

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**EFFECT OF POLYSACCHARIDES OBTAINED FROM LOW
QUALITY TEAS ON GLYCOSIDASE ENZYME ACTIVITY AND
TYPE II DIABETES**

KÜBRA AKALIN
A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

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GEBZE
2019

T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ

DÜŞÜK KALİTELİ ÇAYLARDAN ELDE
EDİLEN POLİSAKKARİTLERİN
GLİKOZİDAZ ENZİM AKTİVİTESİ VE TİP
II DİYABET ÜZERİNE ETKİLERİ

KÜBRA AKALIN
YÜKSEK LİSANS TEZİ
MOLEKÜLER BİYOLOJİ VE GENETİK ANABİLİM DALI

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SUMMARY

Type 2 diabetes mellitus is a global public health problem. In this disease that affects all metabolism, keeping the blood sugar level in balance is the main target of treatment. Due to the increase in costs of treatment, and the side effects of the drugs used, research on substances with natural antidiabetic effects has accelerated. Recent research has shown that conventional plants known to have blood glucose lowering effect are effective in the prevention and treatment of type 2 diabetes. In this study, the effects of tea polysaccharides obtained from low-quality teas on *in vitro* and *in vivo* methods and type 2 diabetes mellitus were investigated. α -glucosidase enzyme inhibition activities of tea polysaccharides were investigated and samples with high inhibition were determined. Tea polysaccharides are shown in cell culture assays that lead to an increase in cellular glucose uptake. Streptozotocin and high-fat diet were administered to the mouse to produce experimental type 2 diabetes mellitus. The model mouse was treated with green and black tea extracts by oral gavage for six weeks. Low-calorie nutrition regimen with green and black tea extract in the fasting blood sugar levels of mice as expected was significantly reduced. High-calorie nutrition regimen combined with black tea extract only in the fasting blood sugar levels were significantly reduced in mice.

Key Words: Type 2 Diabetes Mellitus (T2DM), Tea Polysaccharides (TPS), Streptozotocin (STZ), High-fat diet (HFD), Glucose Uptake, α -Glucosidase Enzyme Inhibition.

ÖZET

Tip 2 diabetes mellitus prevelansı hızla yükselen global halk sağlığı sorunudur. Tüm metabolizmayı etkileyen bu hastalıkta kan şekeri düzeyinin dengede tutulması tedavide başlıca hedeftir. Tedavi masraflarının artışı ve kullanılan ilaçların yan etkileri sebebiyle doğal kaynaklı antidiyabetik etkisi olan maddeler üzerinde araştırmalar hızlanmıştır. Yapılan araştırmalar kan şekeri düşürücü özelliği bilinen geleneksel bitkilerin tip 2 diyabetin önleminde ve tedavisinde etkili olduklarını göstermiştir. Bu çalışmada düşük kaliteli çaylardan elde edilen çay polisakkaritlerin *in vitro* ve *in vivo* yöntemler ile tip 2 diabetes mellitus üzerine etkileri incelenmiştir. Çay polisakkaritlerinin α -glukosidaz enzim inhibisyonu aktivitelerine bakılmıştır ve yüksek inhibisyon gösteren örnekler belirlenmiştir. Çay polisakkaritlerinin hücresel glukoz alımı üzerinde artışa yol açtığı hücre kültürü deneylerinde gösterilmiştir. Deneysel tip 2 diabetes mellitus fare modeli oluşturmak için streptozotosin ve yüksek yağlı diyet uygulanmıştır. Model fare altı hafta boyunca oral gavaj yoluyla çay ekstraktlarıyla tedavi edilmiştir. Yeşil ve siyah çay ekstraktı uygulanan farelerin düşük kalorili beslenme rejimi ile birlikte açlık kan şekeri düzeylerinde beklenildiği gibi anlamlı derecede düşüş tespit edilmiştir. Yüksek kalorili beslenme rejimi ile birlikte sadece siyah çay ekstraktı uygulanan farelerde açlık kan şekeri düzeylerinde anlamlı derecede düşüş tespit edilmiştir.

Anahtar Kelimeler: Tip 2 Diabetes Mellitus (T2DM), Çay Polisakkaritleri (TPS), Streptozotosin (STZ), Yüksek-yagli diyet (HFD), Glukoz Alımı, α -Glukosidaz Enzim İnhibisyonu.

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LIST of ABBREVIATIONS and ACRONYMS

<u>Abbreviations and Acronyms</u>	<u>Explanations</u>
α	:Alpha
β	: Beta
μ	: Micro
μL	: Microliter
μM	: Micromolar
CaCl_2	: Calcium chloride
CO_2	: Carbon dioxide
dL	: Deciliter
KCl	: Potassium chloride
kg	: Kilogram
KH_2PO_4	: Potassium dihydrogen phosphate
M	: Molar
mg	: Milligram
MgCl_2	: Magnesium chloride
MgSO_4	: Magnesium sulphate
mL	: Milliliter
mM	: Millimolar
Na_2CO_3	: Sodium carbonate
NaCl	: Sodium chloride
NaOH	: Sodium hydroxide
nm	: Nanometer
pmol	: Pico mole
U	: Unit
2-DG	: 2-deoxy-D-glucose
2-DG6P	: 2-deoxy-D-glucose-6-phosphate
A1C	: Glycated hemoglobin
ADA	: American Diabetes Association
AKT	: Protein Kinase B
ALP	: Alkaline phosphatase
ALT	: Alanine aminotransferase

AMP	: Adenosine monophosphate
AMPK	: Adenosine monophosphate-activated protein kinase
AST	: Aspartate aminotransferase
AUC	: Area under curve
BM	: Basal medium
BSA	: Bovine serum albumin
BTP	: Black tea polysaccharide
BW	: Body weight
cAMP	: Cyclic adenosine monophosphate
DEXA	: Dexamethasone
DIO	: Diet-induced obesity
DMEM	: Dulbecco's modification of Eagle medium
DMSO	: Dimethyl sulfoxide
DPP-4	: Dipeptidyl peptidase-4
DTNB	: 5,5'-Dithiobis(2-nitrobenzoic acid)
ELISA	: Enzyme-linked immunosorbent assay
ERK	: Extracellular-signal-regulated kinase
FBG	: Fasting blood glucose
FBS	: Fetal bovine serum
FPG	: Fasting plasma glucose
G6PD	: Glucose-6-phosphate dehydrogenase
GCK	: Glucokinase
GLP-1	: Glucagon-like peptide-1
GLP-1R	: Glucagon-like peptide-1 receptor
GLUT-2	: Glucose transporter type 2
GLUT-4	: Glucose transporter type 4
GTP	: Green tea polysaccharide
H&E	: Hematoxylin and Eosin
HDL-C	: High-density lipoprotein cholesterol
HFD	: High fat diet
HIT	: Hamster pancreatic beta cells
IBMX	: 3-Isobutyl-1-methylxanthine
INS-1	: Insulinoma cell line
JNK	: c-Jun N-terminal kinase

KRPH	: Krebs-Ringer-Phosphate-HEPES
L6	: Rat myoblast cell line
LDL-C	: Low-density lipoprotein cholesterol
Met	: Metformin
MIN	: Transgenic C57BL/6 mouse insulinoma cell line
ND	: Normal diet
NPG	: 4-Nitrophenyl α -D-glucopyranoside
OGTT	: Oral glucose tolerance test
PBS	: Phosphate buffered saline
PDX-1	: Pancreatic and duodenal homeobox 1
PI3K	: Phosphoinositide-3-kinase
PKA	: Protein kinase A
PKC	: Protein kinase C
PPARs	: Peroxisome proliferator-activated receptors
RIN	: Rat insulinoma cell line
SGLT-2	: Sodium-glucose co-transporter 2
STZ	: Streptozotocin
T2DM	: Type 2 diabetes mellitus
TC	: Total cholesterol
TG	: Triglycerides
TPS	: Tea polysaccharides
Tyrosin-EDTA	: Trypsinethylenediamine tetraacetic acid solution
β TC	: Beta-tumour cells

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1. INTRODUCTION

Over the last decades, irregularity in eating habits in concur with a sedentary lifestyle has inclined unbearable rise in metabolic diseases. Type 2 diabetes, which is a part of metabolic diseases, comprises approximately eighty percent of diabetes diseases. In recent studies, antihyperglycemic effects of some natural sources that have the potential to be used as antidiabetic drug have been revealed. In the light of these information, in this study, determination the antidiabetic activities of soluble tea polysaccharides obtained from low quality teas has been aimed. The results of these studies provide an important contribution for the development of new treatment approaches for type 2 diabetes. Further, the material used in the study is tea waste, and it is valuable for effective use of these wastes.

2. TYPE 2 DIABETES MELLITUS

Type 2 diabetes (T2DM) is a metabolic disorder with chronic and complex etiology. T2DM is normally characterized by conventional indications such as polydipsia, polyuria, polyphagia, weight loss, but may normally follow long asymptomatic periods. The development of T2DM can be prevented or treated by changes in lifestyle or medication. According to the latest reports, the worldwide rising tide of weight, lack of physical activity and high-energy eating regimens or irregular nutrition has brought about an uncommon increase in the number of patients with T2DM. Despite the incidence and prevalence of T2DM varies by geographic location, the general trend has been an increase in the prevalence of diabetes in every country since 1980. In 2015, 415 million people were estimated to have diabetes, of which ninety percent were T2DM. It is estimated that 642 million people will have diabetes until 2040 [1].

Pathogenesis of T2DM is involved including β cell dysfunction, insulin resistance, and expanded hepatic glucose production. The most characteristic feature of T2DM is insulin resistance in peripheral tissues due to rising blood glucose levels. T2DM is normal in the formation, release, storage of insulin at the beginning of the disease. Despite the increased blood sugar level, insufficiency of cells in using glucose increases insulin production. The increased amount of insulin causes an increase in receptor degradation, and therefore a decrease in the number of receptors. Insulin resistance develops when cells cannot use insulin. In this case, insulin activity at cellular level decreases. In addition, factors such as hypertension, obesity and physical inactivity form insulin resistance. Over time, decreasing insulin production as a result of Type 2 progresses to Type 1 diabetes. As a result, the amount of glucose in blood increases, and the body becomes unable to use the most basic fuel [2].

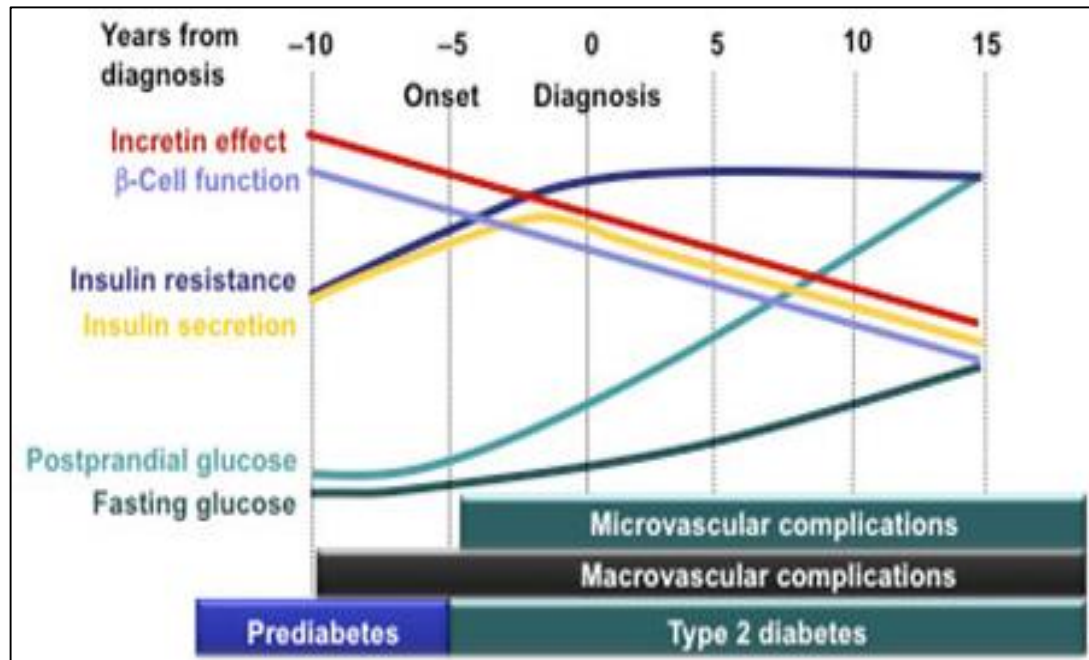


Figure 2. 1: Progression of T2DM. The figure shows the progression of diabetes over time.

While the β cells lose their function in the pre-diabetes period, insulin secretion increases in order to balance the increased blood glucose. When this process is prolonged, insulin resistance develops, and T2DM starts. In the following periods, the rise of blood glucose accelerates and decreases insulin secretion. According to the latest diagnostic criteria of the American Diabetes Association; T2DM is diagnosed at an $A1C \geq 6.5\%$, $FPG \geq 126 \text{ mg/dl}$, $OGTT \geq 200 \text{ mg/dl}$ (Figure 2.1) [3],[4].

In the treatment of T2DM, medical nutrition therapy and change in lifestyle are recommended. Unless these changes regulate blood glucose level, oral antidiabetics are added to the treatment. A wide variety of oral antidiabetic agents such as insulin secretagogue drugs (sulfonylureas, meglitinides), insulin-sensitizing drugs (biguanides, thiazolidinediones), α -glucosidase inhibitors (acarbose), incretin-mimetic drugs (DPP-4 inhibitors, GLP-1 receptor agonists) and sodium-dependent glucose co-transporter 2 (SGLT2) inhibitors to control blood glucose levels have been used in the treatment of T2DM. These drugs can be used alone or in combination, taking into account their advantages and disadvantages [5].

3. ANTIDIABETIC EFFECT OF TPS

Tea that is obtained from tea plant *Camellia sinensis* is one of the most consumed drinks after water [6]. Currently, a widely grown tea plant belongs to only one species, botanical nomenclature belongs to *Camellia sinensis* (L.) O. Kuntze, however, it is divided into many small species, subspecies or varieties by different sources. Tea consumption has a background in excess of two thousand years, and by starting in China, it spreads all over the world. Tea has been consumed by millions of people, and its effects on human being have been documented in numerous writing and records. Tea is not toxic unless its consumption reaches an excessive level, even it can be beneficial to health [7].

Tea passes through some process until it comes to our cup from the field. According to the methods of processing tea have various types; Green tea, White tea, Black tea, Oolong tea, Pu'erh tea, and Roobios, or “Red” tea [8]. To summarize three most known groups; black tea (fully fermented-fully oxidized), green tea (unfermented to non-oxidized) and oolong (semi-fermented-semi-oxidized) tea [9], [10].

The chemical components of tea incorporate polyphenols, alkaloids, volatile oils, polysaccharides, amino acids, lipids, vitamins, and inorganic elements. Also, depending on the fermentation process, the ratio of chemical contents may vary such as the fermentation time increases, the amount of polyphenols in the tea leaves decreases. Black tea is exposed to fermentation process much more than green tea, therefore polyphenol substance of green tea is 30- 40% and black tea is between 3-10% [11].

One of the active compounds contained in tea is polysaccharides. Crude tea polysaccharides are usually obtained from tea leaves, by processing, purity of polysaccharides can be increased. Tea polysaccharides (TPS) is mostly glycoconjugate, which conveys one or more carbohydrate chains covalently attached to a polypeptide backbone. TPS is also made out of heteropolysaccharides in which uronic acids are copious [12]. General characteristics of TPS can be summarized as follows; It is soluble glycoconjugate; mostly composed of xylose, mannose, rhamnose, arabinose, glucose and galactose; molecular weight of TPS in green tea is higher than that of black teas; they can combine with uronic acid, protein, and inorganic elements to form complex structures [13], [14]. The first thing that should be known is that there

are two important features that affect the chemical composition of TPS. The first is the preparation methods, and the second is the differences in the raw tea material [12].

TPS have bioactive properties such as anti-oxidant, anti-tumor, anti-hepatotoxicity, anti-infection, anti-aging, anti-hyperglycemic, anti-obesogenic, anti-inflammatory effects. In addition to all these biological properties, research and application of TPS in the functional food sector have increased due to its low toxicity and great bioactivity. However, molecular mechanisms of TPS activity are not yet understood. If molecular mechanisms are better understood, tea as a natural source will provide a new perspective in both the application of functional foods and the discovery of drugs for the disease [15], [12].

The bioactive compounds which may be an antidiabetic agent from natural sources have effects through different physiological and cellular pathways. These natural components are often characterized by their ability to inhibit enzymes in metabolic pathways including intestinal α -glucosidase and α -amylase inhibition, oxidative stress protection, aldose reductase inhibition, GLUT-4 stimulation, hexokinase activity change, insulin synthesis and release from pancreatic cells, and reduction of G6PD activity [16]. In addition, natural components may regulate the expression levels of some key proteins involved in the PPARs, AMPK, PI3K, AKT, PKC, ERK and JNK signaling pathways [17]. Most studied pathways in terms of TPS includes α -glucosidase inhibition, AMPK signal pathways, and PI3K/Akt signaling pathways.

Chen H. et al. in their study composition, yield, and molecular weight distributions were examined of green tea, oolong tea, black TPS, and significant differences were found between these tea types. Furthermore, they have differences in terms of enzyme activities. Despite all the TPS had α -glycosidase inhibitory activity, they also reported that black tea had higher activity compared to other types [18]. α -Glucosidase inhibitors block intestinal α -glucosidase which is required for enzymatic degradation of polysaccharides, thereby digestion of carbohydrates decelerates and absorption is delayed. As a result, the antihyperglycemic effect occurs [19]. In another study, Chen D. et al. reported inhibition activity Zijuan tea on α -glucosidase *in vitro*, they are *in vivo* studies showed that the tea reduced blood sugar and increased glucose tolerance of hyperglycemic mice [20]. In the study of Zhou et al. mice with alloxan diabetes was applied with brewed tea extracts, crude tea polysaccharides, and tea polysaccharide fractions. Fasting blood glucose (FBG) and glycosylated serum protein

were significantly reduced in all 3 samples [21]. Li et al. investigated hypoglycemic activities of TPS on HFD and STZ induced diabetic mice via the PI3K/Akt signal pathway. HDL-c and TG were remained at a normal level, while LDL-c and TC were decreased in the serum. TPS could upregulate the expressions of PI3K, p-Akt and GLUT-4 proteins in liver cells [22]. Wang H. et al. demonstrated the hypoglycemic mechanism of TP using RIN-5F cells. The results showed that GLP-1R, PKA, PDX-1, INS-1, INS-2, GLUT2 and GCK genes were upregulated at the level of mRNA, and expressed at PDX-1 protein level. This results suggested that TPS has increased glucose-stimulate-insulin-segregation via cAMP-PKA pathway [23].

4. EXPERIMENTAL MODELS OF DIABETES

Experimental models are developed and used to clarify the pathogenesis of diabetes and to develop new treatment strategies. Although there are many diabetes models used in diabetes research, T2DM is summarized under two headings in terms of selective *in vivo* models and *in vitro* techniques.

4.1. *In vivo* Models

A model animal is biological phenomena that is used to understand the effects of an experiment on the whole organism. Some scientific and ethical rules which are established by authorized committees are followed while working with animals. Disease and used model animal are developed in the same direction. Experimental animals such as mice, rats, rabbits, guinea pigs, hamsters, monkeys, pigs, dogs, and cats can be used to develop diabetes model experimentally. Different animal models of diabetes are created by spontaneously, administration of chemical substances, application of proper diet regimen, surgical/genetic manipulations, or their combinations [24].

In the literature, T2DM models are mostly developed using C57BL/6 mice due to being susceptible to feeding on a high-fat diet [25]. The most ideal T2DM animal model is defined by the administration of low-dose STZ and feeding with an intense fat diet [26]. STZ is directly toxic to pancreas beta cells and is commonly used as a chemical inducer in formation of diabetes model. The C57BL/6 mouse is also very sensitive to STZ. High dose of STZ administered mice show the development of type 1 diabetes mellitus, and sudden weight reduction is observed. At low doses, weight loss is not observed in addition to T2DM development. Metabolic disorders are detected in mice which are fed with intense fat diet during the modeling process. Weight gain occurs generally around the abdominal region, and that is characterized by hypertrophy and hyperplasia of adipocytes. During modeling, hyperinsulinemia, hyperglycemia and fatty liver tissue are some markers that have been seen at the onset of metabolic disorders in mice [27]-[30].

4.2. *In vitro* Techniques

The basis of diabetes studies includes inhibition of α -glucosidase and amylase activities *in vitro*. T2DM studies are generally carried out on insulin secretion and glucose uptake to understand effects of antidiabetic agents on cellular metabolism. Studies on the cellular level are carried out by using both primary and continuous cell lines. β -TC, RIN, HIT, MIN6, and INS-1 can be accepted as commercial continuous cell lines which secrete insulin as continuous lines, also 3T3-L1 and L6 cell lines are used in cell culture since insulin resistance affects both adipocytes and skeletal muscles. In order to elucidate the hypoglycemia resulting from insulin resistance, glucose uptake studies are mainly examined in these cell lines. Moreover, to conduct experiments using primary cell lines, pancreatic β cells are isolated from the pancreas of model animals [31].

5. MATERIALS AND METHODS

5.1. Materials

5.1.1. Main Materials

All tea polysaccharides used as test samples were provided by TUBITAK Marmara Research Center (MRC) Food Institute. Cell culture studies were performed by using 3T3-L1 MBX (CRL-3242™) cell line which is purchased from ATCC®. C57BL/6 mice provided by the Experimental Animal Unit of the TUBITAK MRC Institute of Genetic Engineering and Biotechnology (GEBI). All animal protocols were approved by the TUBITAK MRC GEBI Ethic Committee.

5.1.2. Chemicals, Enzymes, Reagents and Kits

4-Nitrophenyl α -D-glucopyranoside (Sigma N1377), α -Glucosidase from *Saccharomyces cerevisiae* (Sigma G5003), Acarbose (Sigma A8980), Dulbecco's Modified Eagle's Medium - high glucose (Sigma D5671), Fetal Bovine Serum (Gibco 16140-071), Trypsin-EDTA (Gibco 25200-072), Antibiotic-Antimycotic Solution (Sigma A5955), Dulbecco's Phosphate-Buffered Saline (Gibco 14190-094), DMSO (Sigma D2650), Cell Proliferation Reagent WST-1 (Roche 11644807001), 3-Isobutyl-1-methylxanthine (Sigma I5879), Dexamethasone (Sigma D4902), Insulin from bovine pancreas (Sigma I6634), Rosiglitazone (Cayman 71740), Metformin hydrochloride (Sigma PHR1084), Glucose Uptake Colorimetric Assay Kit (Sigma MAK083), Lipid (Oil Red O) Staining Kit (Sigma MAK194), Mouse Insulin ELISA kit (ALPCO 80-INSMS-E01), STZ (Sigma S0130), DIO Rodent Purified Diet (TestDiet 58Y1), Mayer's hematoxylin (BIO-OPTICA 05-06002), Eosin Y alcoholic solution (BIO-OPTICA 05-10003), Sub-X Mounting Medium (Leica 3801741), Adhesion microscope slides (Superfrost Plus™ Thermo Scientific™), Paraffin Wax (VWR Chemicals), HEPES (Sigma PHG0001), KH₂PO₄ (EMD Millipore 529568),

MgSO₄ (Sigma M2643), CaCl₂ (Sigma C5670), NaCl (Sigma S5886), KCl (Sigma P5405), Bovine Serum Albumin (Sigma A9418), D-Glucose (Sigma NIST917C).

5.1.3. Instruments & Other Materials

Heater-Magnetic stirrer (FALC), Water bath (Nuve BM400), Precision scales (Sartorius BP221-S), Glass materials (Isolab), Spectrophotometer cuvettes (Isolab I.098.02.001), pH Meter (Hanna), Vortex (FINEVORTEX), Autoclave (Hirayama HVE-50), +4°C and -20°C fridge (Beko, Vestel), -80°C deep freezer (NewsBrunswick), xCELLigence® (ACEA Biosciences RTCA), Table top centrifuge (Eppendorf 5417-R), Animal weighing instrument (Dikomsan ACS-Z), Glucometer and strips (Bayer Contour TS 84239623), Clinical chemistry analyzer and reagents (Mindray™ BS-200), Microplate reader (BioTek Synergy HT), Freezing Container (Thermo Scientific™ Mr. Frosty™), 15 ml and 50 ml Centrifuge Tubes (Eppendorf 5810-R), 1,5 ml and 2 ml Centrifuge Tubes (Axygen), Cell culture flasks (Corning, Nest), dishes and plates (Corning, SantaCruz, Nest), Laminar Flow Cabinet (Telstar Bio II A EN-12469-2000), Light Microscope (Leica), Inverted Microscope (Nikon Eclipse Ti), Serological Pipettes (Corning Costar), Pipette Tips (Eppendorf epT.I.P.S, Isolab), Micropipettor (Thermo ve Gilson), CO₂ Incubators (Sanyo MCO-15AC), Microtome (Leica RM2125 RTS), 1 ml and 5 ml injector (Ayset).

5.2. Methods

5.2.1. Inhibition Assay for α -Glucosidase Activity

The effects of extracts on α -glucosidase activity were determined by using Wei et al. protocol with some modifications [32]. The substrate solution of 4-nitrophenyl α -D-glucopyranoside (NPG) was prepared in 20 mM phosphate buffer with 6.7 mM sodium chloride at pH 6.9. A 75 μ L of α -glucosidase (1.0 U/mL) was pre-incubated with 50 μ L of the different concentrations of the extracts for 5 minutes. Then, a 75 μ L of 3.0 mM of NPG as a substrate was added to start the reaction. The reaction mixture was incubated at 37°C for 20 minutes and terminated by adding 2 mL of 0.1 M

Na₂CO₃. The α -glucosidase activity was determined by measuring the yellow-hued *p*-nitrophenol released from NPG. Absorbance was measured with a spectrophotometer at 405 nm, while the reaction solution without tea extracts was used as a control. The solution without glucosidase was used as a blank, and acarbose was used as positive control. Each experiment was conducted in triplicate. One unit of α -glucosidase activity was characterized as the amount of enzyme required to produce one μ mole of *p*-nitrophenol from NPG (3 mM) in sodium phosphate buffer (20 mM) with 6.7 mM NaCl, pH 6.9 at 37°C.

5.2.2. Cell Culture

The 3T3 L1-MBX cell line with a high differentiation capacity for adipocyte cells and a good response to insulin was used for glucose uptake studies. This cell line is derived from the 3T3-L1 preadipocyte cell line.

The 3T3 L1-MBX cell line was cultured using Dulbecco's Modified Eagle Medium (DMEM) with high glucose supplemented with 10% fetal bovine serum (FBS) and 1% (v/v) penicillin and streptomycin (10000U/mL⁻¹ penicillin G, 10000 mg/mL⁻¹ streptomycin) (basal media) at 37°C humidified atmosphere of 5% CO₂ in air. When the cells reached confluency, they were dissociated by trypsinization with 0.25% trypsin-ethylenediamine tetraacetic acid solution (Trypsin-EDTA). Cells were stained with trypan blue and counted by using hemacytometer for determined the living cell number. After counting the cell number, cells were frozen in DMEM with high glucose supplemented with 45% FBS and 5% dimethyl sulfoxide (DMSO). The cells were stocked early passages up to passage 5 and used for analyses.

5.2.2.1. Determination of Population Doubling Time

The population doubling time of the 3T3 L1-MBX cell line was determined by using the xCELLigence device. Cells were cultured in basal media and harvested when they reached 80-90% confluence. Cells were seeded in 16X E-plates which are specific for the xCELLigence device at a density of 5x10³ and 1x10⁴ cells per well and cultured in 37°C humidified atmosphere of 5% CO₂ in air for 125 hours. Data were collected at intervals of 15 minutes.

5.2.2.2. WST-1 Cell Proliferation Assays

Cell proliferation/viability was quantified using WST-1 reagent. 3T3-L1 cells were seeded at a density of 7500 cells per well in a 96-well plate and grown in basal media. Green tea (crude) extract, black tea (crude) extract and black tea (pure) extract were treated at various doses (0.001 mg/ml up to 5 mg/ml) for 24 hours. WST-1 reagent was added to each well and incubation was carried at 37°C for 4 hours. The absorbance at 450 nm was measured with a microplate reader. Eight replicates were performed for each experimental condition.

5.2.2.3. Adipogenic Differentiation

Adipogenic differentiation optimization studies were done to determine the best adipogenic differentiation. For this purpose, six different adipogenic cocktails were used for differentiation of 3T3 L1-MBX cells into adipocytes according to Zebisch K. et al.'s study [33]. Cells were seeded in 96 well plates at a density of 1500 cells per well and cultured in basal medium (BM) for 48 hours. Two days after confluence, cells were induced to differentiate in six different media for 48 hours. Details of the media used for adipocyte differentiation were given in Table 5.1. After that, the cells were maintained in basal media supplemented with 1 µg/ml insulin for another 48 hours. The culture medium was replaced again with basal media on the 7th day. Differentiation protocols were continued for 14 days by changing the medium every two days.

5.2.2.4. Oil Red O Staining

On the 7th day of the differentiation, each well of each group was stained with Oil Red O Staining Kit and adipocyte differentiation was determined. The cells were washed with PBS and the cells were fixed with 10% formalin solution for 30 minutes. Then, the cells were washed and placed on a 60% isopropanol and incubated for 5 minutes, and Oil Red O dye was added without washing for 15 minutes. Hematoxylin dye was added and kept for one minute to visualize the nucleus. At the end of the staining, stained cells were rinsed with distilled water and examined under an inverted

microscope. As a result of staining, the nucleus of the cells was stained blue with hematoxylin and oil droplets in cytoplasm became visible in red.

Table 5. 1: List of adipocyte differentiation and maintenance medium applied to 6 different experimental groups.

Groups	Adipocyte Differentiation Medium	Maintenance Medium
1 BM		BM
2	BM+1 μ M DEXA+0,5 mM IBMX+ 1 μ g/ml Insulin	BM+1 μ g/ml Insulin
3	BM+1 μ M DEXA+0,5 mM IBMX+ 3 μ g/ml Insulin	BM+1 μ g/ml Insulin
4	BM+1 μ M DEXA+0,5 mM IBMX+ 5 μ g/ml Insulin	BM+1 μ g/ml Insulin
5	BM+1 μ M DEXA+0,5 mM IBMX+ 1 μ g/ml Insulin+2 μ M Rosiglitazone	BM+1 μ g/ml Insulin
6	BM+0,25 μ M DEXA+0,5mM IBMX+1 μ g/ml Insulin+2 μ M Rosiglitazone	BM+1 μ g/ml Insulin

5.2.2.5. Glucose Uptake Assay

3T3 L1-MBX cells were seeded in 96 well plates at a density of 1500 cells per well. The standardized adipogenic differentiation protocol was applied and mature adipocyte cells were obtained on the 9-10th of the differentiation for glucose uptake test. At the start of the test, the cells were initially incubated overnight in serum-free medium with or without tea extracts. After incubating, cells were washed with PBS buffer and 2% BSA containing KRPH buffer was added. At the end of a 40 minutes incubation, cells with activated glucose transporters were incubated for a further 20 minutes by adding insulin. Then cells were incubated for 20 minutes with 2-deoxyglucose which is glucose analog and can be taken into the cell. At the end of incubations, cells were washed 3 times with PBS buffer and extraction buffer was added into wells for 15 minutes at -80°C. Following this step, the plate held for 40 minutes at 85°C incubator. The plate was then allowed to stand on ice for 10 minutes

and the neutralized buffer was added to neutralize the reactions and the cells were collected from the plates and transferred to the microfuge tubes. Each sample were rapidly centrifuged at 13000 g to prepare the cell lysates for a test. A number of redox reactions were carried out in accordance with the protocol given in the kit for quantification of 2-DG in the cell lysates we obtained after this step. A new 96-well plate is added to each well by diluting each lysate (5 μ l) with assay buffer (45 μ l). The assay buffer and enzyme mixture obtained from the kit were prepared in appropriate proportions and added to each well and the plate is incubated for 1 hour at 37°C protected from light. Then, by applying extraction buffer to each well, the plate was wrapped tightly and incubated at 85°C for 40 minutes. At the end of the incubation, the plate was cooled about 5 minutes and then apply neutralization buffer to each well. Lastly, glutathione reductase, substrate-DTNB and recycling mixture from the kit are prepared in appropriate proportions and added to each well and allowed to incubate at 37°C for 40 minutes. Absorbance at 412 nm was measured using a microplate reader.

5.2.3. *In vivo* Experiments

5.2.3.1. Animals

All the procedures were performed on animals in accordance with the protocols and guidelines of the TUBITAK MAM GEBİ Animal Care and Use Ethical Committee of Turkey (project no: 115O902). The animals were housed in a 12/12 hour light/dark cycle at 21 $\pm$ 0.5°C and 50-60% humidity. The mice were fed *ad libitum* with foodstuff and water. C57BL/6J male mice were used as an experimental animal [26]. Mice were fed on chow or high-fat diet (DIO Rodent Purified Diet with 60% Energy From Fat-Dyed Blue).

5.2.3.2. Creating animal model

In the development of *in vivo* diabetes model mice, we have tried to determine the normal state of mice to be used to understand whether they have a healthy glucose metabolism for two weeks. For this reason, blood glucose levels of mice fed normal

diet were measured. Each week, mice were fasted for 6 hours and fasting blood glucose levels were measured (Between W-2 and W0).

A total number of forty-eight (48) mice used in the experiment were selected at random but the difference between body weights were kept less than $\pm 20\%$. The control group mice were fed with a normal diet and test group mice fed with high fat diet. In this way, control and test group mice were fed with different diets. Test group mice were injected 30 mg/kg STZ (twice one month apart) intraperitoneally and maintained on the same diet. Fasting blood glucose levels and body weights of mice were monitored by weekly measurements (Figure 5.1).

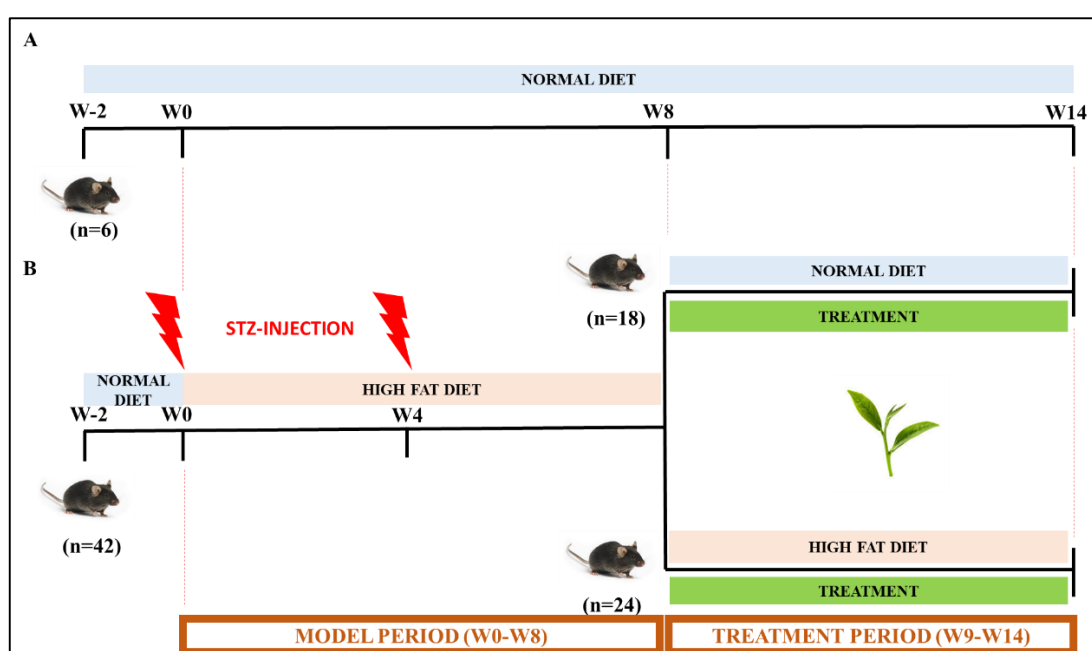


Figure 5.1: Outline of the animal experiments. A) Control group C57BL/6 mice were fed with normal diet throughout experiment without any application. B) Test group C57BL/6 mice were fed with high-fat-diet during the model period and STZ was applied two times.

5.2.3.3. *In vivo* Activity Assays

Experimental groups: At 8th week, test mice were randomly divided into seven groups depend on nutrition and treatment conditions. Treatments for all groups of mice in this study are shown in Table 5. 2.

Treatment procedures: Tea extracts was prepared with pure water and daily administered by gavage (400mg/kg BW). Also, use as positive control (Met-HFD) mice treated with metformin (250 mg/kg BW) daily.

Animal monitoring: Body weight and fasting glucose levels were recorded weekly. Blood glucose was measured with a glucometer using whole blood from the tail vein.

Clinical findings: Animals were observed in terms of common clinical signs during the whole experiment and observed abnormalities were recorded.

Termination of the experiment: All mice were sacrificed by cervical dislocation after blood collection from the ophthalmic vein.

Collection of tissue and blood: At the end of the experiment animals were sacrificed, and tissue samples were immediately collected. For histopathological and further examinations, blood samples, liver, spleen, lung, kidney, pancreas and brown fat tissue were collected and properly stored.

Biochemical analysis of serum: Serum was obtained from blood samples centrifugation at 10000 rpm for 15 minutes, some parts were separated for biochemical analysis with Mindray™ (ALP, ALT, AST, HDL-C, LDL-C, TC) and other parts were stored at -80°C until analysis. Serum insulin levels were measured with the Mouse Insulin Elisa kit (ALPCO).

Histopathological examination: Animal tissues were excised and then were fixed in 10% formalin overnight. The samples were then dehydrated in ascending concentrations (70%, 80%, and 100%) and embedded in paraffin. Sections (5-6 µm thick) were cut, deparaffinized, rehydrated with water, and stained with hematoxylin and eosin (H&E). The H&E sections were examined under a light microscope.

5.2.3.4. OGTT

At the end of the 14th week control, HFD, GTP-HFD, and BTP-HFD groups were exposed to OGTT. These mice have fasted overnight before the test. D-Glucose (Dextrose) was dissolved in pure water and orally administered to the fasted mice (2 g/kg BW) using via gavage. After glucose administration, glucose levels were measured at 0, 30, 60, and 120 minutes with a glucometer.

Table 5. 2: Description of abbreviated names of each experimental groups.

Control	Untreated nondiabetic mice fed with normal diet
ND	Untreated diabetic mice fed with normal diet
HFD	Untreated diabetic mice fed with high fat diet
Met-HFD	Untreated diabetic mice fed with high fat diet and treated with metformin
GTP-HFD	Green tea extract-treated diabetic mice fed with high fat diet
GTP-ND	Green tea extract-treated diabetic mice fed with normal diet
BTP-ND	Black tea extract-treated diabetic mice fed with normal diet
BTP-HFD	Black tea extract-treated diabetic mice fed with high fat diet

5.2.4. Statistical Analysis

All values are presented as the mean $\pm$ standard deviation (means $\pm$ SD). Statistical analysis of the data was analyzed using The Statistical Package for the Social Sciences software 17.0 (SPSS Incorporation, Chicago, Illinois, USA) and Microsoft Excel. Significance was accepted as p-value < 0.05 . The one-way ANOVA test was used to determine multiple comparisons between groups for parametric hypotheses. Wilcoxon test was used to pairwise comparisons and the Kruskal-Wallis test was used to multiple comparisons for nonparametric hypotheses. Pearson and Spearman correlation methods were used for correlation analysis.

6. RESULTS

6.1. Inhibitory Effects of TPS on α -Glucosidase Activity

Tea extracts obtained by the TUBITAK MRC Food Institute were selected in terms of polysaccharide quality and percentage. α -glucosidase enzyme inhibition studies were performed 21 different samples (Table 6.1).

Table 6. 1: Inhibitory effects of TPS on α -glycosidase activity. Data are expressed as the mean $\pm$ SD (n = 3).

Samples/%Inhibition	2,5 mg/ml	1,25 mg/ml	0,125 mg/ml
syh 1 haziran	98,53 $\pm$ 0,34	98,12 $\pm$ 0,60	99,06 $\pm$ 0,72
syh 3 haziran	98,56 $\pm$ 0,02	98,49 $\pm$ 0,79	99,92 $\pm$ 0,14
syh 4 haziran	98,92 $\pm$ 0,17	99,04 $\pm$ 0,35	99,20 $\pm$ 0,14
syh 5 haziran	98,73 $\pm$ 0,59	99,12 $\pm$ 0,75	99,56 $\pm$ 0,33
syh 6 haziran	99,72 $\pm$ 0,23	99,50 $\pm$ 0,24	99,07 $\pm$ 0,16
syh 7 haziran	98,22 $\pm$ 0,42	99,96 $\pm$ 0,73	99,42 $\pm$ 0,03
YSL mayıs sevag	88,10 $\pm$ 7,49	74,70 $\pm$ 1,76	72,63 $\pm$ 9,58
YSL mayıs eski	81,95 $\pm$ 1,51	69,06 $\pm$ 0,86	61,55 $\pm$ 30,06
YSL mayıs santrifüj	87,62 $\pm$ 0,26	85,53 $\pm$ 0,50	76,91 $\pm$ 12,21
Tohum suda	11,65 $\pm$ 0,26	20,15 $\pm$ 2,16	-1,13 $\pm$ 0,80
Tohum enzim	-15,82 $\pm$ 18,09	-3,36 $\pm$ 3,75	44,20 $\pm$ 3,15
Tohum U.S.	4,43 $\pm$ 0,12	4,11 $\pm$ 1,90	-0,80 $\pm$ 1,19
0W	96,86 $\pm$ 0,77	84,52 $\pm$ 1,14	52,05 $\pm$ 4,15
100W	91,64 $\pm$ 1,33	82,53 $\pm$ 0,90	33,37 $\pm$ 0,17
200W	86,71 $\pm$ 0,70	70,55 $\pm$ 2,17	34,06 $\pm$ 9,97
300W	83,68 $\pm$ 1,76	80,24 $\pm$ 1,69	93,79 $\pm$ 5,83
400W	36,51 $\pm$ 2,89	65,11 $\pm$ 2,54	66,13 $\pm$ 20,33
syh mayıs sevag	98,36 $\pm$ 0,34	99,82 $\pm$ 0,60	99,17 $\pm$ 0,55
syh mayıs G	99,27 $\pm$ 0,05	99,76 $\pm$ 0,22	99,28 $\pm$ 0,22
US 0 YSL	98,73 $\pm$ 0,39	46,94 $\pm$ 7,98	95,41 $\pm$ 0,77
US opt YSL	97,07 $\pm$ 0,34	59,68 $\pm$ 22,51	67,07 $\pm$ 5,03

These are different from each other in terms of the extraction method and the collection period of tea. Black tea (coded as a syh) extracts of 3 different concentrations of inhibition percentages are over ~97%. Extracts from seed (coded as a Tohum) do not have a consistent inhibition result. The result obtained from green tea (YSL) is over ~50% but there is less inhibition than black teas. In the 0-400W coded samples obtained by different methods, this method has no significant contribution to enzyme inhibition.

6.2. Preparation of 3T3 L1-MBX and Effect of TPS on Glucose Uptake

6.2.1. Population Folding Time of 3T3 L1-MBX Cell Line

3T3 L1-MBX cells were seeded in 16X E-plates produced specifically for xCelligence, with 5×10^3 and 1×10^4 per well. The population folding times of 3T3 L1-MBX cells in 5×10^3 per well plantings were determined to be 30.27 ± 2.72 hours and 34.45 ± 2.99 hours in 1×10^4 per well (Table 6.2 and Figure 6.1).

Table 6. 2: Population doubling time (PDT) of 3T3 L1-MBX cell line.

Curve1 Doubling Time	
Wells	Doubling Time (h)
5000 cells/well	30,2776 $\pm$ 2,728
10000 cells/well	34,4507 $\pm$ 2,9965

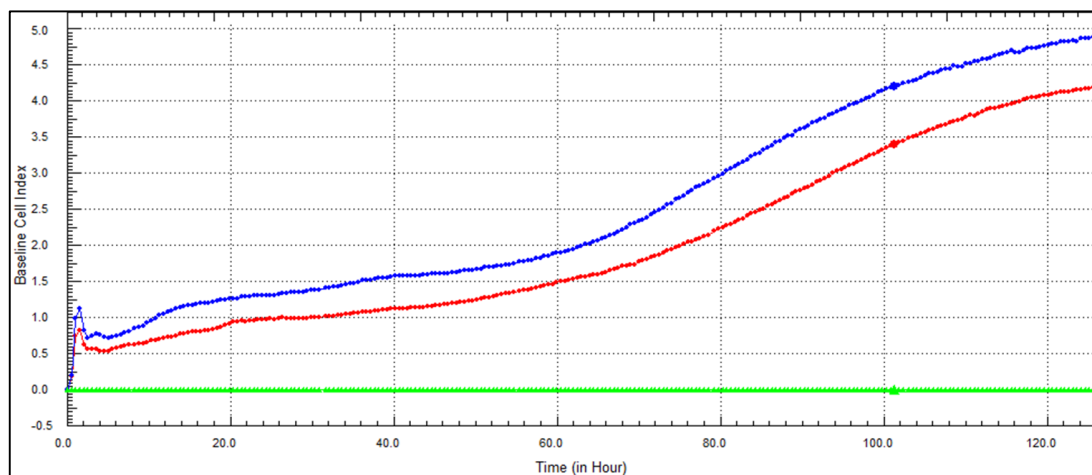


Figure 6. 1: PDT graph and growth curve of 3T3 L1-MBX cells depending on time. Red line in the figure is 5000 cells/well. Blue line in the figure is 10000 cells/well.

6.2.2. Cytotoxic Effects of TPS on 3T3 L1-MBX Cell Line

After 24 hours of cultivation of 3T3 L1-MBX cells, different tea extracts were applied in various doses and the effect of the extracts for 24 hours was determined by WST-1 technique. The cell viability in control group was accepted as 100%. The relative cell viability of tea extracts administrated cell cultures was calculated by comparing the cell viability in control group. When the black tea (crude) dose is above 1.25 mg, it is observed that relative cell viability falls below 50%. Relative cell viability did not fall below 50% at doses applied to green tea (crude) and black tea (pure). No cytotoxic effect was observed for all three types of tea extract below 1.25 mg/ml (Figure 6.2).

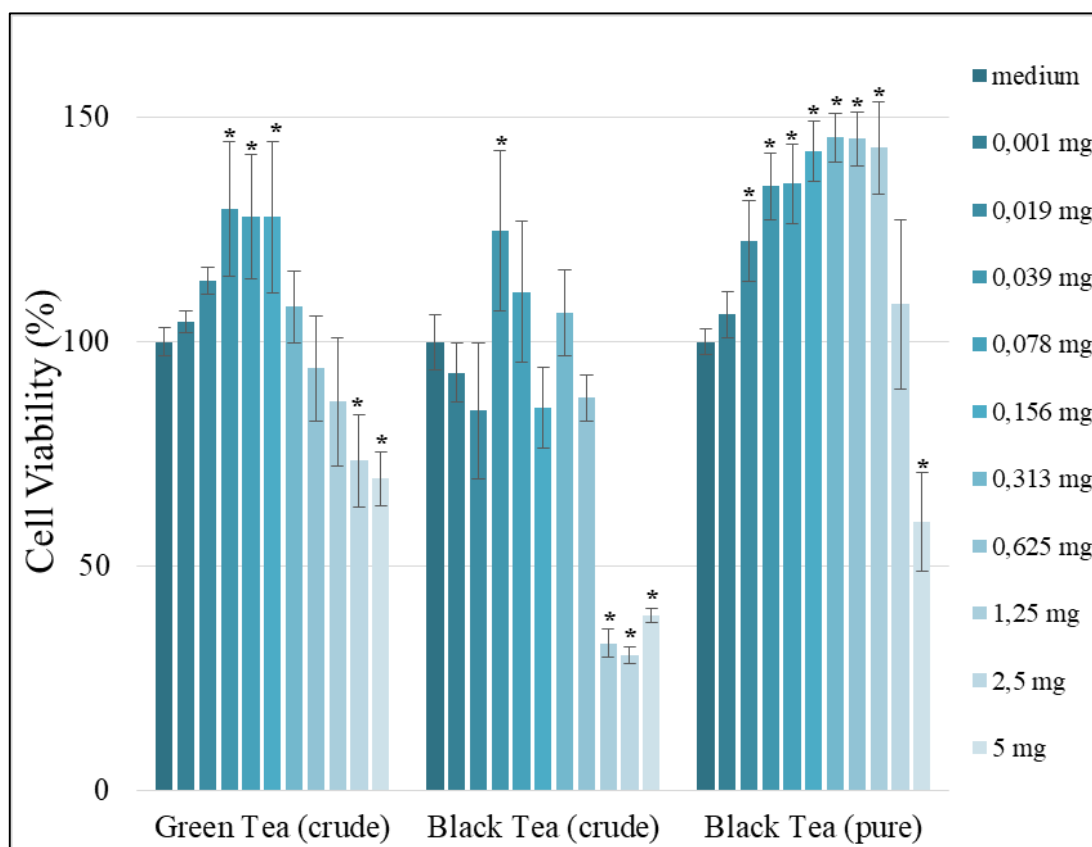


Figure 6. 2: Effects of increased doses TPS on 3T3 L1-MBX relative cell viability.* indicates a significant difference from medium (control) groups. Statistical analysis was performed using one-way ANOVA (* p<0.05).

6.2.3. Differentiation of 3T3 L1-MBX Cell Line

On the 7th day of the experiment, it was observed that adipocyte cells were formed in media groups that applied differentiation media. In control groups, it was observed that cells retain their morphology and no oil droplets were formed (Figure 6. 3a). There was a more effective adipocyte formation in the groups treated with rosiglitazone in differentiation groups (Figure 6. 3e, Figure 6. 3f). Optimized cells using BM+0,25μM DEXA+0,5mM IBMX+1μg/ml Insulin+2μM Rosiglitazone are shown in Figure 6.4.

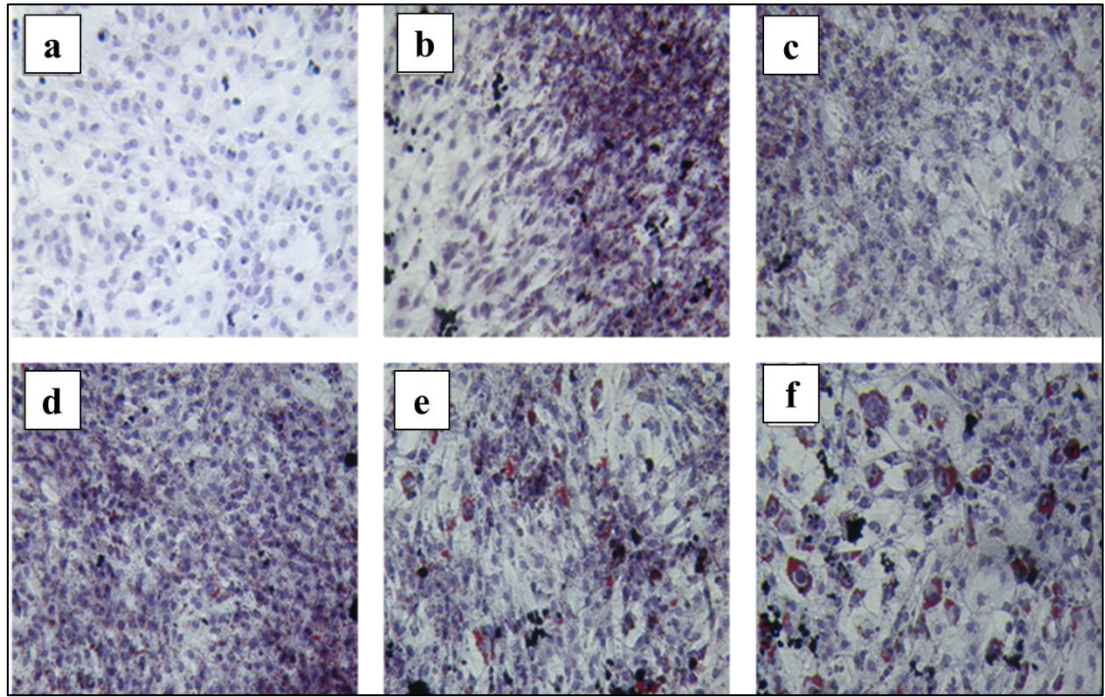


Figure 6. 3: Oil Red O staining was performed on 3T3 L1-MBX cells. a) BM; b) BM+1 μ M DEXA+0,5 mM IBMX+ 1 μ g/ml Insulin; c) BM+1 μ M DEXA+0,5 mM IBMX+ 3 μ g/ml Insulin; d) BM+1 μ M DEXA+0,5 mM IBMX+ 5 μ g/ml Insulin; e) BM+1 μ M DEXA+0,5 mM IBMX+ 1 μ g/ml Insulin+2 μ M Rosiglitazone; f) BM+0,25 μ M DEXA+0,5mMIBMX+1 μ g/ml Insulin+2 μ M Rosiglitazone.

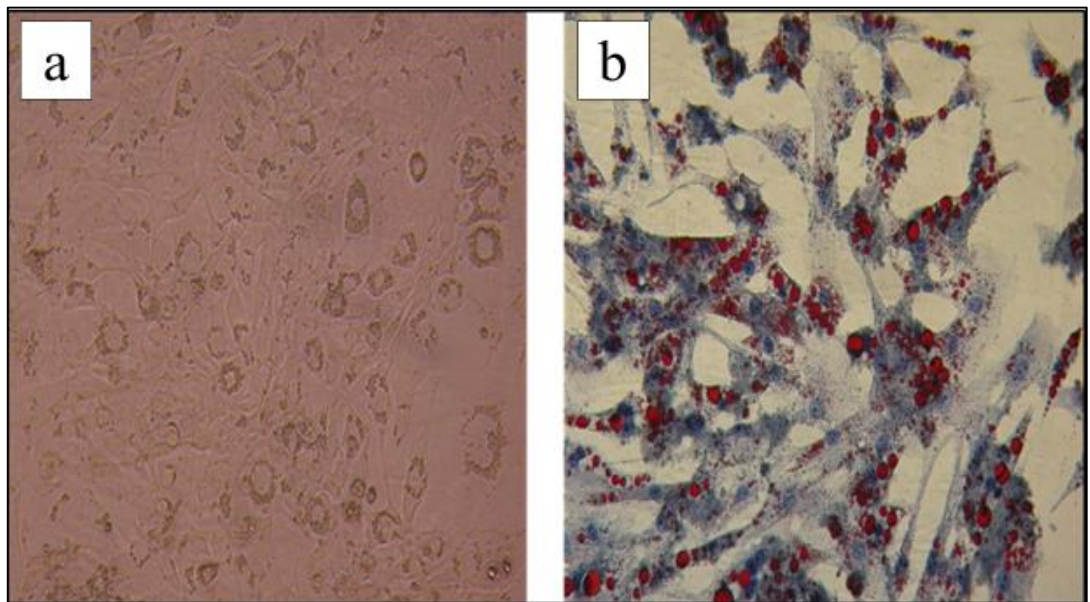


Figure 6. 4: Light microscopy of triglyceride accumulations in 3T3 L1-MBX cells. Results of optimization protocols for differentiation were showed without and with Oil Red O Staining on the right (a) and left (b) pictures respectively.

6.2.4 Effect of TPS Glucose Uptake on 3T3 L1-MBX Cell Line

Glucose uptake test was performed to see if tea extracts had an effect on glucose uptake of cells. Glucose uptake is estimated utilizing the glucose analog, 2-deoxyglucose (2-DG), which is taken up by cells and phosphorylated by hexokinase to 2-DG6P. 2-DG6P can't be further utilized and collects in cells, straightforwardly relative to the glucose uptake by cells. When mature adipocyte cells were ready for glucose uptake test, each tea extract sample was added to the cells in 1mg/ml at different time intervals. The ‘‘ON’’ groups were applied serum-free medium with extract during the overnight and ‘‘20 minutes’’ groups were applied insulin with extract 20 minutes. According to the test results, each application was effective in the intake of glucose at certain levels. The greatest glucose intake was observed in overnight administration of 1 mg/kg black tea extract (pure). For this test group, 5 times elevated glucose intake was recorded according to the control group (Figure 6.5).

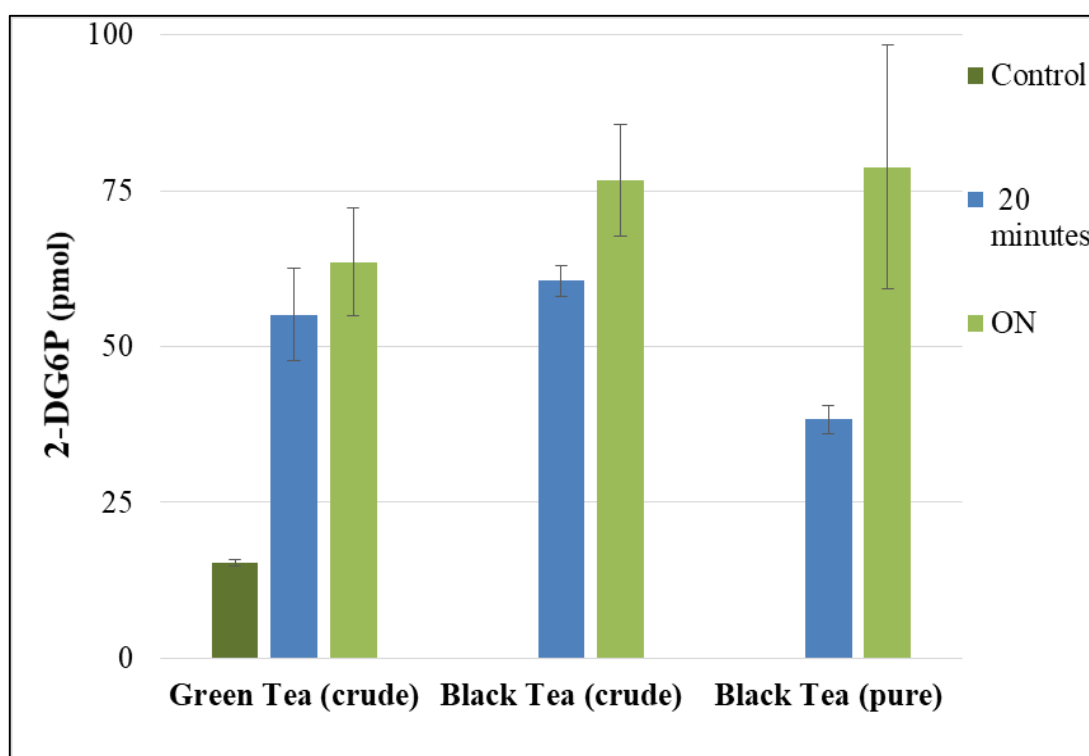


Figure 6. 5: 2-DG uptake in 3T3 L1-MBX cell treated with tea extracts.

6.3. Effect of TPS on C57BL/6 Mice

6.3.1. Body Weight

In vivo studies can be divided into two main phases. One of them is the modelling period and it covers the first eight weeks of the study. Diabetic model mice were created in this period. The second phase of the study can be called as a treatment period which takes 6 weeks following the modeling period. During the second phase, mice were exposed to TPS to identify their therapeutic effect. The body weights of mice were recorded weekly during the entire study.

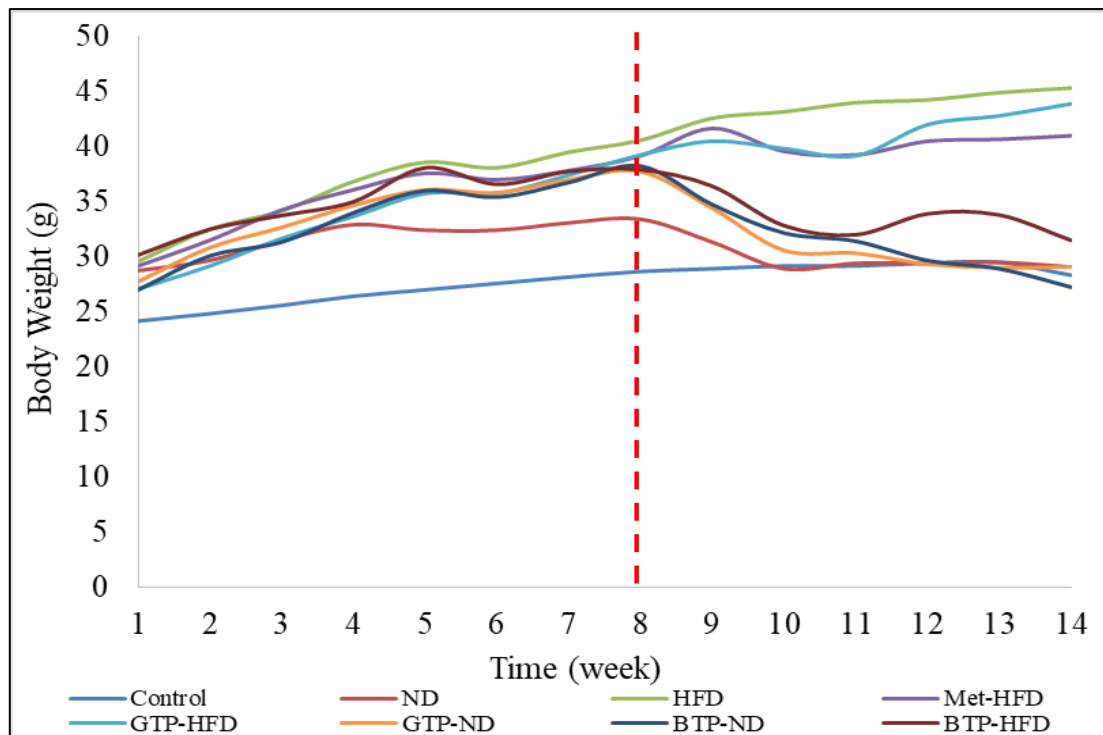


Figure 6. 6: Body weight monitoring graph of mice for 14 weeks. Dashed red line indicates the eighth week to separate the time period between the model period and the treatment period.

Body weight measurements of the control mice were stable for 14 weeks and time-dependent weight gain was normal. As shown in Figure 6.6, the test mice had an average weight of 34.28 g depending on the STZ application and fed with HFD. This weight increase is about 30% higher compared to the control mice. When the test mice were divided into seven different experimental groups after the 8th week, weight loss

was observed in ND, GTP-ND, BTP-ND and BTP-HFD groups. While weight gain was observed in the other groups during the treatment period, the most significant increase was only in the HFD group (Table 6.3 and Table 6.4). Moreover, weight loss in the group treated with black tea and fed with high-fat diet (BTP-HFD) was evaluated as an important result.

Table 6. 3: Descriptive statistical values of body weights of different experimental groups during the model period. The values are from 1 week to 8 weeks.

	Model period (8 Weeks)					
	Mean	Median	Std. Er.	Std. Dev.	Min.	Max.
Control	26,55	27,00	0,33	2,32	22,50	30,50
ND	31,77	31,50	0,41	2,83	25,50	37,00
HFD	36,23	36,75	0,59	4,09	28,50	45,50
Met-HFD	35,32	36,00	0,60	4,12	27,00	46,00
GTP-HFD	33,72	34,50	0,60	4,15	25,50	41,50
GTP-ND	34,07	33,75	0,61	4,22	24,50	43,00
BTP-ND	33,60	34,25	0,78	5,38	24,00	47,50
BTP-HFD	35,22	34,50	0,78	5,38	25,00	46,00

Table 6. 4: Descriptive statistical values of body weights of different experimental groups during the treatment period. The values are from 9 weeks to 14 weeks.

	Treatment period (6 Weeks)					
	Mean	Median	Std. Er.	Std. Dev.	Min.	Max.
Control	29,08	29,50	0,34	2,04	25,00	32,00
ND	29,61	29,50	0,32	1,91	26,00	33,50
HFD	44,04	45,00	0,87	5,19	35,00	52,50
Met-HFD	40,43	40,00	0,75	4,45	31,00	50,00
GTP-HFD	41,23	41,00	0,46	2,62	37,50	46,50
GTP-ND	30,46	30,00	0,52	3,15	26,00	40,00
BTP-ND	30,69	30,75	0,70	4,20	22,50	43,00
BTP-HFD	33,44	35,50	1,58	9,10	18,00	44,00

6.3.2. Fasting Blood Glucose (FBG) Levels

Fasting blood glucose measurements were performed weekly after starving for 6 hours. Fasting blood glucose of control lean mice throughout the entire experiment were 106 ± 18 mg/dL. When FBG follow-up was applied to groups ND, HFD and Met-HFD without tea extracts application, significant decreases were observed in the ND group that was fed with normal diet after 8th week ($p < 0.05$). As the HFD group continued to feed on a fat diet, increased blood sugar levels were observed. Significant changes were observed in all groups treated with tea extracts (Table 6.5 and Table 6.6). In the GTP-HFD group, which applied green tea extract and fed a high-fat diet, blood sugar increases continued. Fasting blood sugar was significantly decreased in the GTP-ND group, which was administered green tea extract but fed with normal diet (Figure 6.7 and Figure 6.8). In the groups treated with black tea extracts, fasting blood glucose values were decreased regardless of their nutritional forms ($p < 0.05$).

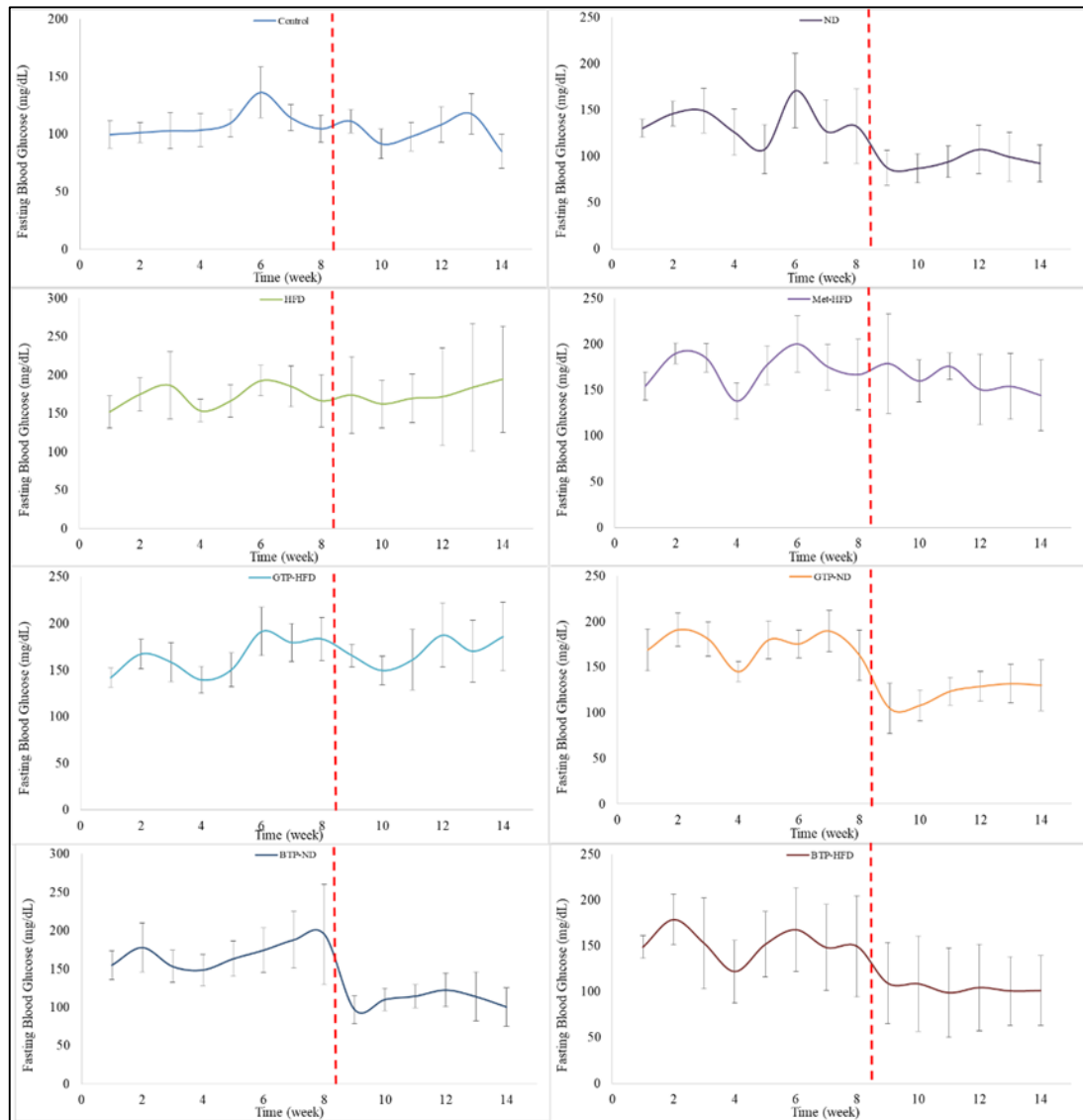


Figure 6. 7: Graphs of FBG measurements of each experimental group. Dashed red line indicates the eighth week to separate the time period between the model period and the treatment period.

Table 6. 5: Descriptive statistical values of FBG of different experimental groups during the model period. The values are from 1 week to 8 weeks.

	Model model period (8 Weeks)					
	Mean	Median	Std. Er.	Std. Dev.	Min.	Max.
Control	109,04	108,00	2,60	17,99	74	180
ND	136,23	138,00	4,93	34,12	67	243
HFD	172,25	174,00	4,43	30,69	125	276
Met-HFD	173,38	172,50	4,41	30,52	102	249
GTP-HFD	163,94	157,00	3,87	26,78	119	236
GTP-ND	174,40	176,00	3,61	25,02	120	225
BTP-ND	169,54	167,50	5,48	37,94	120	332
BTP-HFD	152,40	157,50	6,33	43,83	55	236

Table 6. 6: Descriptive statistical values of FBG of different experimental groups during the treatment period. The values are from 9 weeks to 14 weeks.

	Treatment period (6 Weeks)					
	Mean	Median	Std. Er.	Std. Dev.	Min.	Max.
Control	102,00	102,00	3,06	18,35	65	146
ND	94,58	94,00	3,76	22,57	57	136
HFD	176,14	181,00	9,96	59,75	75	293
Met-HFD	161,06	164,00	6,59	39,00	86	291
GTP-HFD	168,88	167,00	5,57	32,01	97	227
GTP-ND	121,33	122,00	4,08	24,46	71	173
BTP-ND	109,72	111,00	4,01	24,08	57	157
BTP-HFD	104,06	109,00	8,02	46,09	30	183

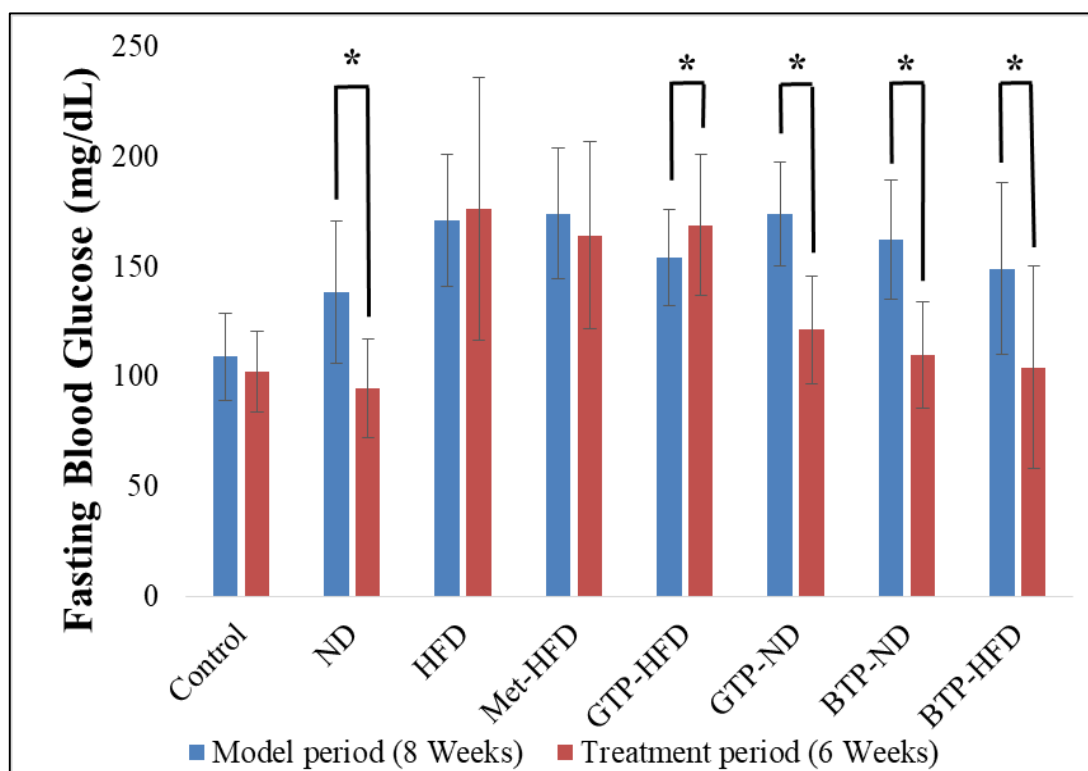


Figure 6. 8: Comparison of model and treatment period in FBG levels. * indicates a significant difference between different periods of FBG. Statistical analysis was performed using the Wilcoxon test (* $p < 0.05$).

6.3.3. Oral Glucose Tolerance Test (OGTT)

The effects of tea extracts on impaired glucose tolerance were investigated. The AUC is obtained by calculating the area under the OGTT test results. The AUC was calculated by the formula: $AUC \text{ (mmol/L} \cdot \text{min)} = [\frac{1}{2} \times (G_{0\text{min}} + G_{30\text{min}}) \times 30] + [\frac{1}{2} \times (G_{30\text{min}} + G_{60\text{min}}) \times 30] + [\frac{1}{2} \times (G_{60\text{min}} + G_{120\text{min}}) \times 60]$. Dysglycemic values are an important predictor of diabetes, and the results of the HFD group have significantly higher AUC values as an indicator of glucose intolerance compared to the control group ($p < 0.05$). While the BTP-HFD group showed a significant decrease compared to the diabetic HFD group, the GTP-HFD group did not (Figure 6.9 and Figure 6.10).

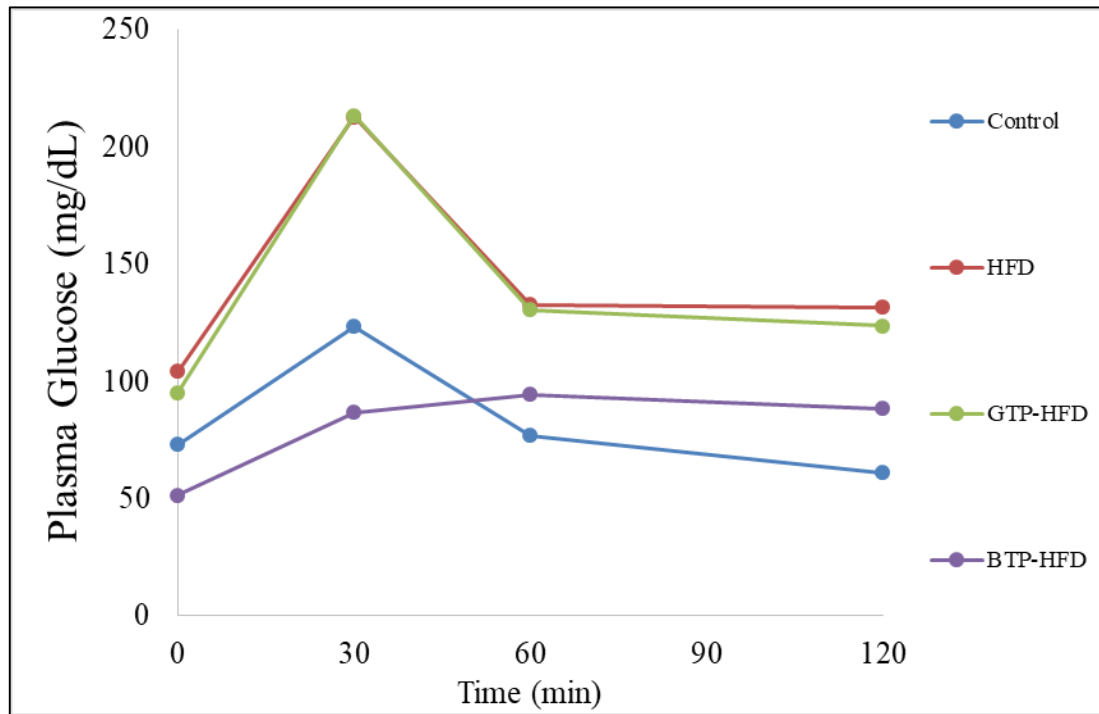


Figure 6. 9: Effects of GTP and BTP on glucose tolerance at 14th week. After fasting overnight, mice were given a glucose solution (2 g/kg) via gavage and changes in blood glucose levels were monitored for the next 120 minutes.

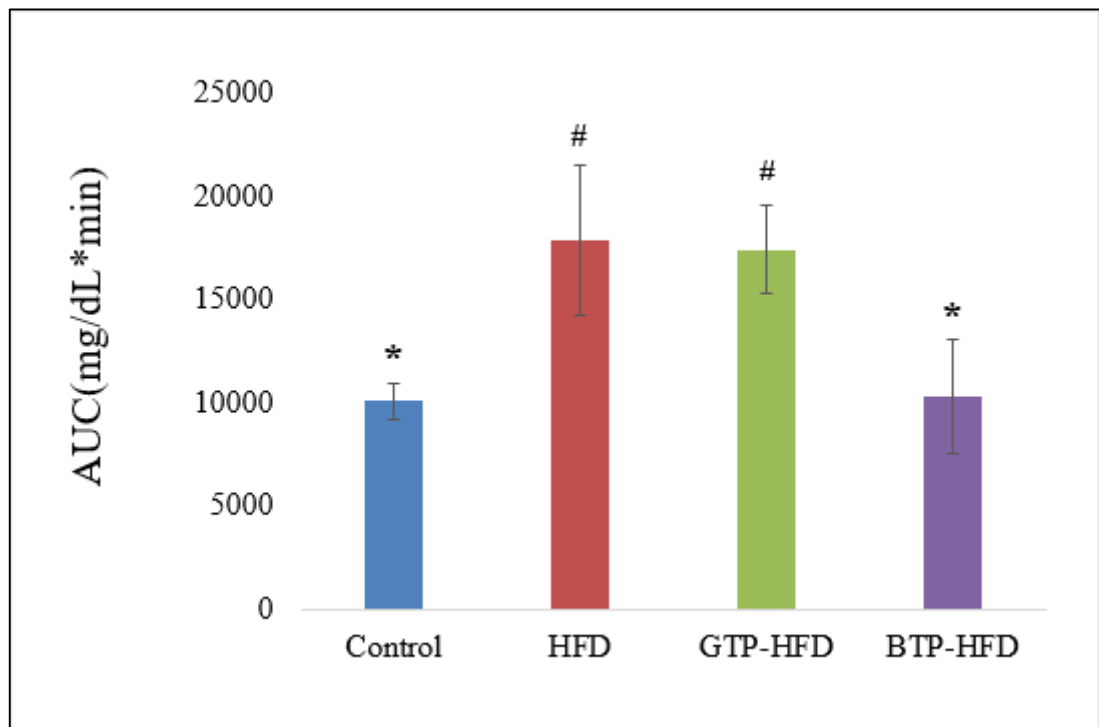


Figure 6. 10: AUC of OGTT in Control, HFD, GTP-HFD, and BTP-HFD groups. * indicates significant difference from HFD group; # indicates a significant difference from the control group. Statistical analysis was performed using the Kruskal-Wallis Test (* $p < 0.05$, # $p < 0.05$).

6.3.4. Serum Insulin Levels

At the end of the 14th week, all mice were sacrificed by cervical dislocation after blood collection from the ophthalmic vein. Serum insulin levels were measured with the Mouse Insulin Elisa kit (ALPCO). Plasma insulin levels measured at the end of the 14th week showed differences between the experimental groups (Figure 6.11). Insulin levels were significantly lower in the ND, GTP-ND, BTP-ND and BTP-HFD groups compared to the control group and diabetic HFD.

Insulin levels at 14 weeks were analyzed according to the percentage of FBG difference between model period and treatment period (Table 6.7). A significant negative correlation exists between the insulin level and % FBG reduction in all the groups (Pearson correlation coefficient (r)= -0.977, $p < 0.01$; Spearman's rho (ρ)= -0.857, $p < 0.01$).

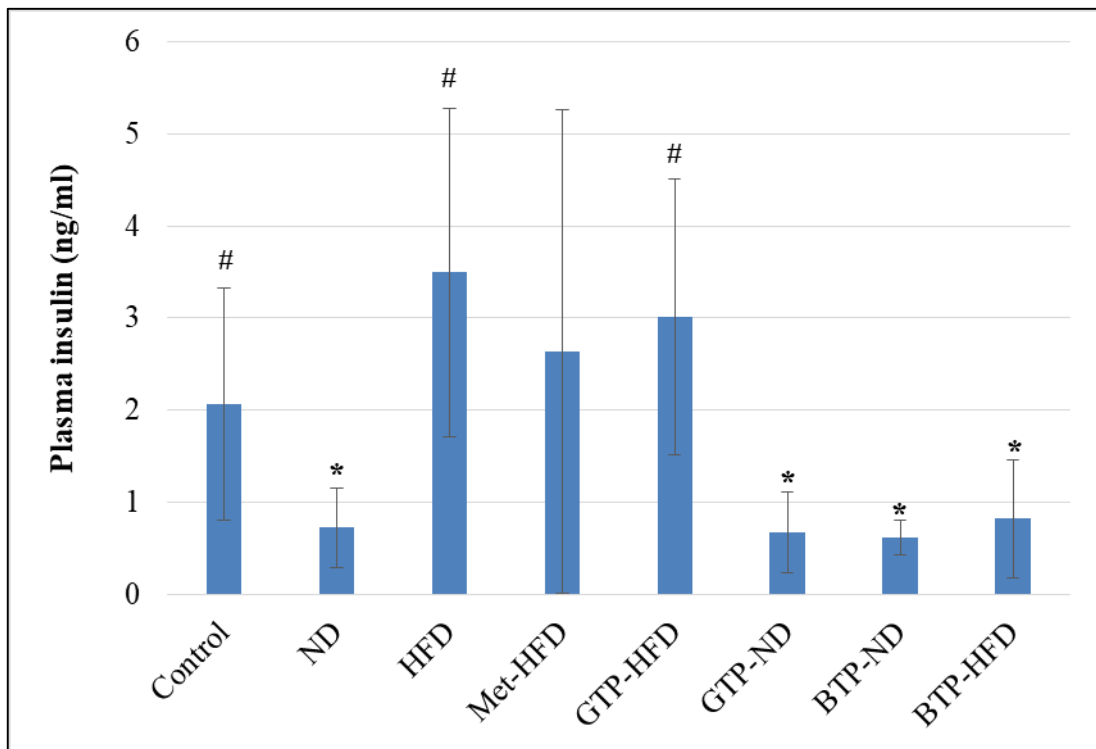


Figure 6. 11: Plasma insulin levels in mice blood collected at the end of the 14th week. * indicates a significant difference from control group; # indicates a significant difference from ND group; ## indicates significant difference from HFD group. Statistical analysis was performed using the Kruskal-Wallis Test (* $p < 0.05$, # $p < 0.05$, ## $p < 0.05$).

Table 6. 7: Correlation between percentage reduction of FBG and insulin levels.

	Parameters	
	Reduction of FBG level (%)	Insülin Levels (ng/ml)
Control	6,46	2,07
ND	30,57	0,73
HFD	-2,26	3,49
Met-HFD	7,10	2,64
GTP-HFD	-3,01	3,01
GTP-ND	30,43	0,68
BTP-ND	35,28	0,62
BTP-HFD	31,72	0,82
r=correlation, Correlation is significant at the 0.01 level.		
Reduction of fasting blood glucose level (%)= $100 \times (\text{FBG}_x - \text{FBG}_y) / \text{FBG}_x$		
x: Average FBG of 1 to 8 weeks, y: Average FBG of 9 to 14 weeks.		

6.3.5. Biochemical Parameters

The hepatotoxic effect of GTP and BTP is shown by serum indicators, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) [34]. Serum lipid profiles, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), of the experimental groups were also examined. In the analysis of blood samples collected at the end of the 14th week, there are differences in a biochemical analysis as shown in Table 6.8.

Table 6. 8: Effect of TPS blood hematological parameters of mice (means $\pm$ SD). Bottom line is reference value range.

Groups	ALP	ALT	AST	HDL-C	LDL-C	TC
Control	59,6$\pm$5,5	38,7 $\pm$ 5,9	116,4 $\pm$ 25,1	42,7 $\pm$ 4,2	0,2 $\pm$ 0,1	62,4 $\pm$ 5,0
ND	57,1$\pm$6,9	33,3 $\pm$ 12,4	88,0$\pm$73,6	42,0 $\pm$ 3,6	0,2 $\pm$ 0,1	60,0 $\pm$ 4,7
HFD	64,1 $\pm$ 14,7	61,7$\pm$32,5	146,9$\pm$41,6	95,5$\pm$22,0	0,3 $\pm$ 0,1	126,3$\pm$23,2
Met-HFD	68,9 $\pm$ 13,1	23,5 $\pm$ 4,0	69,3 $\pm$ 13,6	97,6$\pm$14,0	0,3 $\pm$ 0,1	127,6$\pm$10,9
GTP-HFD	48,0$\pm$2,5	33,3 $\pm$ 12,0	145,5$\pm$62,1	70,4$\pm$18,5	0,3 $\pm$ 0,1	91,5 $\pm$ 21,5
GTP-ND	68,3 $\pm$ 10,9	29,0 $\pm$ 3,0	85,0 $\pm$ 17,5	49,3 $\pm$ 3,6	0,2 $\pm$ 0,1	70,4 $\pm$ 3,4
BTP-ND	73,9 $\pm$ 26,7	25,6 $\pm$ 5,4	104,4$\pm$54,7	47,3 $\pm$ 18,2	0,3 $\pm$ 0,1	69,1 $\pm$ 23,4
BTP-HFD	67,6 $\pm$ 17,2	32,8 $\pm$ 7,3	114,3$\pm$45,7	52,6 $\pm$ 16,6	0,3 $\pm$ 0,1	77,3 $\pm$ 19,8
ALP: 63.1-103.9 (IU/l); AST: 35.7-135.7 (IU/l); ALT: 16.4- 53.2 (IU/l); HDL-C: 40-50 mg/dL; LDL-C: <1.8 mmol/L; TC: 72.9- 113.7						

6.3.6. Histopathology of Liver Tissue

Control liver tissue sample was shown normal morphology however HFD and GTP-HFD were shown abnormal morphology. In these groups, sinusoidal dilatation and hepatocellular vacuolization in liver tissue was visualized at high levels. After treatment of black tea extract, sinusoidal dilation and hepatocellular vacuolization were not found in BTP-HFD, liver tissue morphology was similar to a control group (Figure 6.12).

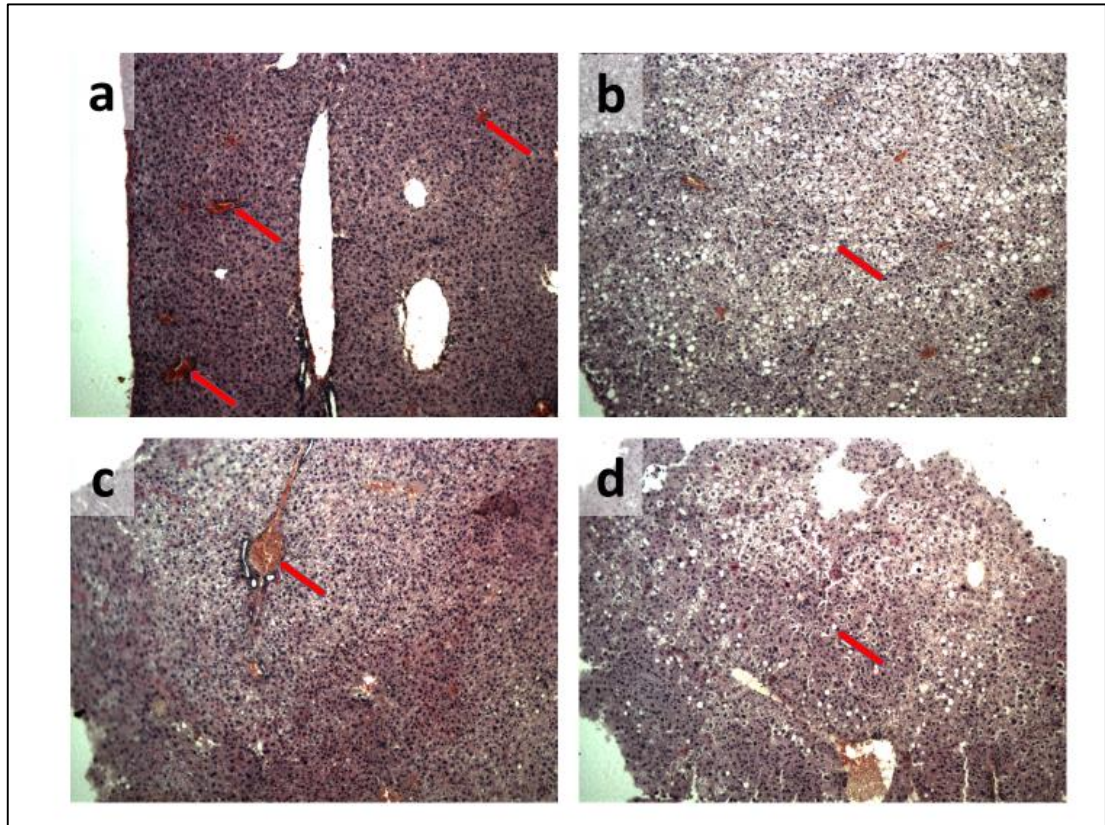


Figure 6. 12: Hematoxylin-eosin-stained liver sections from mice. a) Normal diet showing normal hepatic architecture with central vein and surrounding hepatocytes, sinusoids (Control); b) HFD; c) GTP-HFD; d) BTP-HFD. Figures were taken at the same magnification (10X).

7. DISCUSSION AND CONCLUSION

Nowadays, metabolic diseases are frequently seen because of sedative lifestyles and high-calorie eating habits. T2DM, which is a part of metabolic diseases, is a serious disease requiring progressive stages of insulin-dependent therapy. There are many physiological problems associated with untreated or advanced T2DMs. They reduce the quality of life of patients and also increase the cost of the treatment considerably. T2DM is a disease that can be controlled by early diagnosis and regulation of lifestyle. Despite all preventive and progressive precautions, T2DM patients become drug-dependent in order to provide glycemic homeostasis by age. Keeping blood sugar in balance is a primary factor in the treatment of the disease, and antihyperglycemic is a group of drugs that have a wide range of drugs for this purpose. Further, insulin may be needed in addition to antihyperglycemic agents. In recent years, because of the cost of the drugs used for T2DM and the adverse effects on the patient, the effects of foods with natural antihyperglycemic effect have come to the fore in both academic and industrial research. In particular, it has become very popular in this field to investigate in the generic therapeutic plants used in alternative medicine for many years. The identification of the chemical content and the investigation of their cellular and physiological effects of these plants has been one of the targets of both the pharmaceutical industry and the food industry. The use of phytotherapeutic plants in the production of functional food additives has reached a very wide market coverage in terms of both low toxicity and benefits. In this study, the antihyperglycemic effects of plant-derived substances that have the potential to become a functional food additive in our country have been investigated. As a material, tea, an important industrial farming plant, were used. The fact that tea plants are old in our country and that the standards are not complied with when collecting row teas cause quality tea leaves not to be provided. Dry teas are produced by blending low and high-quality products, which reduces the quality of the teas consumed and obstructs the industrialists from increasing their yield. In the context of all these data, it is necessary to use the potential of the tea plant for the production of other products other than traditional tea products. Chemical extractions methods for the production of polysaccharides were made by the project partners in the Food Institute and 21 tea

extracts according to the polysaccharide ratio and quality were given to our group in order to carry out *in vivo* and *in vitro* experiments. Antidiabetic properties of 21 different tea extracts were tested *in vitro* by α -glucosidase enzyme inhibition technique. The results in Table 6.1 shows that there were significant inhibition percentages in some of the tested tea extracts at different concentrations. These extracts vary depending on both the extraction method and the time of collection of the tea. As a result, black tea extracts (encoded as syh) has the highest inhibition rate. Moreover, inhibition is quite high in green tea extracts (encoded as ysl). Two groups of tea extracts were selected for usage in the ongoing experiment according to show α -glucosidase enzyme inhibition. As suggested in our hypothesis, the potential of being a new antihyperglycemic agent due to the effect of α -glucosidase enzyme inhibition and the effect on T2DM were continued by *in vitro* cell culture experiment. Glucose uptake metabolism was studied in cell culture experiment, and glucose uptake rates of adipocytes were optimized and determined. Cells used in studies on glucose uptake metabolism is 3T3 L1 cell line which is mostly used in insulin-dependent increased intracellular glucose intake. Insulin-dependent glucose molecules that are taken into the cell represent the ability to physiologically reduce blood sugar. Insulin resistance enhancement mechanism is another underlying pathophysiology in T2DM disease and causes increased blood sugar levels. T2DM includes disorders in insulin production as well as disorders developed in response to insulin. Glucose in the blood cannot be taken into cells in tissues showing insulin-dependent sugar use (such as muscle tissue) at the cellular level, therefore hypoglycemia progress. In the cell culture studies, as shown at Figure 6.5, it was observed that both green and black tea extracts triggered a considerable amount of intracellular glucose uptake by time. Black tea polysaccharides obtained from the black tea extract and with a high percentage of purity were subjected to glucose uptake test, and as a result, the highest percentage of glucose intake was observed. In order to understand how the cellular glucose uptake metabolism increases, elucidating of molecular pathways that are triggered by these extracts can be used in the direction of forward-looking studies. *In vivo* experiments were continued with two groups of tea extracts (black and green). Previously, T2DM animal model is created, afterwards, antidiabetic properties in these different experimental groups depending on the diet were determined. Diabetes model was created by screening the most frequently used models. The responses of the animals treated with tea extract for six weeks were compared in terms of different parameters.

After the experiments on the samples of blood and liver tissue collected from animals, the effect of tea polysaccharides on T2DM was determined.

In concluding, it was shown that black tea polysaccharides have a remarkable effect on T2DM in high-fat diet applied mice although there are no effect has been shown in green tea polysaccharides group. Liver damage is observed in the HFD group (Table 6.8 and Figure 6.12). It was determined that black tea extract had a statistically significant effect on blood glucose level (Figure 6.8) and curative effect on liver tissue in T2DM mice fed a normal and high-fat diet types (Figure 6.12). Considering the insulin levels of the HFD group and BTP-HFD group, the anti-diabetic effect of black tea extract might be achieved by improving the action (or sensitivity) of secreted insulin. Considering all these results, it has been observed that especially black tea polysaccharides have a potential for healing effect on T2DM.

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BIOGRAPHY

Kübra Akalın was born in Afyonkarahisar in 1992. After she graduated from Afyonkarahisar Anatolian Teacher Training High School, in 2010, she started her undergraduate studies at the Department of Molecular Biology and Genetics at the Faculty of Science of Istanbul Kültür University. Afterward, she participated in cancer research project in Istanbul Capa Oncology Institute with TUBITAK scholarship. In 2016, she started her master's degree at the Department of Molecular Biology and Genetics of Gebze Technical University, and she worked at MRC GEBI by the scholarship of TUBITAK for her thesis research.

APPENDICES

APPENDIX A: Poster Presentations Within the Scope of the Thesis

Alasalvar C., (2018), “*In vitro* and *in vivo* activity studies of black and green tea polysaccharides on type 2 diabetes”, The International Society for Nutraceuticals and Functional Foods (ISNFF), Vancouver, BC, Canada, 14-17 October.

Akalin K., (2018), “Çay ekstraktlarının anti-diyabetik etkinlikleri üzerine *in vivo* ve *in vitro* çalışmalar”, 7. International Molecular Biology and Biotechnology Congress, Nevsehir, Turkey, 2-5 July.