantly longer telomeres than other experimental groups. [99] Hu et al. underlined that patients with T2DM treated with metformin had a significantly longer telomere length compared with the other experimental groups [99]. Similarly, according to the results obtained from our experimental studies, metformin was more effective in inducing TE activity by reducing the oxidative stress caused by T2DM.

In this study, the effects of vildagliptin on the telomerase enzyme and telomere length were also investigated. A study published in 2021 suggested the finding of a new independent pleiotropic effect of metformin plus vildagliptin combination therapy: the modulation of telomerase activity [108]. However, our results indicated the opposite. Vildagliptin showed an increasing effect on TE activity, but it was lower than the effect of metformin, and it has been observed that the binary combination of these two oral anti-diabetics significantly reduced TE activity compared to the control and monotherapy groups. Therefore, it can be concluded that vildagliptin increased TE activity similar to metformin; however, the combination reduced TE activity. As aforementioned, while Dudinskaya studied patients with diabetes, we used rats in our study as experimental subjects.

The concept of “telomeres” was determined as protective structure which is responsible for the stability and integrity of chromosome ends. Telomeres were composed of thousands of the TTAGGG nucleotide sequence against chromosome degradation. Since the telomere is necessary for DNA replication, it undergoes shortening during each replicative action until the cell reaches apoptotic level. This is why telomeres are used as biomarkers for cellular aging. Evidence indicated that T2DM may affect telomere length. As a result of many studies, it has been reported that T2DM patients have shorter telomeres compared to the healthy individuals. However; geographic region, types of diabetes, BMI, age, sex and species may affect the association between telomeres and T2DM [111]. For that reason, to explain why the results obtained in our study did not support this outcome; differences in the results might have arisen from the lack of study period (8 days) and differences between the experimental subjects (rats vs. humans) used in the study as mentioned above. Further studies should be repeated in humans and with a longer experimental duration.

On the other hand, Sethi I. et. al. were investigated variants of telomere maintenance pathway genes that may associated with pathogenesis of T2DM. They were indicated five variants such as; rs 9419958 (located in the intronic region of the OBFC1 gene), rs4783704 (located in TERF2 gene), rs16847897 (located in Telomerase RNA component (TERC) and in Leucine-rich repeat containing protein 31 (LRRC31)), rs 10936599 (located in TERC and second exon of Myoneurin (MYNN)) and rs74019828 (located in 8th intron of the Casein Kinase 2 Alpha 2 gene (CSNK2A2)). Findings showed that pathogenesis of T2DM is related with these five variants ($p < 0.003$). For that reason, it should be considered that the genes responsible for telomere maintenance and their repair, may also predispose to T2DM [112].

Importantly, Ritskes-Hoitinga et al. had a different perspective regarding the nutritional conditions to be preferred in T2DM studies: they proposed that a restricted diet should be applied since *ad libitum* diet might have a negative impact on rodents' kidney and heart functions, alongside the added risk of cancer [109]. Based on this report, the lack of antioxidant enzyme activity and the statistically insignificant results obtained for the metformin/vildagliptin combination therapy might be caused by the *ad libitum* diet applied in our study. Since the laboratory conditions and the species used might have different effects on the experimental results, detailed research on this topic is required.

The mammalian target of rapamycin (mTOR), an evolutionarily conserved serine/threonine kinase, plays a significant role in integrating cellular and environmental cues that modulate cell metabolism, growth, proliferation, survival, and homeostasis. The distinct roles of mTOR were identified by Reis F. et. al. in tissue regeneration and repair, oxidative stress aging, and cell death that include autophagy and apoptosis [114]. According to Hanna R. K. et. al., metformin potentiates the effects of paclitaxel in endometrial cancer cells through inhibition of cell proliferation and modulation of the mTOR pathway. For that reason obtained positive effects of metformin on TE activity in our study may be associated with not only oxidative stress reducing effect but also mTOR pathway which involved in cell healthspan and longevity [115].

In conclusion, we reported that DPP-4 inhibitor Vildagliptin was found the most effective oral anti-diabetic drug to decrease lipid peroxidation by enhancing GPx and CAT activities in liver. Also, it was observed that metformin is more effective on CAT

rather than GPx, but the influence is lower compared to that of vildagliptin. The highest MDA formation due to lipid peroxidation was observed in combination therapy, likely caused by the lack of CAT and GPx activity. Therefore, it can be suggested that vildagliptin can reduce oxidative stress by inducing the GPx and CAT activity. Meanwhile, metformin was found to be insufficient in activating GPx. The combination also failed to induce enzyme activity. Combining vildagliptin with an anti-diabetic with a substantial effect on GPx instead of metformin might offer a significant efficacy in reducing oxidative stress.

On the other hand, Metformin was found to be effective in enhancing TE activity by reducing the oxidative stress caused by T2DM. Vildagliptin also increased TE activity but to a lesser extent compared to metformin. Finally, the combination of these two oral anti-diabetics significantly reduced TE activity compared to the control and monotherapy groups. Therefore, it can be concluded that the concurrent use of vildagliptin and metformin is not suitable to enhance TE activity.

In order to make a detailed comment, further studies may be repeated by some changes in experimental design such as considering TE maintenance pathway variants genes' opposite effects on T2DM, feeding experimental subjects by restricted diet instead of ad libitum, minimizing the false positive mTOR pathway effects of metformin on healthspan.

REFERENCES

1. Sapra A, Bhandari P. Diabetes Mellitus. In: StatPearls. Treasure Island (FL): StatPearls Publishing; June 26, 2022.
2. Kühl C. Etiology and pathogenesis of gestational diabetes. *Diabetes Care*. 1998;21 Suppl 2:B19-B26.
3. Unger RH, Orci L. Paracrinology of islets and the paracrinopathy of diabetes. *Proc Natl Acad Sci U S A*. 2010;107(37):16009-16012. doi:10.1073/pnas.1006639107
4. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C. Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci*. 2020 Aug 30;21(17):6275. doi: 10.3390/ijms21176275.
5. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature*. 2001;414(6865):782-787. doi:10.1038/414782a
7. McCance DR, Hanson RL, Charles MA, et al. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes [published correction appears in *BMJ* 1994 Oct;309(6958):841]. *BMJ*. 1994;308(6940):1323-1328. doi:10.1136/bmj.308.6940.1323
8. Finch CF, Zimmet PZ, Alberti KG. Determining diabetes prevalence: a rational basis for the use of fasting plasma glucose concentrations? *Diabet Med*. 1990;7(7):603-610. doi:10.1111/j.1464-5491.1990.tb01457.x
9. Schneider H, Shaw J, Zimmet P. Guidelines for the detection of diabetes mellitus--diagnostic criteria and rationale for screening. *Clin Biochem Rev*. 2003;24(3):77-80.
10. Hoffman L, Nolan C, Wilson JD, Oats JJ, Simmons D. Gestational diabetes mellitus--management guidelines. The Australasian Diabetes in Pregnancy Society. *Med J Aust*. 1998;169(2):93-97. doi:10.5694/j.1326-5377.1998.tb140192.x
11. American Diabetes Association. Gestational diabetes mellitus. *Diabetes Care*. 2000;23 Suppl 1:S77-S79.
12. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus

provisional report of a WHO consultation. *Diabet Med.* 1998;15(7):539-553. doi:10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

13. Tominaga M. Diagnostic criteria for diabetes mellitus. *Rinsho Byori.* 1999;47(10):901-908.

14. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2005;28 Suppl 1:S37-S42. doi:10.2337/diacare.28.suppl_1.s37

15. Genuth SM, Palmer JP, Nathan DM. Classification and Diagnosis of Diabetes. In: Cowie CC, Casagrande SS, Menke A, et al., eds. *Diabetes in America*. 3rd ed. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases (US); August 2018.

16. Badaru A, Pihoker C. Type 2 diabetes in childhood: clinical characteristics and role of β -cell autoimmunity. *Curr Diab Rep.* 2012;12(1):75-81. doi:10.1007/s11892-011-0247-2

17. Borg H, Gottsäter A, Fernlund P, Sundkvist G. A 12-year prospective study of the relationship between islet antibodies and beta-cell function at and after the diagnosis in patients with adult-onset diabetes. *Diabetes.* 2002;51(6):1754-1762. doi:10.2337/diabetes.51.6.1754

18. Ahmed AM. History of diabetes mellitus. *Saudi Med J.* 2002;23(4):373-378.

19. Diabetes mellitus. Science Encyclopedia. <http://science.jrank.org/pages/2044/Diabetes-Mellitus.html>. Accessed July 22, 2011.

20. Patlak M. New weapons to combat an ancient disease: treating diabetes. *FASEB J.* 2002;16(14):1853.

21. Maitra A, Abbas AK. Endocrine system. In: Robbins and Cotran Pathologic basis of disease (PA): Saunders; 2005.

22. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus--present and future perspectives. *Nat Rev Endocrinol.* 2011;8(4):228-236. Published 2011 Nov 8. doi:10.1038/nrendo.2011.183

23. Advances and emerging opportunities in diabetes research: a Strategic Planning report of the DMICC. Genetic basis of type 1 and type2 diabetes, obesity, and their complications. www2.niddk.nih.gov/NR. Accessed December 22, 2011.
24. Azevedo M, Alla S. Diabetes in sub-saharan Africa: kenya, mali, mozambique, Nigeria, South Africa and zambia. *Int J Diabetes Dev Ctries*. 2008;28(4):101-108. doi:10.4103/0973-3930.45268
25. Kahn CR. Banting Lecture. Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes*. 1994;43(8):1066-1084. doi:10.2337/diab.43.8.1066
26. Robertson RP. Antagonist: diabetes and insulin resistance--philosophy , science, and the multiplier hypothesis. *J Lab Clin Med*. 1995;125(5):560-565.
27. Fujioka K. Pathophysiology of type 2 diabetes and the role of incretin hormones and beta-cell dysfunction. *JAAPA*. 2007;Suppl:3-8. doi:10.1097/01720610-200712000-00001
28. Garcia-Roves PM. Mitochondrial pathophysiology and type 2 diabetes mellitus [retracted in: *Arch Physiol Biochem*. 2011 Dec;117(5):283]. *Arch Physiol Biochem*. 2011;117(3):177-187. doi:10.3109/13813455.2011.584538
29. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser*. 2000;894:i-253.
30. Karam JH. Pancreatic Hormones and Diabetes Mellitus. In: Basic and Clinical Endocrinology. (CT USA): Appleton & Lange; 1997.
31. Wheatcroft SB, Williams IL, Shah AM, Kearney MT. Pathophysiological implications of insulin resistance on vascular endothelial function. *Diabet Med*. 2003;20(4):255-268. doi:10.1046/j.1464-5491.2003.00869.x
32. Robertson RP, Harmon J, Tran PO, Poitout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes*. 2004;53 Suppl 1:S119-S124. doi:10.2337/diabetes.53.2007.s119
33. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell*. 2012;150(6):1223-1234. doi:10.1016/j.cell.2012.07.029

34. Stoffers DA. The development of beta-cell mass: recent progress and potential role of GLP-1. *Horm Metab Res.* 2004;36(11-12):811-821. doi:10.1055/s-2004-826168
35. Cnop M, Welsh N, Jonas JC, Jöns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. *Diabetes.* 2005;54 Suppl 2:S97-S107. doi:10.2337/diabetes.54.suppl_2.s97
36. Lin CY, Ni CC, Yin MC, Lii CK. Flavonoids protect pancreatic beta-cells from cytokines mediated apoptosis through the activation of PI3-kinase pathway. *Cytokine.* 2012;59(1):65-71. doi:10.1016/j.cyto.2012.04.011
37. Gilbert ER, Liu D. Epigenetics: the missing link to understanding β -cell dysfunction in the pathogenesis of type 2 diabetes. *Epigenetics.* 2012;7(8):841-852. doi:10.4161/epi.21238
38. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes.* 2003;52(1):1-8. doi:10.2337/diabetes.52.1.1
39. İrer SV, Alper G. Experimental Models of Diabetes Mellitus. *Türk Klinik Biyokimya Derg.* 2004;2(3):127-136.
40. Erbaş O. Experimental diabetes model. *FNG & Bilim Tıp Dergisi* 2015;1(1):40-42.
41. Kisaoglu A, Borekci B, Yapca OE, Bilen H, Suleyman H. Tissue damage and oxidant/antioxidant balance. *Eurasian J Med.* 2013;45(1):47-49. doi:10.5152/eajm.2013.08
42. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv.* 2015;5:27986-28006.
43. Mukherji SM, Singh SP. Reaction mechanism in organic chemistry. Madras: Macmillan India Press; 1986.
44. Younus H. Therapeutic potentials of superoxide dismutase. *Int J Health Sci (Qassim).* 2018;12(3):88-93.

45. Azadmanesh J, Borgstahl GEO. A Review of the Catalytic Mechanism of Human Manganese Superoxide Dismutase. *Antioxidants (Basel)*. 2018;7(2):25. Published 2018 Jan 30. doi:10.3390/antiox7020025
46. Lewandowski Ł, Kepinska M, Milnerowicz H. The copper-zinc superoxide dismutase activity in selected diseases. *Eur J Clin Invest*. 2019;49(1):e13036. doi:10.1111/eci.13036
47. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*. 2018;54(4):287-293. doi:10.1016/j.ajme.2017.09.001
48. Hadwan MH. Simple spectrophotometric assay for measuring catalase activity in biological tissues. *BMC Biochem*. 2018;19(1):7. Published 2018 Aug 3. doi:10.1186/s12858-018-0097-5
49. Nandi A, Yan LJ, Jana CK, Das N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. *Oxid Med Cell Longev*. 2019;2019:9613090. Published 2019 Nov 11. doi:10.1155/2019/9613090
50. Margis R, Dunand C, Teixeira FK, Margis-Pinheiro M. Glutathione peroxidase family - an evolutionary overview. *FEBS J*. 2008;275(15):3959-3970. doi:10.1111/j.1742-4658.2008.06542.x
51. Jablonska E, Gromadzinska J, Peplonska B, et al. Lipid peroxidation and glutathione peroxidase activity relationship in breast cancer depends on functional polymorphism of GPX1. *BMC Cancer*. 2015;15:657. Published 2015 Oct 7. doi:10.1186/s12885-015-1680-4
52. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem*. 2006;52(4):601-623. doi:10.1373/clinchem.2005.061408
53. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-A concise review. *Saudi Pharm J*. 2016;24(5):547-553. doi:10.1016/j.jsps.2015.03.013

54. Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. *Oxid Med Cell Longev*. 2020;2020:8609213. Published 2020 Mar 9. doi:10.1155/2020/8609213
55. Devasagayam TP, Boloor KK, Ramasarma T. Methods for estimating lipid peroxidation: an analysis of merits and demerits. *Indian J Biochem Biophys*. 2003;40(5):300-308.
56. Całyniuk B, Grochowska-Niedworok E, Walkiewicz KW, Kawecka S, Popiołek E, Fatyga E. Malondialdehyde (MDA) – product of lipid peroxidation as marker of homeostasis disorders and aging. *Ann Acad Med Siles*. 2016;70:224-228. doi:https://doi.org/10.18794/aams/65697
57. Eken A. Rat Kan ve Doku Örneklerinde Oksidatif Stres Parametreleri. *JCAM*. 69-73.
58. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*. 2001;50(6):537-546.
59. Kshirsagar AD, Shaikh HM. Role of C-Peptide in Development of Diabetic Neuropathy [DN] Animal Model. *American Journal of Drug Discovery and Development*. 2014;4(1):12-21. doi:10.3923/ajdd.2014.12.21
60. Bennett RA, Pegg AE. Alkylation of DNA in rat tissues following administration of streptozotocin. *Cancer Res*. 1981;41(7):2786-2790.
61. Nahdi AMTA, John A, Raza H. Elucidation of Molecular Mechanisms of Streptozotocin-Induced Oxidative Stress, Apoptosis, and Mitochondrial Dysfunction in Rin-5F Pancreatic β -Cells. *Oxid Med Cell Longev*. 2017;2017:7054272. doi:10.1155/2017/7054272
62. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014;2014:360438. doi:10.1155/2014/360438
63. Caf F, Sen Ozdemir N, Gokce Z. Biochemical Content (fatty acids, sterols, total protein, lipophilic vitamins) and Antioxidant activity (GSH, MDA) of Cladocerans (*Daphnia* sp., *Diaphasoma branchyrum*). *MedFAR*. 2020;3(1):34-46.

64. Ates M, Cakir-Sahilli Y, Korkmaz V. Determination of the Level of Malondialdehyde Forming as a Result of Oxidative Stress Function in Fish. *IJSR*. 2018;7(4). 10.21275/ART20181249.
65. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J Biomed Sci*. 2008;4(2):89-96.
66. Moussa SA. Oxidative stress in diabetes mellitus. *Romanian J Biophys*. 2008;18:225-236.
67. Erejuwa OO. Oxidative stress in diabetes mellitus: is there a role for hypoglycemic drugs and/or antioxidants. In: *Oxidative Stress and Diseases*. IntechOpen; 2012. <https://doi.org/10.5772/327412012>
68. Arman SI, Mahmud AA, Mahmud HR, Reza ASMA. Free radical, oxidative stress and diabetes mellitus: A mini review. *Discovery Phytomedicine*. 2019;6(3):99-101. doi:10.15562/phytomedicine. 2019.98
69. Pala FS, Gurkan H. The role of free radicals in ethiopathogenesis of diseases. *Adv Mol Biol*. 2008;1:1-9.
70. Sies H. Oxidative stress: from basic research to clinical application. *Am J Med*. 1991;91(3C):31S-38S. doi:10.1016/0002-9343(91)90281-2
71. Keaney JF Jr, Loscalzo J. Diabetes, oxidative stress, and platelet activation. *Circulation*. 1999;99(2):189-191. doi:10.1161/01.cir.99.2.189
72. Ceolotto G, Bevilacqua M, Papparella I, et al. Insulin generates free radicals by an NAD(P)H, phosphatidylinositol 3'-kinase-dependent mechanism in human skin fibroblasts ex vivo. *Diabetes*. 2004;53(5):1344-1351. doi:10.2337/diabetes.53.5.1344
73. Schrum JP, Zhu TF, Szostak JW. The origins of cellular life. *Cold Spring Harb Perspect Biol*. 2010;2(9):a002212. doi:10.1101/cshperspect.a002212
74. Meiosis. Britannica. <https://www.britannica.com/science/cell-biology/Meiosis#/media/1/101396/99691>. Accessed December 22, 2011.

75. Razgonova MP, Zakharenko AM, Golokhvast KS, et al. Telomerase and telomeres in aging theory and chronographic aging theory (Review). *Mol Med Rep.* 2020;22(3):1679-1694. doi:10.3892/mmr.2020.11274
76. Chatelain M, Drobnjak SM, Szulkin M. The association between stressors and telomeres in non-human vertebrates: a meta-analysis. *Ecology Letters.* 2020;23:381-398. doi: 10.1111/ele.13426
77. Güzelgöl F, Aksoy K. Telomeraz Enziminin Tanı ve Tedavide Kullanım Alanı. *AKTD.* 2011;20(2):69-88.
78. Corey DR. Telomeres and telomerase: from discovery to clinical trials. *Chem Biol.* 2009;16(12):1219-1223. doi:10.1016/j.chembiol.2009.12.001
79. Wang J, Dong X, Cao L, et al. Association between telomere length and diabetes mellitus: A meta-analysis. *J Int Med Res.* 2016;44(6):1156-1173. doi:10.1177/0300060516667132
80. Elks CE, Scott RA. The long and short of telomere length and diabetes. *Diabetes.* 2014;63(1):65-67. doi:10.2337/db13-1469
81. Tamura Y, Takubo K, Aida J, Araki A, Ito H. Telomere attrition and diabetes mellitus. *Geriatr Gerontol Int.* 2016;16 Suppl 1:66-74. doi:10.1111/ggi.12738
82. Baxter M. Treatment of type 2 diabetes: a structured management plan. *Adv Ther.* 2008;25(2):106-114. doi:10.1007/s12325-008-0017-y
83. Nathan DM, Buse JB, Davidson MB, et al. Management of hyperglycaemia in type 2 diabetes mellitus: a consensus algorithm for the initiation and adjustment of therapy. Update regarding the thiazolidinediones. *Diabetologia.* 2008;51(1):8-11. doi:10.1007/s00125-007-0873-z
84. Kurt İ. Glikozile hemoglobin (HbA1c) ölçümü ve diabetes mellitusun uzun dönem glisemik kontrolünde kullanılması. *Gülhane Tıp Dergisi.* 2003;45(4):387-395.
85. Erbey O. Yeni Nesil Anti-Diyabetik İlaçlar: Dipeptidil Peptidaz– 4 İnhibitörleri ve Glukagon Benzeri Peptit – 1 Agonistleri. <https://www.bezelyedergi.net/post/yeni-nesil-anti-diyabetik-i%CC%87la%C3%A7lar>. Accessed Feb 8, 2022.

86. Vilsbøll T, Holst JJ. Incretins, insulin secretion and Type 2 diabetes mellitus. *Diabetologia*. 2004;47(3):357-366. doi:10.1007/s00125-004-1342-6
87. Cheng AY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *CMAJ*. 2005;172(2):213-226. doi:10.1503/cmaj.1031414
88. Katritzky AR, Tala SR, Singh A. Biguanidines, guanylureas and guanylthioureas. *ARKIVOC*. 2010;(viii):76-96. doi:10.3998/ark.5550190.0011.808
89. Budny JA. Book Review: Encyclopedia of Toxicology, Third Edition. *International Journal of Toxicology*. 2015;34(4):366-367. doi:10.1177/1091581815586498
90. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care*. 2015;38(1):140-149. doi:10.2337/dc14-2441
91. Sanchez-Rangel E, Inzucchi SE. Metformin: clinical use in type 2 diabetes. *Diabetologia*. 2017;60(9):1586-1593. doi:10.1007/s00125-017-4336-x
92. Bailey CJ. Metformin: historical overview. *Diabetologia*. 2017;60(9):1566-1576. doi:10.1007/s00125-017-4318-z
93. Rosenstock J, Chuck L, González-Ortiz M, et al. Initial Combination Therapy With Canagliflozin Plus Metformin Versus Each Component as Monotherapy for Drug-Naïve Type 2 Diabetes. *Diabetes Care*. 2016;39(3):353-362. doi:10.2337/dc15-1736
94. Ahrén B. Use of DPP-4 inhibitors in type 2 diabetes: focus on sitagliptin. *Diabetes Metab Syndr Obes*. 2010;3:31-41. Published 2010 Mar 29. doi:10.2147/dmsott.s7327
95. Röhrborn D, Wronkowitz N, Eckel J. DPP4 in Diabetes. *Front Immunol*. 2015;6:386. Published 2015 Jul 27. doi:10.3389/fimmu.2015.00386
96. Keating GM. Vildagliptin: a review of its use in type 2 diabetes mellitus. *Drugs*. 2014;74(5):587-610. doi:10.1007/s40265-014-0199-3
97. Kleppinger EL, Helms K. The role of vildagliptin in the management of type 2 diabetes mellitus. *Ann Pharmacother*. 2007;41(5):824-832. doi:10.1345/aph.1H460

98. Samraj GP. Vildagliptin for the treatment of diabetes. *Therapy*. 2011;8(6), 703–719. doi:10.2217/THY.11.75
99. Hu D, Xie F, Xiao Y, et al. Metformin: A Potential Candidate for Targeting Aging Mechanisms. *Aging Dis*. 2021;12(2):480-493. Published 2021 Apr 1. doi:10.14336/AD.2020.0702
100. Robb-Mackay C, Law D, Matheson C. The effect of metformin on absolute telomere length. 2018. <https://knowledgecommons.lakeheadu.ca/handle/2453/4310?show=full>. Accessed Feb 8, 2022.
101. Anisimov VN, Piskunova TS, Popovich IG, et al. Gender differences in metformin effect on aging, life span and spontaneous tumorigenesis in 129/Sv mice. *Aging (Albany NY)*. 2010;2(12):945-958. doi:10.18632/aging.100245
102. Mohammed I, Hollenberg MD, Ding H, Triggle CR. A Critical Review of the Evidence That Metformin Is a Putative Anti-Aging Drug That Enhances Healthspan and Extends Lifespan. *Front Endocrinol (Lausanne)*. 2021;12:718942. Published 2021 Aug 5. doi:10.3389/fendo.2021.718942
103. Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delattre J. Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes Metab*. 2000;26(3):163-176.
104. Najafi A, Pourfarzam M, Zadhoush F. Oxidant/antioxidant status in Type-2 diabetes mellitus patients with metabolic syndrome. *J Res Med Sci*. 2021;26:6. Published 2021 Jan 28. doi:10.4103/jrms.JRMS_249_20
105. Ávila Dde L, Araújo GR, Silva M, et al. Vildagliptin ameliorates oxidative stress and pancreatic beta cell destruction in type 1 diabetic rats. *Arch Med Res*. 2013;44(3):194-202. doi:10.1016/j.arcmed.2013.03.004
106. Hissa MR, Cavalcante LL, Guimarães SB, Hissa MN. A 16-week study to compare the effect of vildagliptin versus gliclazide on postprandial lipoprotein concentrations and oxidative stress in patients with type 2 diabetes inadequately controlled with metformin monotherapy. *Diabetol Metab Syndr*. 2015;7:62. Published 2015 Jul 11. doi:10.1186/s13098-015-0058-8

107. Garcia-Martin I, Penketh RJA, Janssen AB, et al. Metformin and insulin treatment prevent placental telomere attrition in boys exposed to maternal diabetes. *PLoS One*. 2018;13(12):e0208533. Published 2018 Dec 11. doi:10.1371/journal.pone.0208533
108. Dudinskaya E., Matchekhina L., Strazhesko I. et al. Combined vildagliptin + metformin therapy can increase telomerase activity in patients with type 2 diabetes. Presented at Endocrine Abstracts (2021) 73 AEP196 | DOI: 10.1530/endoabs.73.AEP196
109. Ritskes-Hoitinga M, Tobin G, Jensen TL, Mikkelsen LF. Nutrition of the Laboratory Mouse. In: *The Laboratory Mouse*. 2nd ed. Academic Press; 2012.
110. Tomášová P, Bugáňová M, Pelantová H, et al. Metabolomics Based on MS in Mice with Diet-Induced Obesity and Type 2 Diabetes Mellitus: the Effect of Vildagliptin, Metformin, and Their Combination. *Appl Biochem Biotechnol*. 2019;188(1):165-184. doi:10.1007/s12010-018-2899-8
111. Wang J, Dong X, Cao L, et al. Association between telomere length and diabetes mellitus: A meta-analysis. *J Int Med Res*. 2016;44(6):1156-1173. doi:10.1177/0300060516667132
112. Sethi I, Sharma V, Sharma I, et al. Telomere Maintenance Genes are associated with Type 2 Diabetes Susceptibility in Northwest Indian Population Group. *Sci Rep*. 2020;10(1):6444. Published 2020 Apr 15. doi:10.1038/s41598-020-63510-w
113. Aydin A, Orhan H, Sayal A, Ozata M, Sahin G, İşimer A. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. *Clin Biochem*. 2001;34(1):65-70. doi:10.1016/s0009-9120(00)00199-5
114. Reis F, Maejima Y, Cai Z, Ren J, mTOR Signaling Cardiometabolic Disease, Cancer, and Aging. *Oxidative Medicine and Cellular Longevity*. vol. 2017, Article ID 6018675, 4 pages, 2017. <https://doi.org/10.1155/2017/6018675>
115. Hanna R K, Zhou C, Malloy K M, Gehrig A P, Bae-Jump V L, Metformin potentiates the effects of paclitaxel in endometrial cancer cells through inhibition of cell proliferation and modulation of the mTOR pathway. *Gynecol Oncol*. 2012 May ; 125(2): 458–469. doi:10.1016/j.ygyno.2012.01.009.

APPENDIX - 1



T.C. YEDİTEPE ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu (HADYEK)

ETİK KURUL KARARI

Protokol No	Toplantı Tarihi	Toplantı Sayısı	Karar No	Proje Yürütücüsü
2021-023	02.03.2021	2021/03	2021/03-9	Prof. Dr. Ahmet Aydın
'Metformin Ve Vildagliptin Etken Maddeli Müstahzarların Sıçan Telomeraz Enzimi Ve Oksidatif Stres Parametreleri Üzerine Olan Etkilerinin İncelenerek, Sağlıklı Hücreler İle Karşılaştırılması' isimli proje oy birliğiyle etik açıdan uygun görülmüştür.				
Hayvan Türü / Irkı		Toplam Hayvan Sayısı		Hayvanın Cinsiyeti
Sıçan / Sprague Dawley		35		Erkek

Görevi	Adı Soyadı	Katılım Durumu
Başkan	Prof. Dr. Bayram YILMAZ	
Başkan Vekili	Prof. Dr. Erdem YEŞİLADA	
Üye	Dr. Veteriner Hekim Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	
Üye	Prof. Dr. Rukset ATTAR	
Üye	Prof. Dr. Gamze TORUN KÖSE	
Üye	Doç. Dr. Ediz DENİZ	
Üye	Doç. Dr. Aylin YABA UÇAR	
Üye	Doç. Dr. Burcu GEMİCİ BAŞOL	
Üye	Hakan GÖKSEL	
Üye	Ahmet ŞENKARDEŞLER	

APPENDIX - 2

CV

Personal Informations

Name	Ezgi	Surname	Talo
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Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Doctorate	Pharmaceutical Toxicology	Yeditepe University Institute of Health Science	Feb – 2023
University	Pharmacy	Yeditepe University Faculty of Pharmacy	Jun – 2016

Languages	Grades (#)
English	YDS - B

Work Experience (Sort from present to past)

4.1.1. Position	Institute	Duration (year - year)
Pharmacist	T.C. Pendik State Hospital	2018 -
Pricing & Reimbursement Asst. Spec.	Dem Pharmaceuticals	2017 – 2018
Cosmetology R&D Asst.	Yeditepe Üniversitesi Kozmetik Ar- Ge ve Üretim Birimi	2016 – 2017
Business Development Asst. Spec.	Dem Pharmaceuticals	2016 – 2016
Laboratory Asst.	Yeditepe University Faculty of Pharmacy	2016 – 2016
R&D Formulation Asst.	Onko & Koçsel Pharmaceuticals	2015 - 2016

Computer Skills

Program	Level
Microsoft Office	Excellent
SPSS	Average
Prezi	Good

Proceedings presented in international scientific meetings and published in proceedings book.

<i>Investigation of Effects of Oral Anti-diabetics on Oxidative Stress Parameters and Comparison with Healthy Cells</i> 4th East Mediterranean Congress of Animal Science – LASA – December 2021
<i>Investigation of Effects of Oral Anti-Diabetics on Lipid Peroxidation and Enzymatic Defense System</i> 11th International Congress of the Turkish Society of Toxicology – TTD – November 2022
<i>Investigation of Effects of Oral Anti-Diabetics on Telomerase (TE) Activity in Rats</i> 11th International Congress of the Turkish Society of Toxicology – TTD – November 2022
<i>Formation of Spots and Anti-Spot Strategies</i> IVEK 3rd International Convention of Pharmaceuticals and Pharmacies – İVEK – April 2017

Others (Projects / Certificates / Rewards)

Toxicological Evaluation of Benzothiazole Derivatives Carrying Piperazine Ring, Marmara Pharmaceutical Journal 21/2: 243-250, 2017.
Lead Levels in the Food and its Biological Derivatives in Turkey A Meta- Analysis Study, 10th Congress of Toxicology in Developing Countries, (CTDC10), 12th Congress of the Serbian Society of Toxicology (12th SCT), 18-21 April, 2018.
Certificate of Animal Use in Experimental Research – Animal Experiments Local Ethics Committee – April 2021
Natural Cosmetic Production Training – TEAK – April 2021
Güvenlik Bilgi Formu Hazırlayıcısı – KİM CERT – May 2017
2nd Poster Communication Award – 4th EMCLAS – December 2021