

REPUBLIC OF TURKEY
YUZUNCU YIL UNIVERSITY
INSTITUTE OF HEALTH SCIENCE

**EFFECT OF THE *Silybum marianum* EXTRACT IN
EXPERIMENTAL DIABETIC RATS ON THE SERA
8-HYDROXY-2'-DEOXYGUANOSINE, TOTAL OXIDANT
AND ANTIOXIDANT CAPACITY**

Gulala Ahmed Mohammed SHINKI
DEPARTMENT OF BASIC PHARMACEUTICAL SCIENCES
BIOCHEMISTRY SCIENCE
MASTER THESIS

SUPERVISOR

Assoc. Prof. Dr. Tahir KAHRAMAN

This study with the project number (TYL-2016-5107) was supported by
Yuzuncu Yil University Presidency of Scientific Research Projects.

VAN-2016

REPUBLIC OF TURKEY
YUZUNCU YIL UNIVERSITY
INSTITUTE OF HEALTH SCIENCE

**EFFECT OF THE *Silybum marianum* EXTRACT IN
EXPERIMENTAL DIABETIC RATS ON THE SERA
8-HYDROXY-2'-DEOXYGUANOSINE, TOTAL OXIDANT
AND ANTIOXIDANT CAPACITY**

Gulala Ahmed Mohammed SHINKI
DEPARTMENT OF BASIC PHARMACEUTICAL SCIENCES
BIOCHEMISTRY SCIENCE
MASTER THESIS

Prof. Dr. Semiha DEDE
Chairman

Assoc Prof. Dr. Tahir KAHRAMAN
Supervisor and Member

Assist. Prof. Dr. Müslüm KUZU
Member

Thesis Admission Date
07/ 10 / 2016

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisor Assoc. Prof. Dr. Tahir KAHRAMAN, for the continuous support of my MSc study and related research, for their excellent guidance. Their guidance helped me in all the time of research and writing of this thesis. My gratitude is extended to all my teachers at Yuzuncu Yil University, Department of Basic Pharmaceutical Sciences, Assoc. Prof. Dr. Tahir Kahraman and Faculty of Veterinary Medicine Prof. Dr. Semiha DEDE, Prof. Dr. Nihat MERT, Prof. Dr. Handan MERT, and who have taught me to finally learn how to relax, to establish better studying techniques and for their support and encouragement during the tough time of this two years. I would like to expand my special gratitude to Prof. Dr. Rizgar Mageed for his unforgettable help in theoretical part of this project. I would like to expand my gratitude to both teachers Assist. Prof. Dr. Oruç ALLAHVERDİYEV, Lecturer Mehmet BERKÖZ for their helps in practical part of this project I would also like to thank Prof. Dr. Sıddık KESKİN who helped me at statistic issue of this thesis I am very thankful to the Presidency of Scientific Research Projects at Yuzuncu Yil University for supporting this thesis. I would like to thanks Dr. Saed Ali and Mr. Abubakir Mala Abdulla for their helps. I would like to thanks SOLGAR Company for providing free Milk thistle extract. Also I would like to very thank my friends Ms. Zwain Nawzad, Mr. Karzan, for their helps, and facilitation in practical and laboratory affairs. Finally, I would like to thank my family, my parents and to my brothers and sisters, for supporting me spiritually throughout writing this thesis and my life in general. I would not have been able to complete this thesis without their continuous love and encouragement.

CONTENTS

ACCEPTANCE AND APPROVAL	II
ACKNOWLEDGMENTS	III
CONTENTS.....	IV
SYMBOLS AND ABBREVIATIONS.....	VI
FIGURES.....	VIII
TABLES	IX
1. INTRODUCTION	1
2. LITERATURE REVIEW	3
2.1. Diabetes Mellitus (DM).....	3
2.1.1. Diabetes mellitus definition.....	3
2.1.2. Pancreas	3
2.1.3. Insulin	3
2.1.4. Insulin resistance.....	5
2.1.5. Criteria for the diagnosis of diabetes	5
2.1.6. The glycohemoglobin (HbA1c) test	5
2.1.7. Types of diabetes	6
2.1.8. Diabetes complications.....	7
2.2. 8-Hydroxy-2'-deoxyguanosine (8-OHdG).....	10
2.2.1. DNA structure.....	10
2.2.2. Free radicals.....	13
2.2.3. Free radicals and DNA-damage in diabetes	13
2.2.4. DNA damage and its causes	14
2.2.5 The cause of 8-hydroxy-2'-deoxyguanosine formation and its clinical significance..	14
2.2.6. Mechanism of 8-hydroxy-2'-deoxyguanosine formation.....	15
2.2.7. Oxidative stress.....	17
2.2.8. Oxidative stress and diabetes	17
2.3. Antioxidants.....	18
2.3.1. Endogenous antioxidants defense system.....	18
2.3.2. Exogenous antioxidants	19
2.3.3. Antioxidants in diabetes.....	20
2.4. <i>Silybum marianum L.</i>	21

2.4.1. <i>Silybum marianum</i> L. component	21
2.4.2. Biochemical effects of <i>Silybum marianum</i> L.	22
2.4.3. <i>S marianum</i> L. effects on diabetes	25
3. MATERIAL AND METHOD	26
3.1. Materials	26
3.1.1. Experimental animals	26
3.1.2. Equipment and apparatus.....	26
3.1.3. Chemical agents.....	27
3.2. Methods	27
3.2.1. Experimental animal design	27
3.2.2. Blood sample collection.....	29
3.2.3. Biochemistry routine assay determination in serum.....	29
3.2.4. Glycohemoglobin (HbA1c) level determination in whole blood	31
3.2.5. Total oxidant capacity (TOS) determination in serum	31
3.2.6. Total antioxidant capacity (TAS) determination in serum	32
3.2.7. 8-Hydroxy 2-deoxyguanosine determination in serum	33
3.3. Statistical Analysis.....	34
4. RESULTS	35
5. DISCUSSION AND CONCLUSION	45
SUMMARY	59
ÖZET	60
REFERENCES	61
CURRICULUM VITAE.....	74
ATTACHMENT.....	75
Attachment 1. Acceptance letter from experimental animal ethics committee	75
Attachment 2. The acceptance letter for results of the thesis by experimental animal committee in Yuzuncu Yil University	76

SYMBOLS AND ABBREVIATIONS

ADA	: American diabetes association
AGE	: Advanced glycation end product
ALT	: Alanine aminotransferase
AST	: Aspartate aminotransferase
β-cells	: Beta cells
CKD	: Chronic kidney disease
CVD	: Cardiovascular disease
CAT	: Catalases
DNA	: Deoxyribonucleic acid
DM	: Diabetes mellitus
DKA	: Diabetic ketoacidosis
DN	: Diabetic nephropathy
DPN	: Diabetic peripheral neuropathy
DR	: Diabetic retinopathy
ELISA	: Enzyme-linked immunosorbent assay
FPG	: Fasting plasma glucose
FFA	: Free fatty acid
GDM	: Gestational diabetes mellitus
GPx	: Glutathione peroxidase
GR	: Glutathione reductase
GST	: Glutathione s transferase
HbA1C	: Glycohemoglobin
HCC	: Hepatocellular carcinoma
HDL	: High density lipoprotein
IDDM	: Independent diabetes mellitus
IGT	: Impaired glucose tolerance
LDL	: Low density lipoprotein
MDA	: Malondialdehyde
MODY	: Maturity-onset diabetes of the young

NIDDM	: No independent diabetes mellitus
NAFAD	: Non-alcoholic fatty liver disease
OGTT	: Oral glucose tolerance test
PKC	: Protein kinase C
RNS	: Reactive nitrogen species
ROS	: Reactive oxygen species
<i>S. marianum</i>	: <i>Silybum marianum</i> L. extract
STZ	: Streptozotocin
SOD	: Superoxide dismutase
TBARS	: Thiobarbituric acid reactive substance
TAS	: Total antioxidant status
TOS	: Total oxidant status
T₁ D	: Type 1 diabetes
T₂ D	: Type 2 diabetes
T₁ DM	: Type 1 diabetes mellitus
T₂ DM	: Type 2 diabetes mellitus
USA	: United States of America
VLDL	: Very low density lipoprotein
WHO	: World health organization
2-h PG	: 2-h plasma glucose
8-OHdG	: 8-hydroxy-2'- deoxy guanosine
8-oxodG	: 8-oxo-7,8-dihydro-2-deoxyguanosine
Equiv/L	: Equivalent per liter
mL	: Milliliter
mg	: Milligram
µg	: Microgram
µl	: Microliter
µmol	: Micromole
mmol/L	: Mill moles per liter
mg/dL	: Milligrams per deciliter
U/L	: Units per liter

FIGURES

Figure 1. Insulin structure	4
Figure 2. DNA helix shape	11
Figure 3. A segment of one strand of a DNA molecule.....	12
Figure 4. Purines; adenine, guanine. Pyrimidines; cytosine, thymine	12
Figure 5. 8- OHdG formation	15
Figure 6. Reaction of 2-deoxyguanosine with hydroxyl radicals	16
Figure 7. <i>Silybum marianum</i> L. plant.....	21
Figure 8. Silybin, and 2,3-dehydrosilybin structure	22
Figure 9. <i>Silybum marianum</i> functions.....	22
Figure 10. Standard curve according to the optical density of the standard 8- OHdG	34
Figure 11. The average of serum glucose level (mg/dL) in study groups	39
Figure 12. The average of blood HbA1c (%) levels in study groups.	39
Figure 13. The average of serum T-cholesterol level (mg/dL) in study groups	40
Figure 14. The average of serum triglycerides level (mg/dL) in study groups.....	40
Figure 15. The average of serum HDL-cholesterol level (mg/dL) in study groups	41
Figure 16. The average of serum LDL-cholesterol level (mg/dL) in study groups.....	41
Figure 17. The average of serum VLDL-cholesterol level (mg/dL) in study groups	42
Figure 18. The average of serum ALT levels (U/L) in study groups	42
Figure 19. The average of serum AST levels (U/L) in study groups.....	43
Figure 20. The average of serum TOS levels (μmol/L) in study groups	43
Figure 21. The average of serum TAS levels (μmol/L) in study groups	44
Figure 22. The average of serum 8-OHdG levels (ng/dL) in study groups.....	44

TABLES

Table 1. The level of biochemistry routine assay, HbA1c, 8-OHdG, TAS, TOS in study groups	35
--	----



1. INTRODUCTION

Diabetes mellitus (DM) is one of the most challenging diseases of the 21st century. The incidence of diabetes is increasing globally, expected to increase to 366 million by 2030 (El-Wassef et al., 2012). The disease is recognized as one of the leading causes of morbidity and mortality in the world (Seghrouchni et al., 2002). DM is a long-term condition even in developed countries (Huseini et al., 2006).

DM is classified into a number of types, type 1 diabetes mellitus (insulin-dependent diabetes), type 2 diabetes mellitus (non-insulin dependent diabetes), gestational diabetes mellitus and diabetes due to other causes, such as, neonatal diabetes and maturity-onset diabetes of the young (MODY) (ADA, 2016).

DM is a chronic metabolic syndrome (El-Wassef et al., 2012), characterized by hyperglycemia due to insufficient secretion of insulin from the pancreas, or due to lack of insulin action, which is not properly formed, or both. Diabetes is associated with complications, of which there are two types. Microvascular complications (neuropathy, retinopathy, and nephropathy), and macrovascular complications include atherosclerosis leading to strokes, heart attacks, and peripheral vascular diseases (Ghule et al., 2012; Koulis et al., 2015; Ullah et al., 2015; Akhavan et al., 2016; Hwang et al., 2016).

One of the major theories to start diabetic complications is the increase in oxidative stress due to the increased production of reactive oxygen species (ROS). Hyperglycemia increases ROS production, which in turn decreases cellular antioxidant defenses and causes DNA lesions and mutations (Xavier et al., 2014). Oxidative stress in diabetes leads to tissue damage, with lipid peroxidation, inactivation of proteins, and protein glycation as intermediate mechanisms for its complications including retinopathy, nephropathy, and coronary heart disease (El-Far et al., 2015).

Unluckily perhaps, most hypoglycemic drugs, such as, glibenclamide, chlorpropamide, gliclazide, tolbutamide, and glipizide have limited effects and different side effects such as disturbances to the gastrointestinal tract, skin rashes, weight gain, hypoglycemia and liver damage. Thus, looking for new classes of compounds is

essential to overcome these complications. Recently, scientific interests have focused on the use of antioxidants such as flavonoids in lowering the negative effect of oxidative stress and free radicals in patients with diabetes. Flavonoids contain a large family of plant-derived poly phenolic compounds that have been shown to have different biological and pharmacological activities such as anti-cancer, anti-oxidants, anti-inflammatory, anti-microbial, anti-allergic, and anti-malarial properties. They are important ingredient of human diet and are extracted from plants. They have antioxidant activities, radical scavenging ability, and cell function modulation, *Silybum marianum* L. Gaertner (*S. marianum*), is one of these flavonoids (El-Far et al., 2015). There is a demand to develop safe or riskless and effective treatment for diabetes. In this regard, plants provide the best options for research of the desired safe and effective medication, ever since ancient times (Patel et al., 2012).

S. marianum L. is common names: Milk thistle, Holy thistle, Marian thistle. Milk thistle is widely used in Europe for hepatic and biliary disorders, and against nephrotoxicity, also it is protecting the liver from some hepatotoxins including Amanita mushrooms, and alcohol. The primary active ingredient in the *S. marianum* L. is silymarin, which is a strong antioxidant (Kaur et al., 2011; Abu-Zaiton 2013).

The aim of this study was to evaluate of medicinal plants used for their antidiabetic activities, and *S. marianum* L in particular, specifically the study was focused on examining the effect of this plant on 8-hydroxy-2'- deoxy guanosine (8-HdOG), total oxidant (TOS) and antioxidant capacity (TAS) activities, in addition effect of this plant on liver enzymes, and lipoprotein levels in experimental diabetes in rats.

The current study includes a discussion about diabetes, diabetes definition, diabetic complications, free radicals and DNA-damage in diabetes, *S marianum* L. plant and its components and the effect of *S. marianum* L. on 8-OHdG. Furthermore, there is a section on the practical work, which also includes discussion about experimental animals, methods of biochemistry routine assays of serum, glycohemoglobin (HbA1c%) in whole blood, total antioxidant status (TAS) and total oxidant status (TOS) in serum. 8-OHdG determination is also in serum discussed in the results section.

2. LITERATURE REVIEW

2.1. Diabetes Mellitus (DM)

2.1.1. Diabetes mellitus definition

DM is a common chronic disease characterized by elevation of blood glucose levels (Kaatabi et al., 2015), due to insufficiency in production, or action of insulin which is produced by the pancreas (Ramachandra et al., 2012; Ullah et al., 2015). DM is a multi-systemic metabolic disorder showing the common underlying feature of hyperglycemia (Shinde et al., 2010). Oxidative stress associated with hyperglycemia is now recognized as the driving force for the development of diabetic complications (Fowler, 2008; Zemestani et al., 2015).

2.1.2. Pancreas

The pancreas is an organ that lies behind the stomach (Ogden, 2010). The endocrine pancreas contains a core unit known as islet of Langerhans which regulates blood glucose homeostasis. This regulation is accomplished the pancreas secreting two main endocrine hormones: insulin and glucagon. The production of insulin by beta cells of pancreas occurs in response to raising blood glucose levels, while glucagon secretion from alpha cells of pancreas is prompted by low blood glucose level (Ridha and Moog, 2015; Quilichini and Haumaitre, 2015; Campbell and Hoffman, 2016; Mellado-Gil et al., 2016). Glucagon induces the liver to secrete glucose into blood stream when the level of glucose is low (Ogden, 2010; Ridha and Moog, 2015). Insufficient capacity of pancreatic β cells to secrete enough insulin leads either to hyperglycemia, the hallmark of DM, or to hypoglycemia (Mellado-Gil et al., 2016).

2.1.3. Insulin

Insulin is a hormone that helps glucose get inside the body's cells. Cells are use glucose for energy (Ogden, 2010). Insulin is a protein with two polypeptide chains: A (21 amino acid residues), and B (30 amino acid residues) (Joshi et al., 2007). These two chains are linked together by disulphide bridges. The A chain has an N-terminal helix joined to an anti-parallel C-terminal helix. The B chain, in contrast, has a central helical

segment. The two chains are joined by two disulphide bonds, which join the N- terminal and C-terminal helices of the A chain to the B central helix chain. In pro-insulin, a connecting peptide links the N-terminus A chain with the C-terminus of the B chain (Figure 1). Insulin monomers combine to form dimers and hexamers. The hexamer contains three insulin dimmers associated in a three-fold symmetrical pattern (Wilcox, 2005; Joshi et al., 2007).

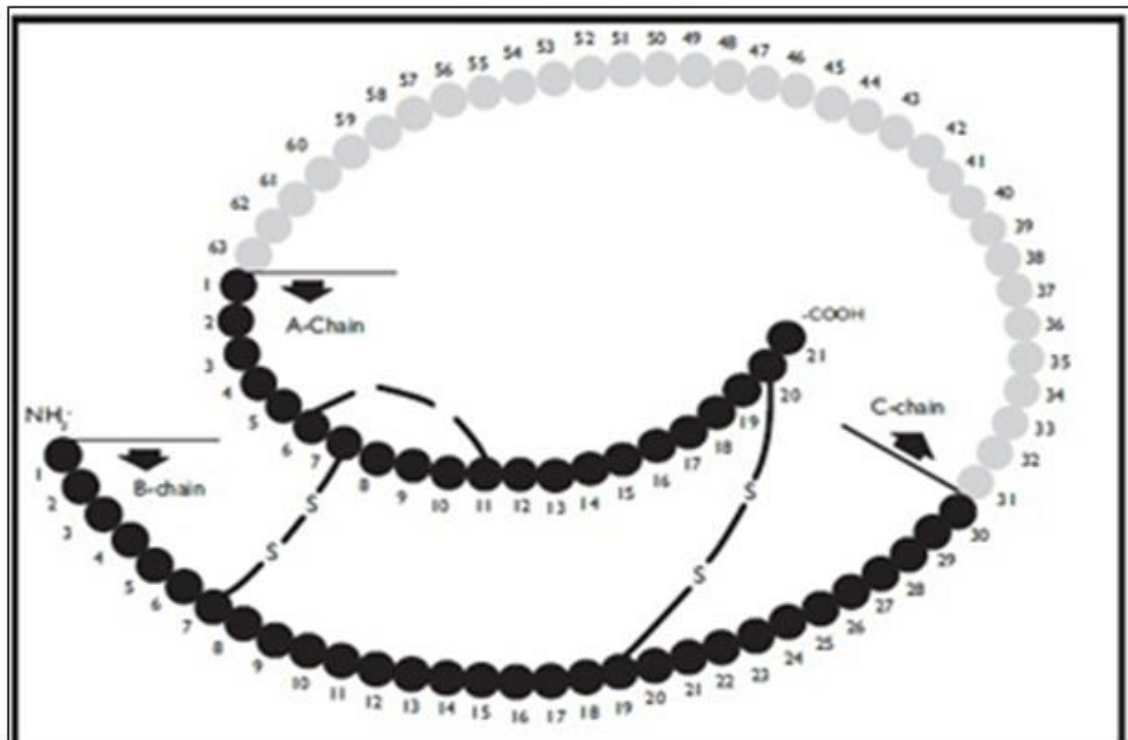


Figure 1. Insulin structure (Joshi et al., 2007).

Insulin as a hormone has many important functions in the body. Thus insulin is keeps normal blood glucose levels by helping cellular glucose uptake. Insulin also plays an important role in carbohydrate metabolism is association with glucagon. Insulin is necessary for carrying glucose in to the cells and for lowering glucose levels in blood stream. Therefor insulin has regulator role on blood sugar level (Wilcox, 2005; Unal et al., 2012). When insulin is not able to do its job, glucose accumulates in blood stream as in the case of individuals with diabetes. Over time, high blood glucose level leads to complications may damages the eyes, kidneys, nerves, heart and blood vessels (Ogden, 2010; Xavier et al., 2014; ADA, 2016).

2.1.4. Insulin resistance

Insulin resistance can be defined as a state of decreased responsiveness to normal levels of circulating insulin (Savage et al., 2005). In insulin resistance, the pancreas keeps making insulin to lower blood glucose, but the body does not respond to the insulin as it should. After years of this situation, the insulin-producing cells in the pancreas often stop working to produce insulin (Ogden, 2010). Insulin resistance plays important roles in the pathogenesis of type 2 diabetes mellitus (Al-Daghri et al., 2016).

2.1.5. Criteria for the diagnosis of diabetes

Diagnosis criteria of DM based on both classic symptoms and laboratory tests according to the American Diabetes Association (ADA, 2016). These define DM patients with classic symptoms as having fasting plasma glucose (FPG) levels of ≥ 126 mg/dL (7 mmol/L); (Fasting=stop caloric intake for at least 8 hrs.), or patients having classic symptoms with two-hour plasma glucose levels of ≥ 200 mg/dL (11 mmol/L) during an oral glucose tolerance test (OGTT) 2 hours after administering glucose orally. This test should be carried out according to WHO protocols using a glucose load of 75 g anhydrous glucose dissolved in water. The glycosylated hemoglobin (HbA1c) test should be performed with HbA1c level $\geq 6.5\%$ is conforming the diagnosis of diabetes (Ogden, 2010; ADA, 2016). In a patient with classic symptoms of hyperglycemia a random plasma glucose level ≥ 200 mg/dL (11.1 mmol/L) is diagnosis for diabetes (ADA, 2016).

2.1.6. The glycohemoglobin (HbA1c) test

Red blood cells express a protein hemoglobin. Hemoglobin carries oxygen from the lungs to all the cells of the body via the heart. Hemoglobin can bind to sugars, such as glucose as a result becoming glycated hemoglobin, or A1c glycohemoglobin, or HbA1c. If there is more glucose in the blood stream, it means that there will be more hemoglobin bind to glucose. Once they are bound together, hemoglobin and glucose stay for a long time throughout the life of the red blood cell. This test gives information about the average level of blood glucose for the previous 2 to 3 months. Thus, this test

measures the amount of glycohemoglobin in red blood cells. HbA1c test is typically used for the diagnosing of diabetes (Ogden, 2010).

2.1.7. Types of diabetes

Type 1 diabetes (T1 DM): This type of diabetes is known as insulin-dependent diabetes mellitus (IDDM) (Ullah et al., 2015). T1 DM accounts for about 5–10% of all diabetes patients (ADA, 2015). It is a chronic disease caused by the autoimmune destruction of beta cells of the Langerhans (Gingras et al., 2015). In this type of diabetes, the pancreas stops making insulin or makes only a small amount of it, blood glucose excessively raises to hyperglycemia and is associated with severe late complications, including end-stage renal failure. T1 DM begins most often in young people under the age of 30 years (Ogden, 2010). T1 DM, however can develop at any age, but is one of the most common chronic diseases of childhood (Forlenza et al., 2015). In T1 DM, pathogenesis involves mostly autoantibodies attack the body's own beta cells of the Langerhans, and destroy these insulin-producing cells and vessels which lead to an absolute lack of endogenous insulin production (Robertson et al., 1992; Ridha and Moog, 2015). T1 DM signs are frequent urination, fatigue, constant hunger thirst, edginess, changes in mood, losing weight, nausea, weakness and vomiting (Ogden, 2010).

Type 2 diabetes mellitus (T2 DM): This type of diabetes is non-insulin dependent diabetes mellitus (NIDDM) (Ullah et al., 2015). The globally-estimated levels of patients affected with T2 DM is about (90-95%) of all cases of diagnosed diabetes (Xavier et al., 2014; Merecz et al., 2015; Hsieh et al., 2016). It is a chronic metabolic disorders characterized by hyperglycemia due to insulin resistance, or insufficient insulin, or both. It has significant effects on health and quality of life of affected patients (Ogden, 2010; Liu et al., 2014; Zemestani et al., 2015). T2 DM is also called adult-onset diabetes, mostly affecting people over 40 years of age, although it can also affect younger people including children (Ogden, 2010; Xavier et al., 2014; ADA, 2016). The beginning of T2 DM or its onset is slow and it is often a symptomatic for a long time with glucose levels raising gradually over time. It is possible that this type of diabetes remains undetected for many years, thus, leading to severe complications (Ballotari et al., 2016). T2 DM signs are frequent urination, constant hunger and thirst,

fatigue, tingling or numb hands, dry feet, itchy skin, blurred vision and weakness (Ogden, 2010).

Gestational diabetes mellitus (GDM): This type of diabetes is diagnosed in the second, or third trimesters of pregnancy due to insulin resistance in pregnant women who do not already have diabetes (Murphy et al., 2016; Rahmannedhad et al., 2016). During that time, the placenta, produces high levels of hormones for the fetus to grow. These hormones block insulin and not let insulin act as it should. This is called insulin resistance. If pregnant women do not make sufficient insulin to overcome insulin resistance then these women develop gestational diabetes (Ogden, 2010). In many populations, more than 8% of pregnant women can be affected by GDM, and is associated with short and long-term negative effects for the mother and the fetus (Capobianco et al., 2016). The fetus will be at risk of macrosomia (Olagbuji et al., 2015), and the mother will be at risk of getting diabetes T2 DM, or obesity in the future (ADA, 2016; Rahmannedhad et al., 2016; Sun and Zhao, 2016; Van Ryswyk et al., 2016). The fetus will be more likely to have hypoglycemia, which is due to fetal hyperinsulinemia, as a results from maternal hyperglycemia (Sugiyama et al., 2014).

Specific types of diabetes: There are other types of diabetes, for example those caused by exocrine pancreas disease, exposure to certain drugs or chemically- induced diabetes. Monogenic defects that cause β -cell dysfunction lead to diabetes such as neonatal diabetes and maturity-onset diabetes of the young (MODY) which is estimated to account for (5%) of all diabetic patients. These types of diabetes are characterized by the onset of hyperglycemia before the age of 25 years. MODY is characterized by impaired insulin secretion with minimum or no defects in insulin action. It is inherited in an autosomal dominant pattern. (ADA, 2016).

2.1.8. Diabetes complications

Diabetes can lead to other diseases and conditions and these are generally referred to as complications. Patient with diabetes are at an all-time on risk of complications due to chronic hyperglycemia with metabolic abnormalities (Ogden, 2010; Johnson et al., 2013; Ullah et al., 2015; Rodríguez et al., 2016). Such complication can affects vessels and there are two types of complication, microvascular

complications (neuropathy, retinopathy, nephropathy) and macrovascular complications which include atherosclerosis leading to strokes, heart attacks and peripheral vascular diseases (Ghule et al., 2012; Koulis et al., 2015; Ullah et al., 2015; Akhavan et al., 2016; Hwang et al., 2016). The microvascular and macrovascular complications of diabetes increase mortality and morbidity in type 1 and type 2 diabetic patients (Koulis et al., 2015; Olamoyegun et al., 2015; Takakura et al., 2016). Generally, complications of DM can be classified into two groups. First metabolic acute complications: these consist of short-term and include, ketoacidosis, hypoglycemia and hyperosmolar non ketonic coma. Second is systemic late complications: and these are long term-chronic complications and includes diabetic nephropathy, neuropathy, retinopathy, atherosclerosis and infections (Ullah et al., 2015).

Metabolic acute complications

Diabetic ketoacidosis (DKA): Is an acute disorder of glucose and lipids metabolism and it is considered the most common acute life-threatening hyperglycemic complication in patients with diabetes (Desse et al., 2015; Salardi et al., 2016). When the body can't get benefits from glucose to use for energy, it starts to burn fat, as a result ketone bodies will be formed (Ogden, 2010; Anderson, 2015).

Diabetic Coma: A diabetic coma is a life-threatening complication that causes unconsciousness. The causes of these conscious disturbances are cerebral dysfunction in association with severe hypertonic dehydration and electrolyte disturbances in patients with DM (Neufang, 2011).

Hypoglycemia: Is a common short-term complication of glucose lowering status in DM patients (Borzi et al., 2016; Samson et al., 2016), which is associated with coma (Reifegerste and Hartleib, 2016). Clinically, severe episodes of hypoglycemia are associated with un-equal increased in plasma insulin concentrations after a loading of glucose (Vaurs et al., 2016). Incidents of hypoglycemia are a fact of life for majority of people with T1 DM and many with advanced T2 DM can sometime be fatal (Cryer, 2007).

Late systemic complications

Cardiovascular disease (CVD): Mainly (coronary heart disease and stroke), is leading causes of disability and death in industrialized countries (Luepker et al., 1994). Diabetes is thought to be a major risk factor for CVD disease (Akhavan et al., 2016) which, in turn, is the main cause of death in people with diabetes (Fowler, 2008; Huang et al., 2010).

Stroke: Stroke is a global health concern and is the leading cause of neurological disability and death worldwide. It has negative physical and psychosocial effects on patients (Shou et al., 2015). When blood flow to the brain is blocked it causes a stroke, since the brain can't get its requirement of oxygen and this leads to damage to parts of the brain. Fat and cholesterol are buildup in the blood vessels leading to blood flow cut off to the brain (atherosclerosis). This is known as ischemic stroke (Ogden, 2010). DM is considered a major risk factor of stroke (Cheng et al., 2014; Lei et al., 2014).

Diabetic retinopathy (DR): DR is a common microvascular complication in patients with diabetes (Fowler, 2008). This causes visual disability and blindness (Huynh et al., 2014; Olamoyegun et al., 2015; Wang et al., 2016). DR is a chronic progressive sight-threatening complication of the retinal microvasculature and neuronal cells linked with prolonged hyperglycemia (Lombardo et al., 2016).

Diabetic nephropathy (DN): Is now the most common cause of end-stage renal failure, and forms a major portion of progressive kidney disease (Huynh et al., 2014; Takakura et al., 2016; Ziqiang et al., 2016). In early-stage DM, hyperglycemia leads to damage to the glomerulus filtration membrane leading to the occurrence of proteinuria. In addition, continually-recurring proteinuria leads to damage to the kidney's structure, and fibrosis (Ziqiang et al., 2016). Serious nephropathy can develop to chronic kidney disease (CKD) and end-stage renal disease. These patients either enter long-term hemodialysis or require renal transplantation (Rodríguez et al., 2016).

Diabetic neuropathy: Diabetic neuropathy is one of the major microvascular complications of types of diabetes one and two. Diabetic neuropathy is associated with vascular and neuronal damage usually peripheral and autonomic, which results in impairment of sensation, movement or impairment of controlling organs and glands. In more severe cases, the complication can result in ulcerations and amputation of limbs (Ahmad et al., 2016; Rodríguez et al., 2016). Diabetic neuropathy can progress to peripheral nerve dysfunction in distal regions such as the hands and feet (Huynh et al., 2014). This is called diabetic peripheral neuropathy (DPN) (Dixit et al., 2015). The cause of DPN is the oxidative stress cycle, which causes damage to nerve cells (Yin et al., 2015).

2.2. 8-Hydroxy-2'-deoxyguanosine (8-OHdG)

8-OH-dG was first reported in 1984 as a major form of oxidative DNA damage product by heated sugar (Kasai, 1997). 8-OHdG is, thus, a products of oxidative DNA damage (Fraga et al., 1990). Pathogenically, oxidative DNA damage is linked to a variety of aging-associated degenerative diseases such as cancer, coronary heart disease and diabetes. Recently, 8-OHdG was identified as a new sensitive biomarker of in vivo oxidative DNA damage in diabetes (Cooke et al., 2001; Kanauchi et al., 2002). 8-OHdG is one of approximately 20 known oxidative DNA damage products, which have been identified in DNA isolated from various organs, such as the liver, brain, kidneys, intestine and testes (Fraga et al., 1990). 8-OHdG is formed by hydroxyl radicals, singlet oxygen and direct photodynamic action. It can be detected in tissues, serum, urine and in other biological fluids (Kasai et al., 1986).

2.2.1. DNA structure

DNA is commonly takes the shape of a right-handed double helix, which consists of two polydeoxynucleotide chains twisted around each other. Every chain is an alternating polymer of deoxyribose sugars and phosphates that are linked together through phosphodiester linkages. One of four bases protrude from each sugar. These bases are cytosine (C), thymine (T), adenine (A) and guanine (G) (Figure 2, 3, 4).

The A and G, known as purines contain two rings, while T and C, named pyrimidines contain only one ring. Each chain has a 5 to 3 polarity with the two chains of the double helix oriented in an antiparallel manner and run in opposite directions. Hydrogen bonds forms the chain and is specific: Adenine of one chain is paired with thymine on the other, whereas guanine is paired with cytosine. This strict base-pairing reflects the fixed locations (Watson et al., 2004).

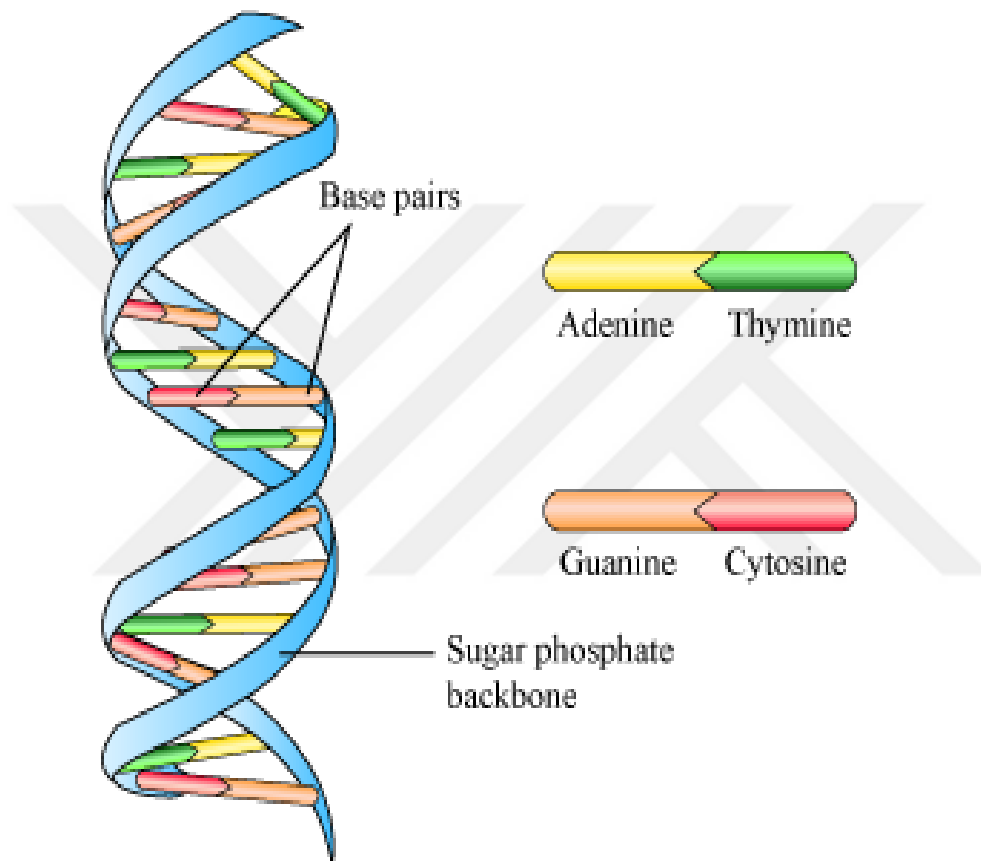


Figure 2. DNA helix shape (Anonym 1).

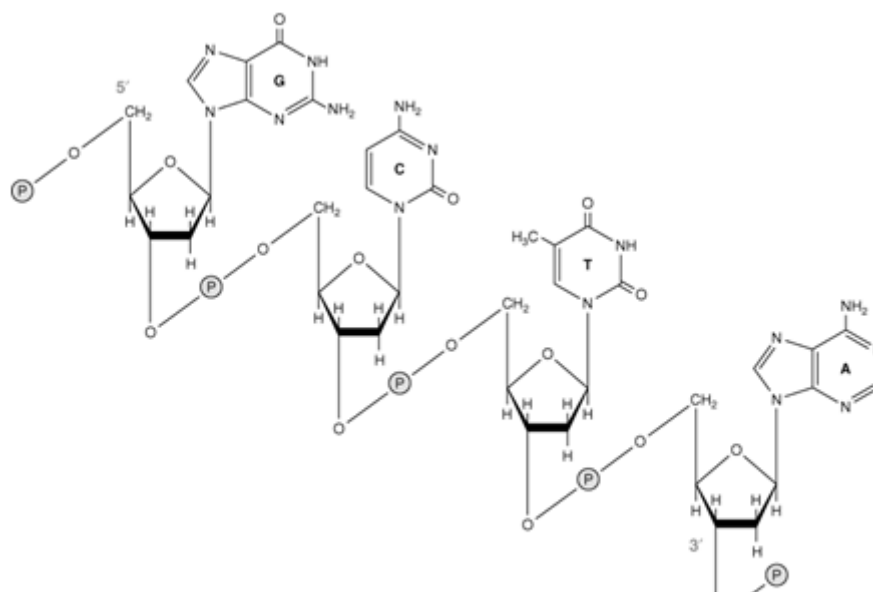


Figure 3. A segment of one strand of a DNA molecule in which the purine and pyrimidine bases guanine(G), cytosine (C), thymine (T), and adenine (A) are held together by a phosphodiester backbone (Watson et al., 2004).

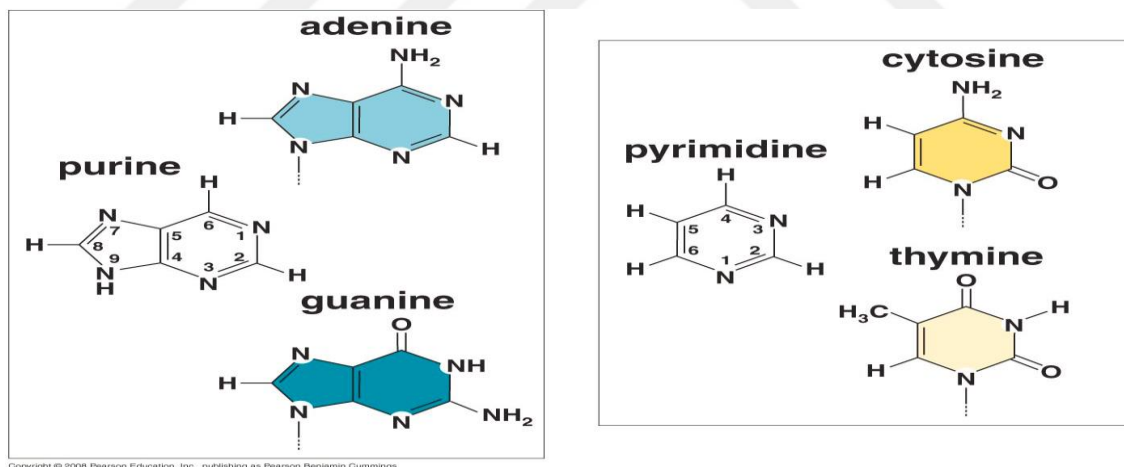


Figure 4. Purines, adenine, guanine, Pyrimidines, cytosine, thymine structures (Watson et al., 2004).

Until now, the majority of research has focused on guanine (G) and 2'-deoxyguanosine (dG); the most easily oxidized of the four DNA bases and the four nucleosides, respectively. Their main oxidation products, 7, 8-dihydro-8-oxoguanine (8-oxoG) and 8-OHdG respectively, are considered to have the possibility to lead to the determination of the degree of oxidative DNA damage (Kelly et al., 2007).

2.2.2. Free radicals

Free radicals are generally unstable, reactive and short-lived chemical species. Holding one or more unpaired electrons (Sanocka and Kurpysz, 2004), elevate the chemical reactivity of these molecules. Free radicals induce damage to cells by passing the unpaired electron onto nearby cellular structures resulting in oxidation of cell components and molecules (Bansal and Bilaspuri, 2010; Ullah et al., 2015). Free radicals have biological roles inside the cell activating different signaling pathways like the mitogen activation protein kinase and extracellular-signal-regulated kinase pathways that change gene expression. In addition, free radicals coordinate with superoxide dismutase (SOD) to induce cell death (Ullah et al., 2015).

Free radical can be expanded with other additional compounds which are not radicals but with oxidant properties. This group include hydrogen peroxide H_2O_2 , ozone O_3 , nitrous acid HNO_2 , etc. despite their lack of an unpaired electron, can react with other molecules to form free radicals and damage the cell (Knak Jensen, 2003; Wojtunik-Kulesza et al., 2016). A major part of ROS are products of the aerobic metabolism in the mitochondria (Knak Jensen, 2003). Free radical's examples are the hydroxyl radical ($\cdot\text{OH}$), superoxide anion ($\text{O}_2^{\cdot-}$), transition metals such as copper and iron, peroxynitrite ($\text{ONOO}^{\cdot}$), and nitric oxide ($\text{NO}^{\cdot}$) (Betteridge, 2000).

Normally, the generation of free radicals is important as they are necessary for host defenses against phagocytic cells with ROS used against invading pathogens. Moreover, they regulate cell growth, apoptosis and differentiation. In contrast to these mentioned roles which are generally good to the host, these molecules are able to oxidase signaling molecules, DNA, macromolecules and cell structures such as lipid membranes of healthy host cells, causing damage to cells (Petersen and Smith, 2016).

2.2.3. Free radicals and DNA-damage in diabetes

Free-radicals have long been believed to be a key element in the cellular dysfunction and destruction that are central to the pathogenesis of many aspects of diabetes. For example, in diabetes type 1, it has been indicated that inflammatory cytokines play a great role in the insulin producing β -cells destruction through

producing oxygen-derived free radicals that cause DNA-damage and apoptosis in β -cells of both rodents and humans. In both T1 DM, and T2 DM diabetes, the role of free radicals in diabetic complications is well established. The most important feature of diabetes, hyperglycemia, is increases free radical production, due to increased mitochondrial oxidative metabolism that is secondary to a high intracellular glucose concentration. Glucose also leads to advanced glycation end products, in turn which initiates free radical cascades and are toxic to cells, including β -cells (Tyrberg et al., 2002).

2.2.4. DNA damage and its causes

DNA damage results in a change in the chemical structure of DNA. Several types of DNA damage exist such as a break in a strand of DNA, or a chemically-changed base (Kumari et al., 2008). Living cells undergo DNA damage as a result of oxidative metabolism, replication errors and exposure to environmental factors such as ultraviolet light, ionizing radiation, chemotherapies and radiation therapies for treatments of cancer (Kai, 2016; Kumari et al., 2008). Many of the risk factors associated with atherogenesis, such as smoking and DM, may directly induce DNA damage. DNA damage can also occur in the mitochondria (Mahmoudi et al., 2006).

2.2.5. The cause of 8-hydroxy-2'-deoxyguanosine formation and its clinical significance

8-OHdG is a common form of free radical stimulated oxidative lesions. Therefore, 8-OHdG has been used to a great degree as a biomarker for oxidative stress and carcinogenesis. Studies have indicated that urinary 8-OHdG is an acceptable biomarker for risk assessment of different cancers and degenerative diseases (Cooke et al., 2008; Valavanidis et al., 2009). 8-OHdG, the biomarker, has been used to estimate the DNA damage in humans after exposure to cancer-causing agents, like tobacco smoke, asbestos fibers, polycyclic aromatic hydrocarbons and heavy metals. Recently, 8-OHdG has been used widely in many fields of studies, not only as a biomarker for estimating of endogenous oxidative DNA damage, but also as a risk factor for other diseases including cancer (Valavanidis et al., 2009). In addition, it has already been proposed that plasma and urinary 8-oxodG can be used as a useful biomarker for

determining of oxidative DNA damage in diabetic patients (Kushwaha et al., 2011). Formation of 8-OHdG in tissue DNA can be used as a marker for the predicting target organ carcinogenesis. In fact, many recent investigations adopted this approach to assess the target organ specificity of carcinogenesis (Nishimura, 2011).

2.2.6. Mechanism of 8-hydroxy-2-deoxyguanosine formation

HO• interact with nucleobases of the DNA strand, like guanine, to form C8-hydroxyguanine (8-OHGua), or its nucleoside form deoxyguanosine (8-hydroxy-2-deoxyguanosine). Firstly, HO• addition leads to the generation of radical adducts, then the 8-OH-dG is formed with abstraction of one electron (Figure 5) (Valavanidis et al., 2009).

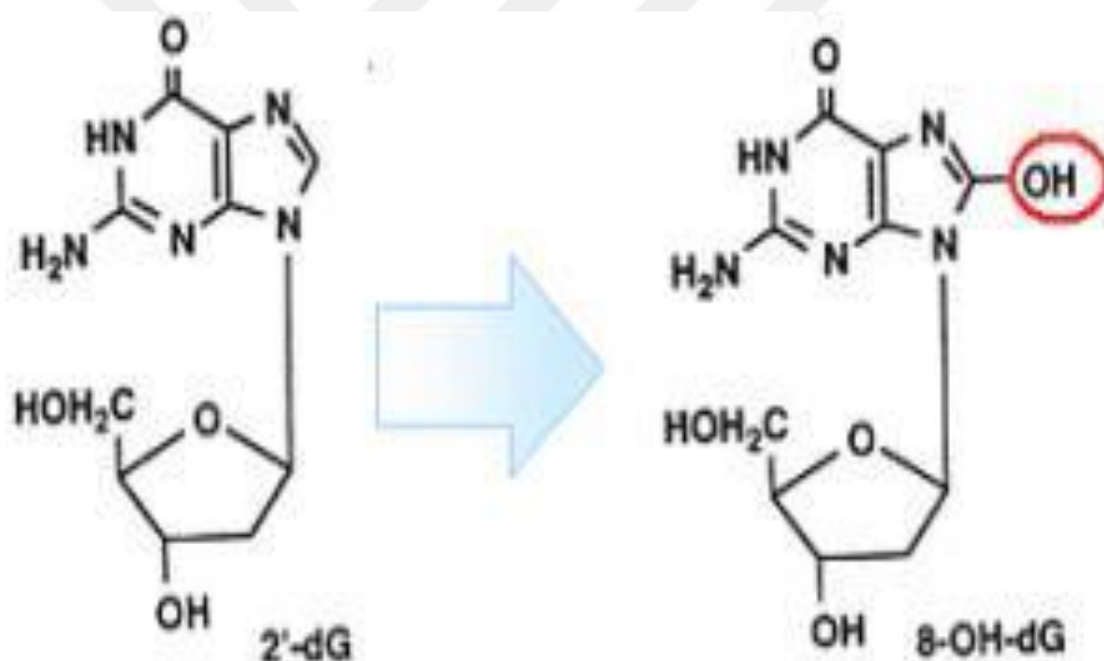


Figure 5. 8-OHdG formation (Valavanidis et al., 2009).

8-OHdG undergoes keto-enol tautomerism, which favors the oxidized product 8-oxo-7, 8-dihydro-2-deoxyguanosine (8-oxodG). 8-OHdG and 8-oxodG are interchangeable names used for the same compound in scientific literature (Valavanidis et al., 2009).

Mutations that may arise due to the formation of 8-oxodG, mostly involves base changes at the GC base pairing, changing the GC to TA base transversion (Halliwell and Aruoma, 1991; Greenberg, 2004; Kamiya et al., 2009; Valavanidis et al., 2009). Reaction of hydroxyl radicals with 2-deoxyguanosine and radical adducts followed by reduction to 7-hydro-8-hydroxy-2-deoxyguanosine and the oxidation to 8-OHdG or 8-oxo-7-hydro-2-deoxyguanosine (8-oxodG) which its tautomer (Figure 6) (Valavanidis et al., 2009).

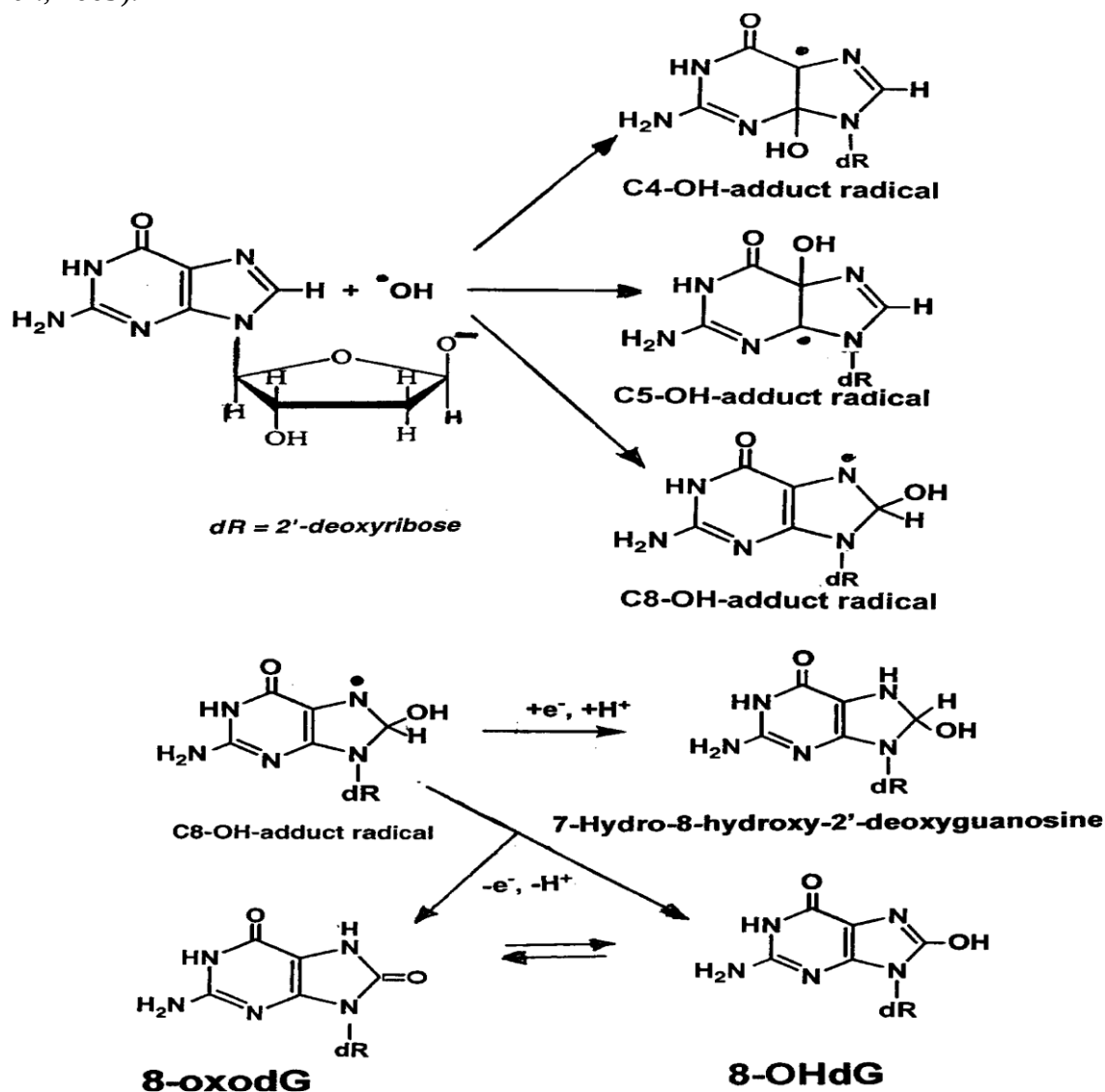


Figure 6. Reaction of 2-deoxyguanosine with hydroxyl radicals, radical adducts followed by reduction to 7-hydro-8-hydroxy-2-deoxyguanosine, and by oxidation to 8-OHdG or its tautomer 8-oxo-7-hydro-2-deoxyguanosine (8-oxodG) (Valavanidis et al., 2009).

2.2.7. Oxidative stress

Oxidative stress is described as the imbalance between the production of reactive oxygen species (ROS; free radicals) and antioxidant defenses. This imbalance causes tissue damages and the products have a role in the development and progressions of many chronic diseases such as diabetes (Betteridge, 2000; Khan et al., 2015). Oxidative stress is associated with an increased rate of cellular damage brought about by oxygen and oxygen derived oxidants ordinarily-known as ROS (Sikka et al., 1995).

ROSs have very important roles in physiology, but high levels can lead to oxidative stress (Radomska-Leśniewska et al., 2016). Uncontrolled production of ROS that exceeds the capacity of antioxidant causes protein oxidation, lipid peroxidation or denaturation, oxidation of nuclear acid and many other macromolecular changes. These lead to serious cellular damage. Indeed ROS-related damage has been identified to occur in many diseases, such as metabolic syndrome, diabetes, cancers, cardiovascular diseases and Alzheimer's disease (Sikka et al., 1995; Bansal and Bilaspuri, 2010; Auberval et al., 2015; Zalewska et al., 2015). Obesity, hyperglycemia and hyperlipidemia are associated with elevated oxidative stress induced by high level production of ROS, which is likely due to increased mitochondrial dysfunction and superoxide production due to fat accumulation (Auberval et al., 2015).

Increased oxidative stress caused by hyperglycemia has a critical role in the pathogenesis of diabetes and its complications (Kushwaha et al., 2011; St, 2012). Oxidative stress is also known to elevate the conversion of deoxy guanosine to 8-oxo, 2-deoxyguanosine in DNA (Hinokio et al., 1999).

2.2.8. Oxidative stress and diabetes

Oxidative stress play a key mediatory role in the development of diabetes, its progression and complications because it increases the production of free radicals and impairs antioxidant defenses (Rolo and Palmeira, 2006; Barathmanikanth et al., 2010; Zephy and Ahmad, 2015). Production of oxidative stress caused by hyperglycemia plays a critical role in the pathogenesis of both types of DM, and its micro and macro

vascular complications (Kushwaha et al., 2011; Pourvali et al., 2016; Toorang et al., 2016).

Oxidative stress is induced by reactive oxygen and nitrogen species produced through several biochemical pathways associated with hyperglycemia (glucose autooxidation, polyol pathway, prostanoid synthesis, and protein glycation end products AGEs, raised protein kinase C activity and over activity of hexosamine pathway). It is critically involved in the impairment of β cell function during the development of T2 DM (Son et al., 2004; Zephy and Ahmad, 2015; Safi et al., 2016). Moreover, many studies have demonstrated that raised oxidative stress can lead to insulin resistance by suppressing insulin signals and adipokines deregulation (Safi et al., 2016).

In T1 DM pathology, oxidative stress has been suggested to play a role in the destruction of β -cells in the islets of Langerhans in the pancreas (Pourvali et al., 2016). In T2 DM contributing factors such as obesity, physical inactivity, and dietary habits are contribute to promote an oxidative stress environment that leads to chronic extracellular hyperglycemia because of insulin resistance, or impaired glucose tolerance (Zephy and Ahmad, 2015).

2.3. Antioxidants

Antioxidant is any substance, present at low levels compared with that of an oxidizable substrate, can significantly delay or suppresses oxidation of that substrate. The antioxidant defense system protects the cell from ROSs toxicity (Betteridge, 2000; Radomska-Leśniewska et al., 2016). Generally, antioxidants are classified into groups depending on their source.

2.3.1. Endogenous antioxidants defense system

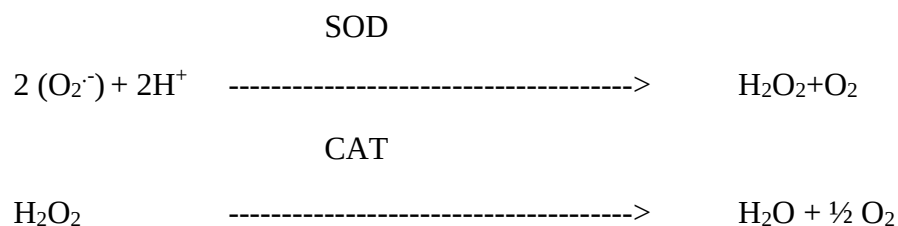
This system consists of enzymatic, known as natural antioxidants, and nonenzymatic antioxidants (Khan et al., 2015; Radomska-Leśniewska et al., 2016). The major classes of antioxidant enzymes are the SOD, catalases CAT and glutathione peroxidase (GPx) and glutathione reductase (GR) hemeoxygenase. Non-enzymatic antioxidants are known as synthetic antioxidants or dietary supplements. Including

vitamins, and minerals such as vitamins A, C, E, zinc, taurine, hypotaurine (Bansal and Bilaspuri, 2010). Glutathione; α -lipoic acid; carotenoids; trace elements like copper, zinc and selenium; coenzyme Q₁₀; and cofactors like folic acid, uric acid, albumin, and vitamins B₁, B₂, B₆ and B₁₂ (Johansen et al., 2005; Radomska-Leśniewska et al., 2016; Pourvali et al., 2016).

2.3.2. Exogenous antioxidants

Obtained through dietary intake, exogenous antioxidants include two groups according to their origin, either nature-identical or synthetic. Natural antioxidants are obtained mainly from plants but also from microorganisms, fungi and animals. Nature-identical antioxidants are substances that are identical to the natural product, but synthesized by they constitute a large group of pure, well-characterized, relatively cheap, available and reproducible substances (Radomska-Leśniewska et al., 2016; Pourvali et al., 2016) ROS can be eliminated by these agents through a number of enzymatic and non-enzymatic antioxidant mechanisms (Bansal and Bilaspuri, 2010).

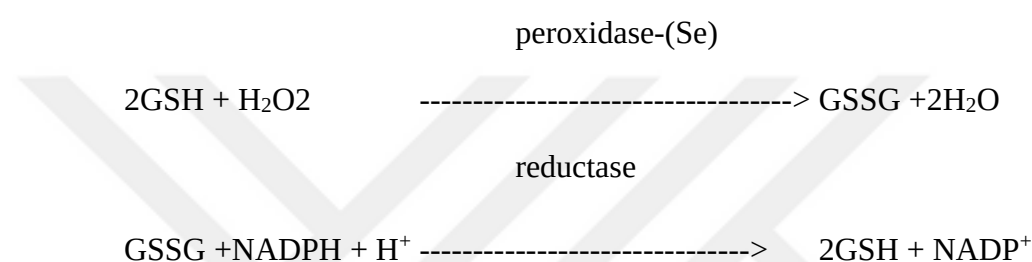
In general, antioxidants are compounds which react with, discard, scavenge, or suppress ROS formation, or act against ROS actions (Sikka et al., 1995). Amongst the fully-known biological antioxidants are those are specific to SOD and its two isozymes and CAT. Spontaneously produced SOD, O₂^{•-} anion to form O₂ and H₂O₂, when CAT that is present in peroxisomes in many tissues convert H₂O₂ to O₂ and H₂O when present in high concentrations.



GPx present in the cytosol and mitochondria have a major role in removing hydrogen peroxide generated by SOD with the oxidation of glutathione (GSH) (Betteridge, 2000). GPx, a selenium-containing antioxidant enzyme with glutathione as

the electron donor, removes peroxy (ROO[•]) radicals from various peroxides including H₂O₂ (Sikka et al., 1995; Johansen et al., 2005).

Another important enzyme is glutathione reductase, which regenerates glutathione from GSSG when glutathione is used as a hydrogen donor by GPx during the elimination of H₂O₂ (Sikka et al., 1995; Johansen et al., 2005). GR then reforms reduced GSH from oxidized GSH (GSSG) as in the following formula (Sikka et al., 1995).



2.3.3. Antioxidants in diabetes

The antioxidant defense system, involved protecting beta cells that produce insulin, is known to be very weak in patient with diabetes. This renders these beta cells highly sensitive to oxidative stress, thus, promoting cell death and disease. The use of exogenous antioxidant compounds has been identified as an effective strategy to delay of diabetes (Auberval et al., 2015). Recently Johansen and colleagues reviewed ROS in diabetes and suggested that these compounds have more than one effects on protein levels and the activity of enzymes, and suggested they further increase oxidative stress by suppressing the natural defense system (Johansen et al., 2005). Reductions in the status of antioxidant system is associated with diabetic oxidative stress and can further increase the damaging effects of free radicals, thus, reducing antioxidants raises diabetes complications (Pourvali et al., 2016).

2.4. *Silybum marianum* L.

The *S. marianum* L. belongs to the Asteraceae family. It is an annual or biannual herbal plant. The common name of *S. marianum* L. is Milk thistle (Abu-Zaiton, 2013; Rady et al., 2014). This plant grows to 5-10-foot-high has big prickly leaves, big purple flowering heads and strong spinose leaf margin. The seeds of Milk thistle contain concentrated extract of *Silymarin* (Bhattacharya, 2011). *Silymarin* has been used for more than 2000 years as a natural remedy for treating hepatitis, cirrhosis and to protect liver from toxic substances. *Silymarin* acts as an anti-oxidative, anti-lipid per-oxidation, anti-fibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating in experimental liver diseases (Ramasamy and Agarwal, 2008). Milk thistle's common name comes from the white markings on the leaves and its milky white sap, used traditionally by nursing mothers to increase milk (Figure 7) (Kaur et al., 2011).



Figure 7. *Silybum marianum* L plant (Anonym 2).

2.4.1. *Silybum marianum* L. component

S. marianum L. contains approximately (65–80%) *Silymarin* flavonolignans (*Silymarin* complex) with small amounts of flavonoids and approximately (20–35%) fatty acids and other polyphenolic compounds (Ramasamy and Agarwal, 2008). The active ingredient in milk thistle is *Silymarin*, which is composed of flavonolignans that include silydianin, silychristin, and silybin (Cavaretta, 2015). The major component of the *silymarin* complex is silybin that is synonymous with silibinin other substances present in lesser amount are flavonolignan 2, 3-dehydrosilybin and flavonoids taxifolin and quercetin (Figure 8) (Svobodová et al., 2007a).

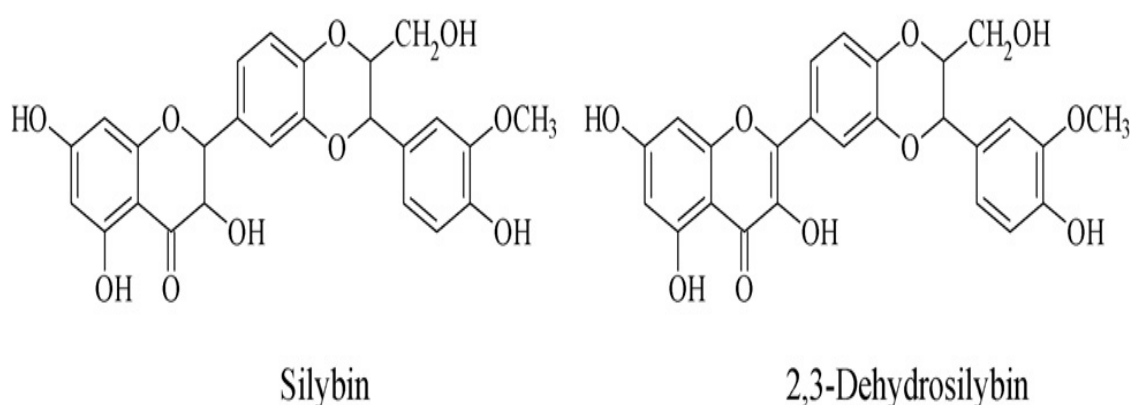


Figure 8. Silybin, and 2, 3- dehydrosilybin structure (Svobodová et al., 2007a).

2.4.2. Biochemical effects of *S. marianum L.*

S. marianum L. has large numbers of effects including antioxidant, anti-inflammation, immunomodulatory, antiviral and hepatoprotective effects. It reduces the incidence of certain forms of cancer (Abu-Zaiton, 2013). It also has other therapeutic properties including antibacterial, antiallergic, antimutagenic, antineoplastic, antithrombotic and vasodilatory actions (Figure 9) (Akkaya and Yilmaz, 2012).

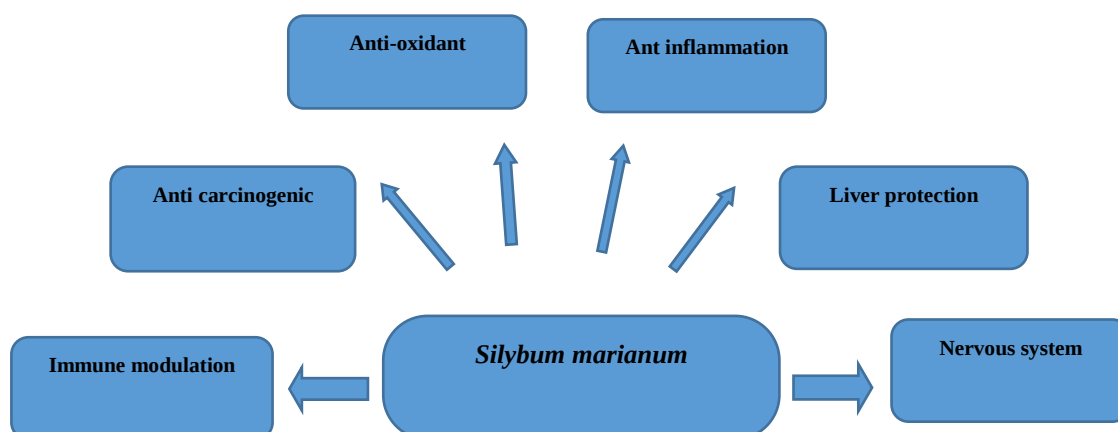


Figure 9. *Silybum marianum L.* functions (Akkaya and Yilmaz, 2012).

S. marianum L. effects as an antioxidant activity helps preventing tissue damage. It scavenges free radicals that damage cells exposed to toxins (Kaur et al., 2011). *S. marianum L.* scavenging free radical is due to the flavonoids, flavonolignans and other polyphenolic components found in its structure (Aslan and Can, 2014). Because *S.*

marianum L. includes high polyphenolic contents it has hepatoprotective effects against pro oxidant products and this leads to a decrease in free radicals (Abu-Zaiton, 2013; Aslan and Can, 2014). It also increases glutathione in the liver and increases the level of antioxidant enzyme SOD in cell cultures. *Silymarin* which is from *S. marianum L.* inhibits the synthesis of leukotrienes (mediators of inflammation, which can result in psoriasis), (Kaur et al., 2011). *S. marianum L.* has membrane-stabilizing and antioxidant activities, thus, can reduce inflammation and have immunomodulatory effects and also has therapeutic benefits in chronic inflammatory hepatic disorders, including hepatitis, and hepatoprotective influences, regeneration (Kaur et al., 2011; Abu-Zaiton, 2013). *Silibinin* produces a significant increase in the formation of ribosomes and DNA synthesis as well as induces liver tissue re-formation by raising the level of protein synthesis in the injured liver. It also inhibits fibrogenesis and is used for the treatment of jaundice, alcohol abuse, fibrosis, cirrhosis in hepatotoxicity by mushroom poisoning and by industrial pollutions (Bhattacharya 2011). The plant can also reduce incidence of diabetes. Importantly, in clinical studies *S. marianum L.* extracts did not cause any significant side effect (Soto et al., 2003; Bhattacharya, 2011; Abu-Zaiton, 2013; Aslan and Can, 2014; Rady et al., 2014; Ajiboye et al., 2015).

Silymarin the active extract from the seeds of *S. marianum L.* can also regulates and coordinates the expression of various genes involved in inflammation, cell survival, differentiation and growth (Ramasamy and Agarwal, 2008). A study by Kaur and colleagues demonstrated that *S. marianum L.* enhances immunity defence in vitro. These effects of *S. marianum L.* are associated with an increase in interferon gamma, interleukin (IL)-4 and IL-10 cytokines production (Kaur et al., 2011).

Silymarin and *silibinin* exert anti-oxidant activity and assist redox homeostasis in various in vitro and in vivo models. *Silymarin* raises the activities of antioxidant enzymes like CAT, SOD, GPx, GR, and glutathione S transferase(GST), with reduction of malondialdehyde (MDA) level, a marker of lipid peroxidation (Ramasamy and Agarwal, 2008).

In addition to the above cited therapeutic effects of *S. marianum L.*, it can also reduce the incidence of certain cancer (Abu-Zaiton, 2013). A study carried out by Aslan and Can suggested that *S. marianum* stops the reproduction of ovary and breast cancer

cells (Aslan and Can, 2014). *Silymarin* and/or *silibinin* modulate the lack of balance between apoptosis and cell survival via interference with the expressions of cell cycle regulators and proteins required for apoptosis (Ramasamy and Agarwal, 2008).

According to the further studies carried out *Silymarin*, it has been shown to be capable of suppressing the proliferation of tumor cells in various cancers including prostate, ovarian, breast, lung, skin, and bladder. Many reports state have suggested that *silymarin* suppresses proliferation of cells by inhibiting cell cycle progression at different stages of the cycle. Further studies demonstrated that *silymarin* has anti-angiogenic activity, which is one of the essential approaches for treating various cancer through its anti-metastatic activity. Cancer metastasis, a primary cause of cancer deaths and currently a key approach for the clinical management of cancer (Ramasamy and Agarwal, 2008; Bhattacharya, 2011). *S. marianum L.* can reduce inflamed gall bladder while at the same time clearing out inactivity that might be present (Kaur et al., 2011). Some components of *S. marianum L.* silybin and 2, 3-dehydrosilybin have chemo preventive effects and the ability to moderate UVA-induced damage. A number of studies have shown the two compounds have chemopreventive effects against UVB-induced photocarcinogenesis in animal models. Furthermore, silybin has also been shown to suppress UVB damage in human keratinocytes. *S. marianum L.*, its flavonolignans silybin and 2,3-dehydrosilybin have also shown to prevent hydrogen peroxide-induced oxidative stress in human keratinocytes (Svobodová et al., 2007b).

S. marianum L. has a long history of having phototherapy activities used as hepatoprotectant. Indeed, the compound is currently used as herbal product for treating patients with chronic liver disease. Several in vitro studies have highlighted the bioactivity of this plant as anti-inflammatory and antiviral agent acting against hepatitis C virus (HCV) (Calani et al., 2012).

A results by Toklu and colleagues (Toklu et al., 2007) demonstrated that burn-induced skin damage is a caused by oxidative injury and that damage can be improved by treatment of silymarin. Furthermore, the morphological changes in the injured skin resulting from the activity of thromboplastin and the burn trauma were improved after treatment of *silymarin*. *Silymarin* decreased lactate dehydrogenase LDH and the proinflammatory cytokine TNF- α in serum levels (Toklu et al., 2007).

2.4.3. *S. marianum* L. effects on diabetes

S. marianum L. extract demonstrated remarkable anti-diabetic activities, it has a long folk history in controlling disease in diabetic patients (Abu-Zaiton, 2013). In general *S. marianum* plants have a long established history of having antioxidant properties, and used in the control of oxidative metabolic disturbance in diabetes (Shakeel and Yar, 2014). Oxidative stresses are increasingly implicated in the pathogenesis of diabetic complications which may either cause direct pancreatic β -cell damage or lead to metabolic abnormalities that can induce diabetes. The effect of antioxidant nutrients on glycemic control in diabetic patients has been reported in experimental and clinical studies (Huseini et al., 2006).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Experimental animals

A total of 28 male rats of 7 weeks' old weight 250-300 gram were obtained from Research Animal Unit of Yuzuncu Yil University Faculty of Medicine. They divided into four groups (7 rats per cage): control group (C), diabetes group (D), healthy group with *S. marianum* extract given (S), diabetes group with *S. marianum L.* extract given (DS). All animals were housed in cages with free access to drinking water and standard diet, under controlled conditions of humidity (50%), lighting (12 h light/dark cycle) and temperature (22±2°C).

This method was accepted by Experimental Animal Committee belonging to Yuzuncu Yil University (Order Number: 2016/01 Date: 28.01.2016). This research accepted by Yuzuncu Yil University Presidency of Scientific Research Projects (TYL-2016-5107).

3.1.2. Equipment and apparatus

Automatic micropipette (Socorex)

Biochemical auto analyses (Architect Plus, Ci16200, by Abbott USA)

Blue, and yellow pipette tips

Centrifuge (Universal 320 R)

Centrifuge tubes

Cylinder

ELISA (Anthos- Zenyth-200 rt)

Eppendorf tube

Falcon tubes 15 ml

Freezer (Arçelik)

Gavages,

Gloves

Glucometer and strips (eBsensor Taiwan),

Normal and insulin injector syringe

Refrigerator (Arçelik Turkey)
Sensitive balance (Bosch S 2000)
Spectrophotometer (Boaco S-22 UV/VIS)
Tubes (EDTA, Gel)
Vortex (Mixer VM20)

3.1.3. Chemical agents

Alfamine (Ketamine HCl)
ALT kit (Roche Germany)
AST kit (Roche Germany)
Blood glucose kit (Roche. Germany)
Citrate buffer (Merck Turkey)
HDL kit (Roche Germany)
HbA1c measurement kit (Roche. Germany)
LDL kit (Roche Germany)
Olive oil (Taris, Turkey)
S. marianum L. extract (Milk thistle 100 mg, Silymarin 80 mg, Solgar, USA)
Streptozotocin (Sigma, USA)
Total antioxidant statues kit (Rel assay, Turkey)
Total oxidant statues kit (Rel assay, Turkey)
Total cholesterol kit (Roche Germany)
Triglycerides kit (Roche Germany)
8-Hydroxy 2-deoxyguanosine kit (Rel assay, Turkey).

3.2. Methods

3.2.1. Experimental animal design

A total of 28 male rats weighting (250 - 300 g) were quarantined for 7 days and had access to normal basal diet. Following quarantine, at 7 weeks of age, all animals were randomly distributed into four groups (7 rats per group).

Control group (C): This group is containing 7 healthy male rats, during the period of the study were given standard diet and water. In the morning at 8:00 AM the foods were collected from rats, at 10:00 AM after fasting of two hours the rats were given 1 mL olive oil using intragastric gavage.

Diabetes group (D): This group including 7 male rats were exposed to single dose of 45 mg/kg of streptozotocin in cold citrate buffer, intraperitoneally injected, after 72 hours, random blood glucose measured by glucometer (eBsensor, Taiwan). The result of more than 270 mg/dL for blood glucose considered as diabetes, this group were given standard diet and water only (Sajedianfard et al., 2013).

***S. marianum L.* extract given (S):** This group is including 7 healthy male rats, during the two weeks of the study the rats were given standard diet and water, during this period of the study the diets were collected from rats at 8:00 AM every day, and at 10.00 AM the rats were given *S marianum L.* extract (Milk thistle 100 g silymarin 80 mg, Solgar, USA, 80%), 200 mg/kg dissolved in 1 mL olive oil using intragastric gavage daily (Sajedianfard et al., 2013).

Diabetes group and *S marianum* given (DS): This group including 7 male rats were induced diabetes by intraperitoneally injecting single dose of 45/mg/kg of streptozotocine (Sigma, Germany) dissolved in citrate buffer. After 72 hours the blood glucose for rats (blood from tail puncture has been measured) were using glucometer (eBsensor, Taiwan) the result of blood glucose more than 270 mg/dL considered as diabetes (Sajedianfard et al., 2013). During the two weeks of the study the rats were given standard diet and water, during this period every day in the morning at 8:00 AM the foods were collected from rats, and at 10:00 AM the rats were given *S marianum L.* extract (Milk thistle 100 g silymarin 80 mg, Solgar, USA, 80%), 200 mg/kg dissolved in 1 mL olive oil using intragastric gavage daily (Sajedianfard et al., 2013).

3.2.2. Blood samples collection

After 14 days of the experimental periods treatment , under anesthetic (Alfamine) condition blood was taken from heart into both vacutainer tubes gel tube with EDTA tube 2 mL of drawn blood collected in EDTA tube to be used for HbA1c assay, and the remaining (5 mL) of the drawn blood was collected into another vacutainer tubes with gel and centrifuged to (3000 rpm in 4°C) for ten minutes to separate serum, the serum were transferred to eppendorf tubes for biochemistry testes, TAS, and TOS all samples were stored at -20 °C prior to analysis and for 8-hydroxy 2-deoxyguanosine test serum stored a -80°C prior to analysis.

3.2.3. Biochemistry routine assay determination in serum

Biochemistry analysis in serum were performed for glucose, high density lipoprotein HDL-cholesterol, very low density lipoprotein VLDL- cholesterol, low density lipoprotein LDL-cholesterol, total cholesterol, triglycerides, alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

Blood glucose determination: Blood glucose level was determined by using automatic biochemical analyzer (Architect Plus, ci16200, by Abbott Company, USA).

Principle of test: The principle of determination of blood glucose based on the enzymatic method. Glucose is phosphorylated by hexokinase in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphatedehydrogenase (G-6-PDH) specifically oxidizes G-6-P to 6-phosphogluconate with the helps reduction of nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide reduced (NADH). One micromole of NADH is produced for each micromole of glucose consumed. The NADH can be detected.

Total cholesterol level determination: Total cholesterol level was determined by using automatic biochemical analyzer (ARCHITECT PLUS, ci16200, by Abbott Company, USA).

Principle of test: Homogenous enzymatic colorimetric. Cholesterol esters hydrolyzed into cholesterol, and free fatty acid enzymatically then free cholesterol oxidized with cholesterol oxidase to cholest-4-ene-3-one and hydrogen peroxide. The hydrogen peroxide combines with hydroxybenzoic acid and 4-aminoantipyrine to form a chromophore (Quinoneimine dye) which is quantitated which is directly proportional to cholesterol level.

Triglyceride level determination: Triglyceride level was determined by using automatic biochemical analyzer (Architect Plus, ci16200, by Abbott Company, USA).

Principle of test: Enzymatic colorimetric. First triglyceride hydrolyzed into yield glycerol in presence of lipase after several steps. Quinoneimmune dye will be formed its intensity proportional to triglyceride level.

HDL-cholesterol level determination: HDL-cholesterol level was determined by using automatic biochemical analyzer (Architect Plus, ci16200, by Abbott Company, USA).

Principle of test: Homogenous enzymatic colorimetric first step elimination of all other lipoprotein, and dissolving HDL-cholesterol selectively using a specific detergent. And chromogenic coupler to develop purple blue color for the quantitative determination of HDL.

The VLDL-cholesterol and LDL-cholesterol levels estimation: The VLDL-cholesterol and LDL-cholesterol levels were estimated by calculation, according to the methods described by Fried Ewald by the following formula: $VLDL = \text{triglyceride}/5$, and $LDL = \text{total cholesterol} - \text{HDL cholesterol} - VLDL$ (El-Far et al., 2015).

AST level determination: AST level was determined by using automatic biochemical analyzer (Architect Plus, ci16200, by Abbott Company, USA).

Principle of test: The test principle enzymatic AST present in the sample catalyzes transfer of the amino group from L-aspartate to 2-oxoglutarate, in the presence of pyridoxal-5'-Phosphate, forming oxaloacetate and L-glutamate. Oxaloacetate in the

presence of NADH and malate dehydrogenase (MDH) is reduced to *L*-malate. In this reaction, the NADH is oxidized to NAD. Which gives AST level.

ALT level determination: ALT level was determined by using automatic biochemical analyzer (Architect Plus, ci16200, by Abbott Company, USA).

Principle of test: The test principle enzymatic ALT present in the sample catalyzes transfer of the amino group from *L*-alanine to α -ketoglutarate, forming pyruvate and *L*-glutamate pyruvate in the presence of NADH and lactate dehydrogenase (LD) is reduced to *L*-lactate. In this reaction NADH is oxidized to NAD. Which gives ALT level.

3.2.4. Glycohemoglobin (HbA1c) level determination in whole blood

HbA1c determined from blood in EDTA tube at same day using (cobas C311) auto analyzer supplied by (Roche, Germany).

Principle of test: Principle is Turbidimetric inhibition immunoassay method HbA1c react with anti HbA1c form antigen-antibody complexes, the second antibody (polyhaptens) react with anti-HbA1c antibody form antibody-polyhaptens complex which can be measured.

3.2.5. Total oxidant capacity (TOS) determination in serum

TOS activity has been determined, in serum by commercial kit (Ref; RL024, lot number; AL150570) from (Rel assay Turkey) using ELISA.

Principle of test: In the sample the ferrous ion–chelator complex oxidizes to ferric ion by oxidants which present in the sample. The oxidation reaction is prolonged by enhancer molecules, which are present in the reaction medium abundantly. a colored complex with chromogen will be formed in an acidic medium by The ferric ion. The color intensity, measured by ELISA, is represent the total amount of oxidant molecules found in the sample. The assay is calibrated with hydrogen peroxide H_2O_2 and the results are given in micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol } H_2O_2 \text{ equiv/L}$).

Procedure of assay: First of all, 200 microliters of reagent 1 placed in ELISA plate well and 30 microliter of the standard (or sample) added to well. The initial absorbance was read at 530 nm for the first absorbance point. Later 10 microliters of reagent 2 add to the well and incubated 10 mint at room temperature. Then read the absorbance a second time at 530 nm.

Calculating the results

Result = (Δ Absorbance sample / Δ Absorbance standard) X 10 (Standard concentration)

Sample absorbance = (Second absorbance of sample - First absorbance of sample)

Absorbance standard = (Second absorbance of standard - First absorbance of standard)

Standard concentration = 10 μ mol /L.

3.2.6. Total antioxidant capacity (TAS) determination in serum

TAS, activity has been determined, in serum by commercial kit (REF; RF0017, Lot number; MH15050A) from (Rel assay Turkey) using ELISA.

Principle of test: Antioxidants in the sample reduce dark blue-green colored ABTS radical to the colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution, which is traditionally named as Trolox equivalent that is a vitamin E analog.

Procedure: Place 200 microliter reagent 1 in cell and add 12 microliter standard (or sample). Read the initial absorbance at 660 nm for the first absorbance point. Add 30 microliter reagent 2 to the cell and incubate 10 min at room temperature or 5 min at 37°C read the absorbance a second time at 660 nm. Calculating the results.

Calculating the Results

Result = [(Δ Abs H₂O) - (Δ Abs sample)] / [(Δ Abs H₂O) - (Δ Abs Std)]

Δ AbsH₂O= (Second Abs - First Abs)

Δ Abs standard = (Second Abs Std - First absorbance Std)

Δ Sample absorbance = (Second Abs of sample- First Abs of sample)

Unit = μ mol/L

3.2.7. 8 -Hydroxy-2-deoxy guanosine (8-OHdG) determination in serum

Rat 8-OHdG ELISA kit (REF; E20160415006, lot number; 20160415) using ELISA.

Test principle: Uses competitive method for 8-OHdG detection in sample. The microtiter plate provided in this kit has been pre-coated with antibody. Then add biotinylated antigen incubate for one hour 37°C. During the reaction, 8-OHdG in the sample or standard competes with a fixed amount of 8-OHdG on the solid phase supporter for sites on the biotinylated detection Ab specific to 8-OHdG to form immune complex. Excess conjugate and unbound sample or standard are washed from the plate by the unbound biotinylated antigen. Then add avidin HRP and incubate for another 1 hour 37°C to conjugate HRP after washing catalyzes TMB (tetramethyl benzidine) to be blue and change color to be yellow with acid affect it has absorbance peak under 450nm wavelength. The concentration of 8-OHdG in the samples is then determined by comparing the optical density OD of the samples to the standard curve. The absorbance has the negative correlation with concentration of antigen in the sample.

Procedure:

1. 50 µL of the prepared samples and standards were added to the wells.
2. Add 50 µL working solution of biotinylated antigen to each well. Cover with the plate sealer provided. Gently tap the plate to ensure thorough mixing. Incubate for 60 minutes at 37°C.
3. Wash: Aspirate each well and wash, repeating the process 5 times wash by filling each well with wash buffer.
4. HRP conjugate: Add 50 µL of HRP to the standard and sample well. Cover with a new plate sealer. Incubate for 60 minutes at 37°C.
5. Second wash: Repeat wash process for five times.
6. Coloration: Add 50 µL of colored substrate A to each well then add 50 µL of colored substrate B sway and mix. Protect from light for about 10 minutes for coloration.
7. Stop: Add 50 µL of stop solution to each well. Color turn from blue to yellow immediately.

8. OD measurement: Determine the (OD value) of each well, under 450 nm. Make measurement within 10 minutes after feeding the stop solution.

9. Standard curve according to the optical density of the standard 8- OHdG were calculated as follows: This curve was determined by 8- OHdG concentrations in the samples of formula, (Figure10).

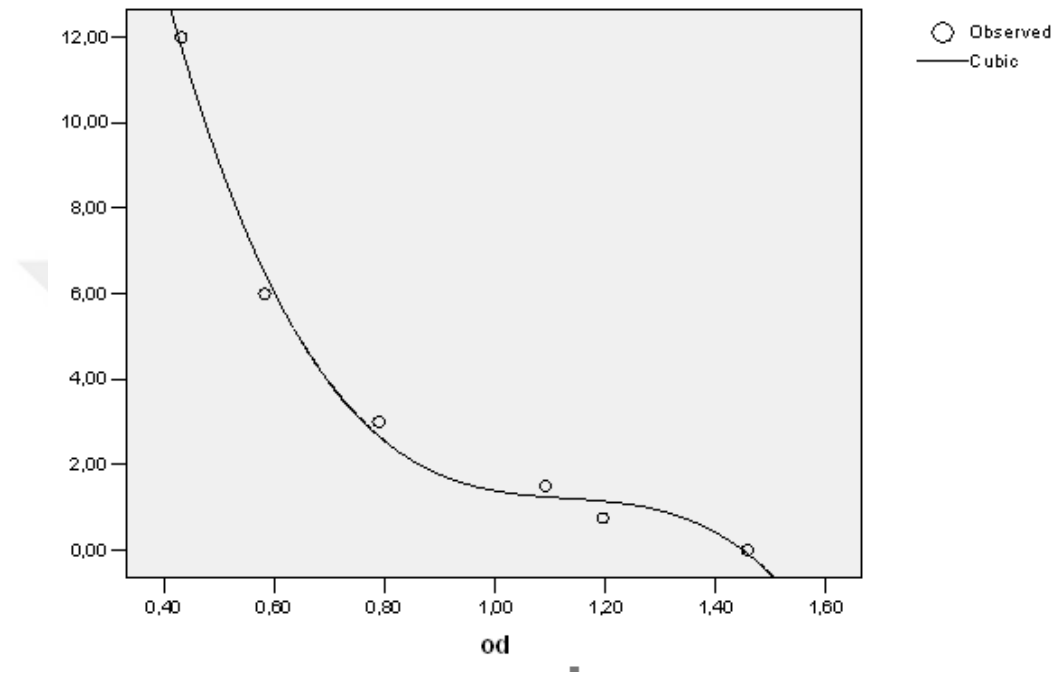


Figure 10. Standard curve according to the optical density of the standard 8-OHdG

3.3. Statistical Analysis

The results obtained at the end of this study; control, diabetes, *S. marianum*, and diabetes + *S. marianum*. All data of group was evaluated with SPSS (13) software package, nearly tests. Raw values obtained from the analysis, were presented as mean $\pm$ standard error (SE) of the group. For the statistical significance the level of 5% was used.

4. RESULTS

In this study, the effect of *S. marianum* L. extract on the level of glucose, total cholesterol, triglyceride, HDL- cholesterol, LDL- cholesterol, VLDL- cholesterol, AST, ALT were studied in serum of experimental groups, and (%HbA1c) studied in whole blood of experimental, also its effect on 8-OHdG, TAS, and TOS activity were studied in serum of experimental groups, all results were shown in Table 1.

Table 1. The level of biochemistry routine assay, HbA1c, 8-OHdG, TAS, TOS in study groups

	Control (C) (X±SE) N=7	Diabetes (D) (X±SE) N = 7	<i>S. marianum</i>(S) (X±SE) N=7	Diabetes + <i>S. marianum</i>(DS) (X±SE) N=7
Glucose mg/dL	177±17 a*	645±23 b	213±18 a	376±50 c
HbA1c %	3.90±0.04 a	7.83±0.23 b	3.98±0.04 a	6.35±0.33 c
Total Cholesterol mg/dL	53.0±1.1 ac	75.6±3.6 b	47.9±1.1 c	54.9±1.2 a
Triglyceride mg/dL	78.9±6.8 a	105.6±8.6 b	49.9±5.4 c	49.3±2.3 c
HDL- Cholest. mg/dL	24.5± 0.8 a	27.6± 0.6 b	24.4±1.2 a	27.9±0.5 b
LDL- Cholest. mg/dL	12.7±2.0 a	26.8±3.7 b	13.5±1.8 a	17.1 ±1.0 a
VLDL-Cholest. mg/dL	15.8±1.4 a	21.1±1.7 b	10.0±1.1 c	9.9±0.5 c
ALT U/L	34.0±1.0 a	1148.0±80.0 b	38.0±1.0 a	139.0±46.0 a
AST U/L	125±9.1 a	1985±247.0 b	129±7 a	264±36.3 a
Tot. Oxidant Status µmol /L	5.480±0.305 a	8.058±0.886 b	3.690±0.147 c	4.818±0.435 ac
Tot. Antioxidant Status µmol /L	0.768±0.046 a	0.581±0.032 b	0.793±0.038 a	0.857±0.030 a
8-OHdG ng/dL	15.53±0.86 a	19.33±1.87 b	15.75±0.61 a	16.41±0.57 ab

*Different letters between the lines is statistically significant of ($p<0.01$) for all tests except only 8-OHdG different letters between the lines is statistically significant of ($p<0.05$) for 8-OHdG.

X=mean, SE=Standard error

As showed in (Table 1 and Figure 11) serum glucose levels were 177 ± 17 , 645 ± 23 , 213 ± 18 , 213 ± 18 for control group (C), diabetes group (D), *S. marianum* group (S), and diabetes+ *S. marianum* group (DS) respectively. Serum glucose levels were significantly increased ($p<0.01$) in the D group when compared to C group. Glucose levels in DS group is decreased significantly ($p<0.01$) compared to D groups. No significant difference ($p>0.01$) can be seen in glucose level between C group and (S) group.

In (Table 1 and Fig 12) showed that the (%HbA1c) levels in whole blood were 3.90 ± 0.04 , 7.83 ± 0.23 , 3.98 ± 0.04 , 6.35 ± 0.33 for C group, D group, S group, and DS group respectively. HbA1c level in D group was increased significantly ($p<0.01$), compared to C group, (%HbA1c) levels showed significantly decrease ($p<0.01$) in DS group, compared to D group (%HbA1c) There was no significant difference ($p<0.01$) between control group and S group in comparison.

Cholesterol levels in serum (Table 1 and Fig 13) were 53.0 ± 1.1 , 75.6 ± 3.6 , 47.9 ± 1.1 , 54.9 ± 1.2 for C group, D group, S group, and DS group respectively. Cholesterol levels in D group significantly ($p<0.01$) increased compared to C group. Cholesterol levels in DS group at dose 200 mg/kg decreased significantly ($p<0.01$), compared to the D group. The cholesterol levels in S group decreased, but not significant ($p>0.01$) compared to C group.

Triglyceride levels in serum (Table 1 and Fig 14) were 78.9 ± 6.8 , 105.6 ± 8.6 , 49.9 ± 5.4 , 49.3 ± 2.3 for C group, D group, S group, and DS group respectively. Triglyceride level in D group increased significantly ($p<0.01$) compared to the C group. The level of triglyceride in DS group decreases significantly ($p<0.01$), compared to the D group, and the level of triglyceride in S group decreases significantly ($p<0.01$), compared to the C group.

HDL-cholesterol levels in serum (Table 1 and Fig 15) were 24.5 ± 0.8 , 27.6 ± 0.6 , 24.4 ± 1.2 , 27.9 ± 0.5 for C group, D group, S group, and DS group respectively. HDL-cholesterol levels in D group increased significantly ($p<0.01$) compared to the C group. The level of HDL-cholesterol in DS group increased but no significantly ($p<0.01$), compared to the D group, and there was no significant difference between C group, and S group.

LDL-cholesterol levels in serum (Table 1 and Fig 16) were 12.7 ± 2.0 , 26.8 ± 3.7 , 13.5 ± 1.8 , 17.1 ± 1.0 for C group, D group, S group, and DS group respectively. LDL-cholesterol levels in D group increased significantly ($p < 0.01$) compared to the C group. The level of LDL-cholesterol in DS group decreases significantly ($p < 0.01$), compared to the D group, and there was no significant difference of level LDL-cholesterol between the S group, and C group.

VLDL level in serum (Table 1 and Fig 17) were 15.8 ± 1.4 , 21.1 ± 1.7 , 10.0 ± 1.1 , and 9.9 ± 0.5 for each group of C group, D group, S group, and DS group respectively. VLDL-cholesterol levels in D group increased significantly ($p < 0.01$) compared to the C group. The level of VLDL-cholesterol in DS group decreases significantly ($p < 0.01$), compared to the D group, and there were significant differences ($p < 0.01$) of VLDL-cholesterol level between the S group, and C group.

ALT levels in serum has been showed in (Table 1 and Fig 18) were 34.0 ± 1.0 , 1148.0 ± 80 , 38.0 ± 1.0 , 139.0 ± 46.0 for C group, D group, S group, and DS group respectively. ALT level D group was increased significantly ($p < 0.01$), compared to C group, ALT levels showed significantly decrease ($p < 0.01$) in DS group, compared to D group ALT There was no significant difference ($p > 0.01$) between control group and S group.

AST levels in serum has been showed in (Table 1 and Fig 19) were 124.7 ± 9.1 , 1984.6 ± 247 , 129.4 ± 7 , 264.1 ± 36.3 for C group, D group, S group, and DS group respectively. AST activity increased significantly ($p < 0.01$) in the D group compared to C group, while the DS group noticed significantly decrease ($p < 0.01$) in level of AST compared to D group. also there was no significant ($p > 0.01$) difference between healthy S group treated with *S. marianum* extract and the C group ($p > 0.01$).

TOS levels in serum has been showed in (Table 1 and Fig 20) were 5.480 ± 0.305 , 8.058 ± 0.886 , 3.690 ± 0.147 , 4.818 ± 0.435 for C group, D group, S group, and DS group respectively. In this study TOS level increased significantly ($p < 0.01$) in the D group compared to C group, while noticed in the DS group level of TOS significantly decreased ($p < 0.01$) when compared to D group. There was significant ($p < 0.01$) difference between S group and the C group.

TAS levels in serum has been showed in (Table 1 and Fig 21) were 0.768 ± 0.046 , 0.581 ± 0.032 , 0.793 ± 0.038 , 0.857 ± 0.030 for C group, D group, S group, and DS group respectively. TAS level decreased significantly ($p < 0.01$) in the D group compared to C group, while noticed the level of TAS significantly increased ($p < 0.01$) in DS group when compared to D group. There was no significant ($p > 0.01$) difference between S group and the C group.

8-OHdG levels in serum has been showed in (Table 1 and Fig 22) were 15.53 ± 0.86 , 19.33 ± 1.87 , 15.75 ± 0.61 , 16.41 ± 0.57 for C group, D group, S group, and DS group respectively. 8-OHdG activity increased significantly ($p < 0.05$) in the in D group compared to C group, there was slightly decrease in 8-OHdG level in the DS group, but not significant ($p > 0.05$) compared to D group. There was no difference between C group and S group.

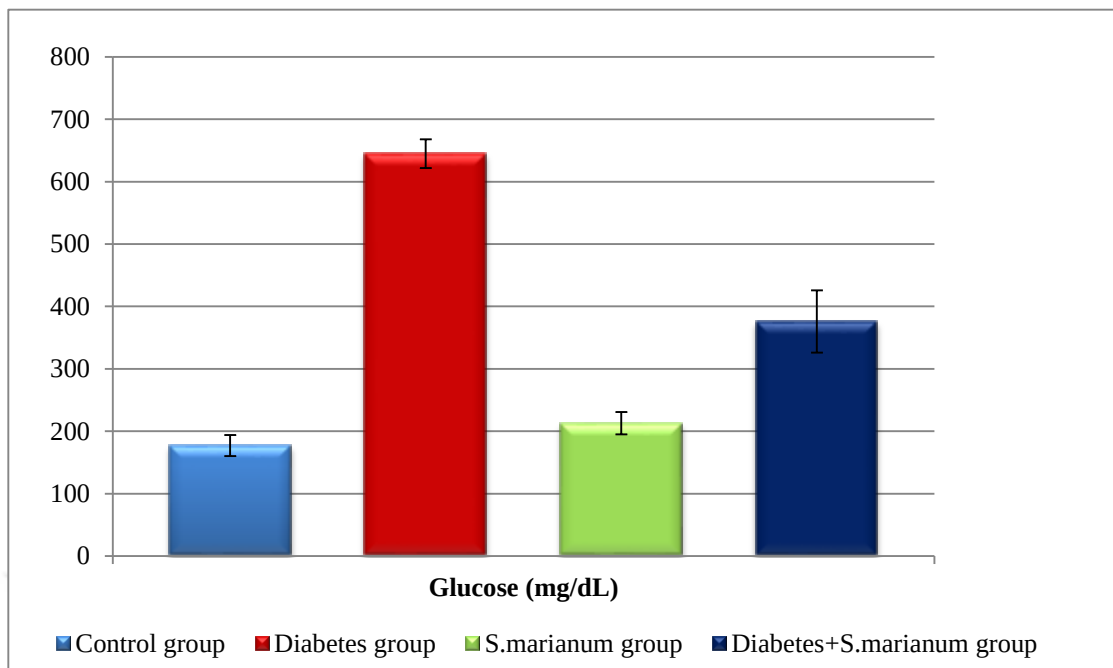


Figure 11. The average of serum glucose level (mg/dL) in study groups

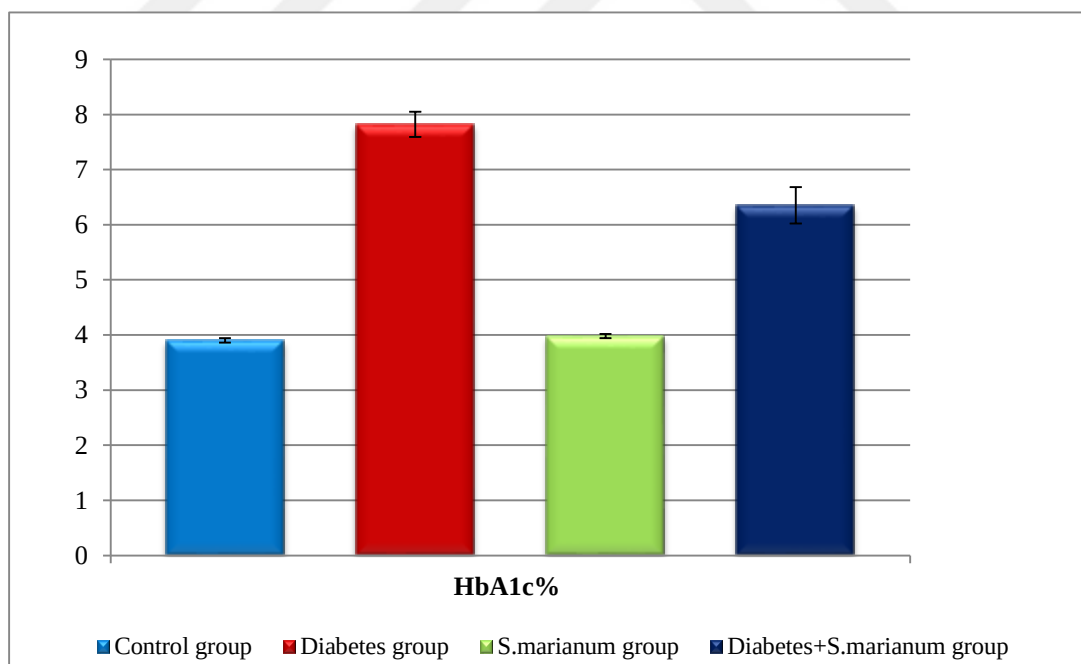


Figure 12. The average of blood (%HbA1c) levels in study groups

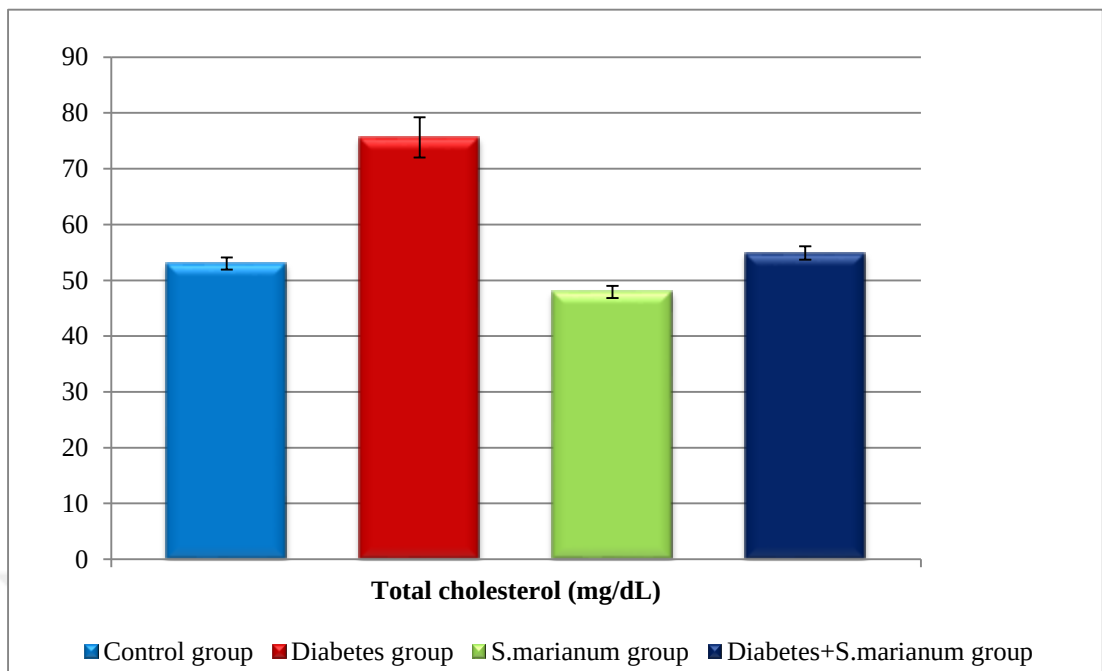


Figure 13. The average of serum total cholesterol level (mg/dL) in study groups.

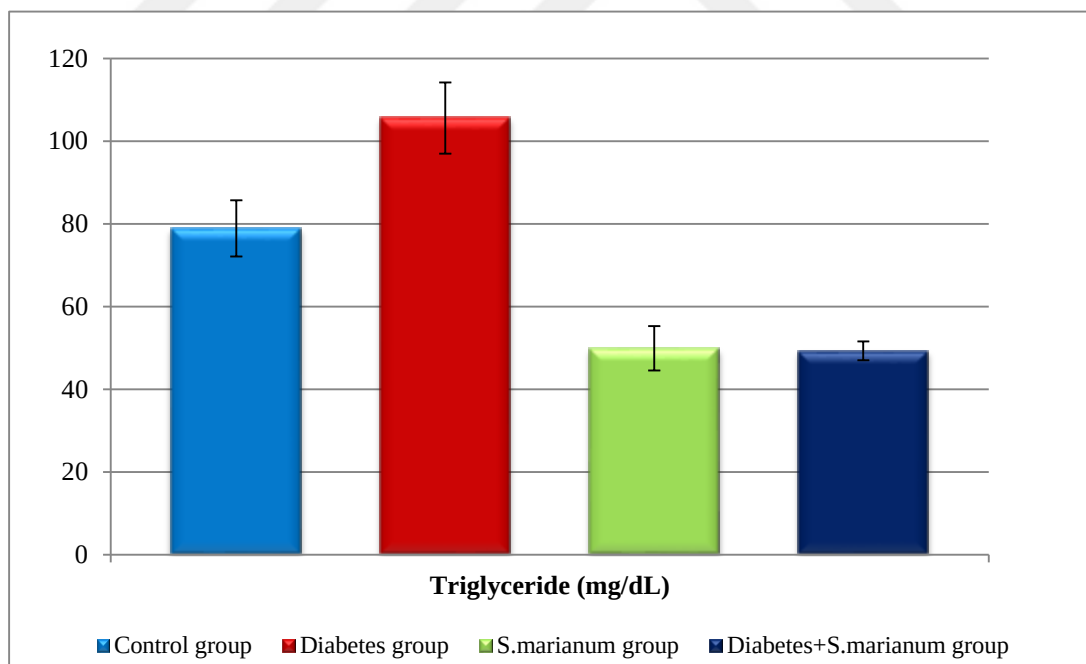


Figure 14. The average of serum triglycerides level (mg/dL) in study groups.

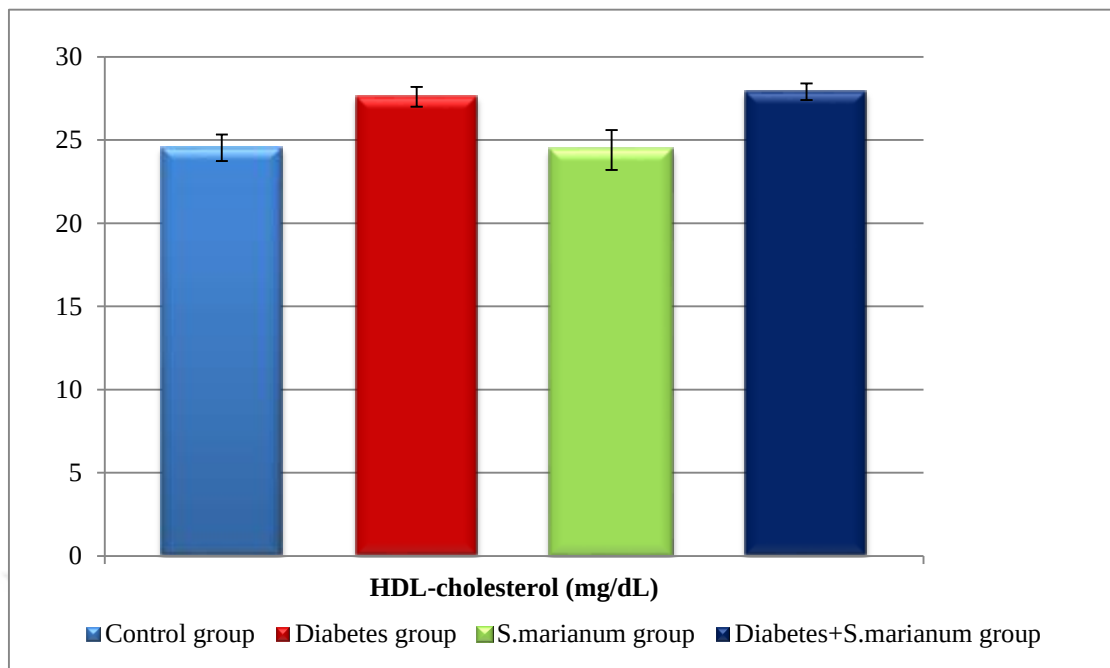


Figure 15. The average of serum HDL-cholesterol level (mg/dL) in study groups

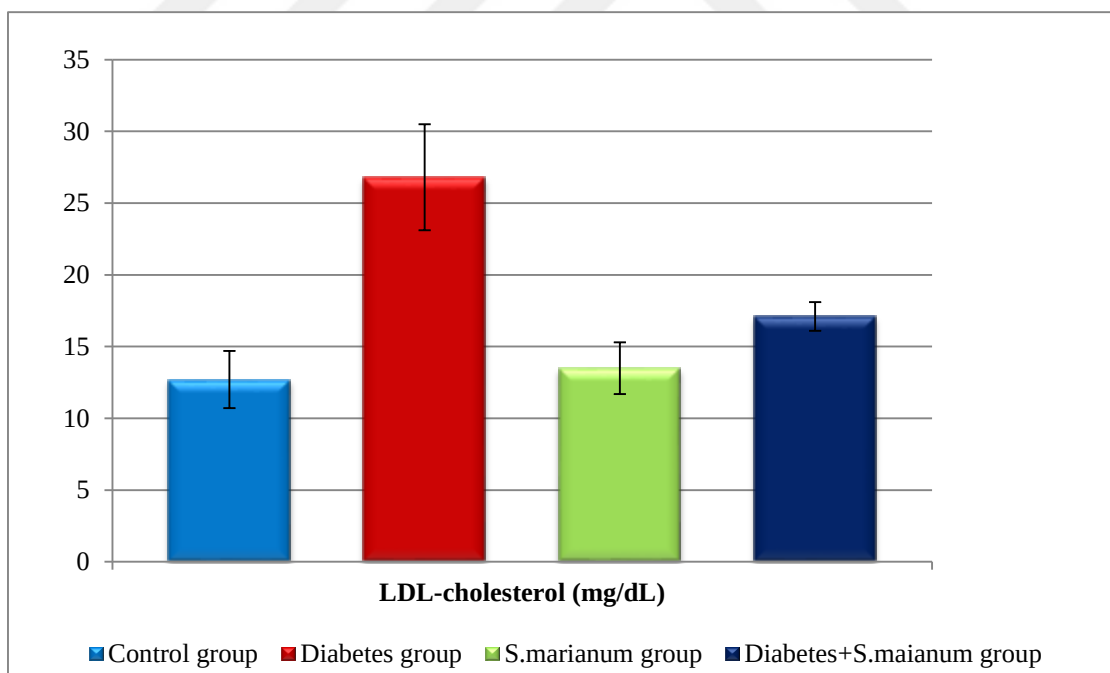


Figure 16. The average of serum LDL-cholesterol level (mg/dL) in study groups

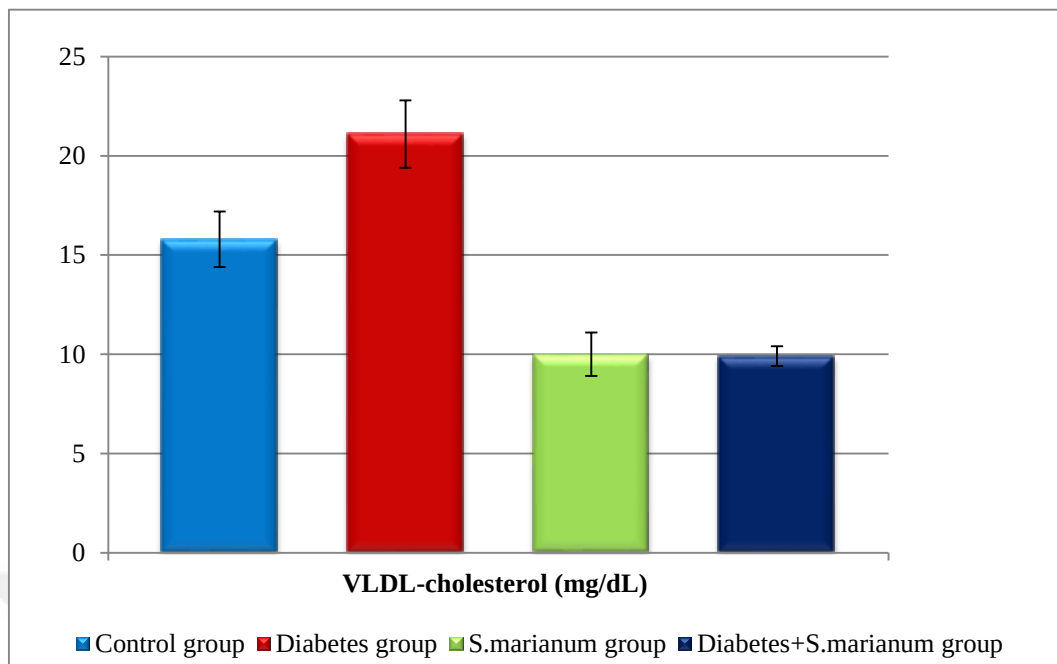


Figure 17. The average of serum VLDL-cholesterol level (mg/dL) in study

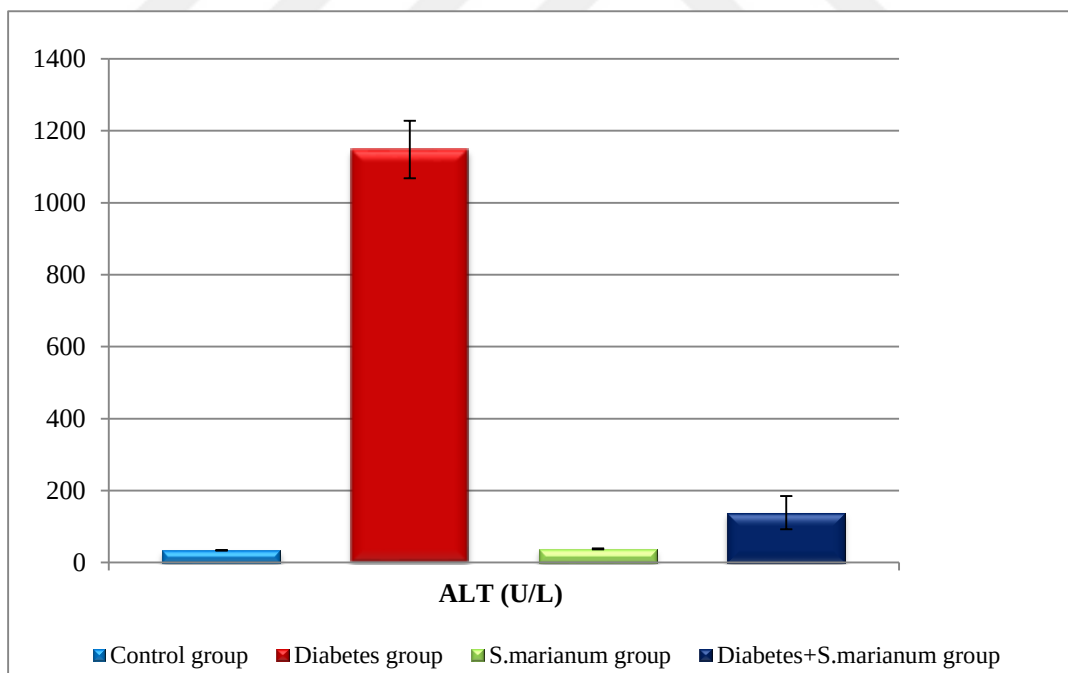


Figure 18. The average of serum ALT level (U/L) in study groups

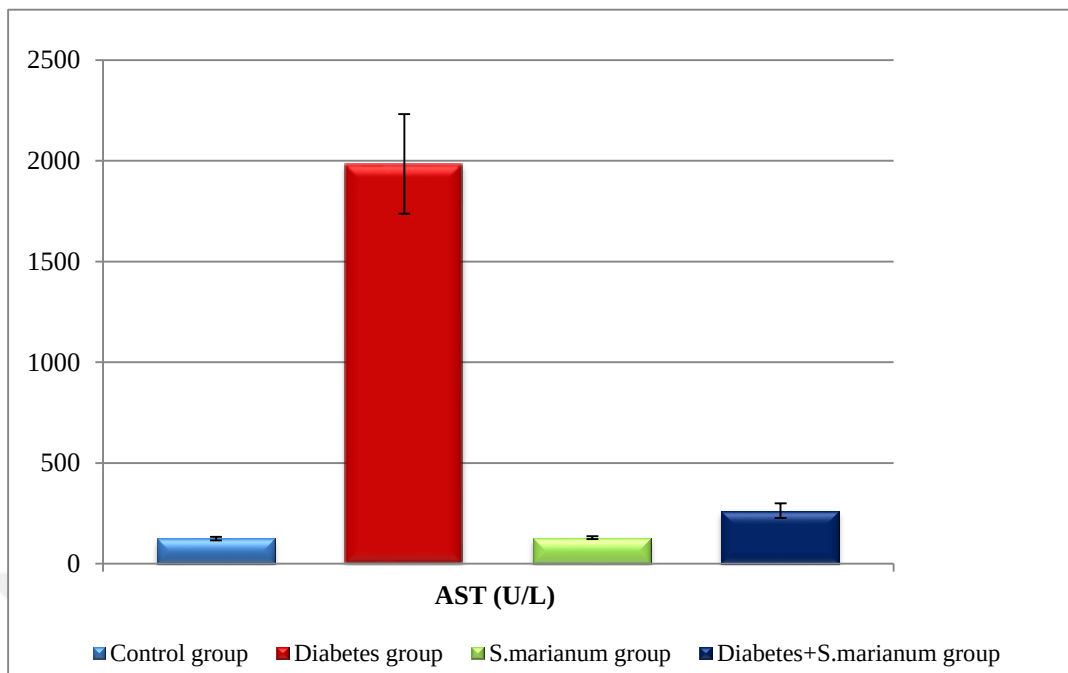


Figure 19. The average of serum AST level (U/L) in study groups

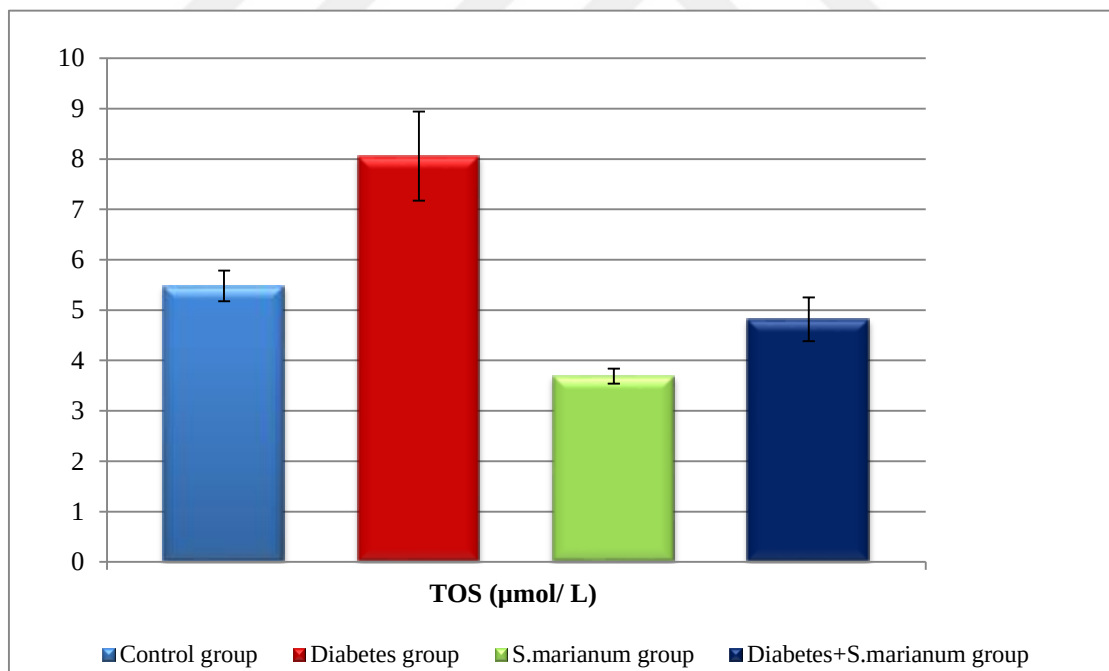


Figure 20. The average of serum TOS levels (μmol/ L) in study groups.

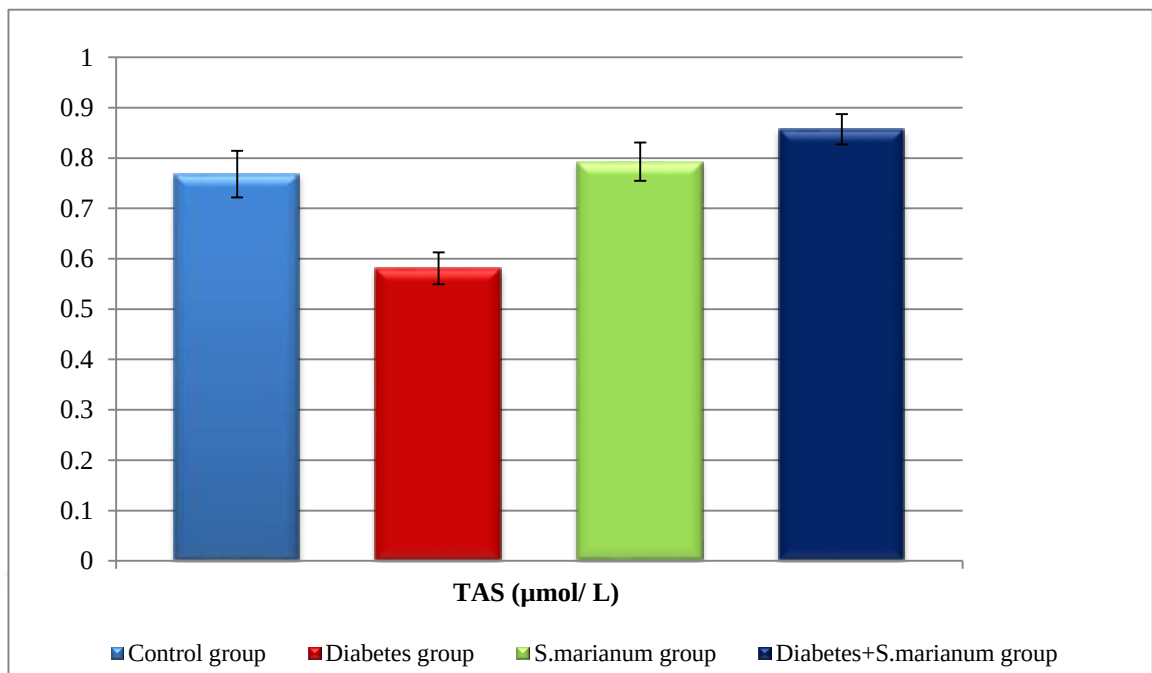


Figure 21. The average of serum TAS levels (μmol/ L) in study group.

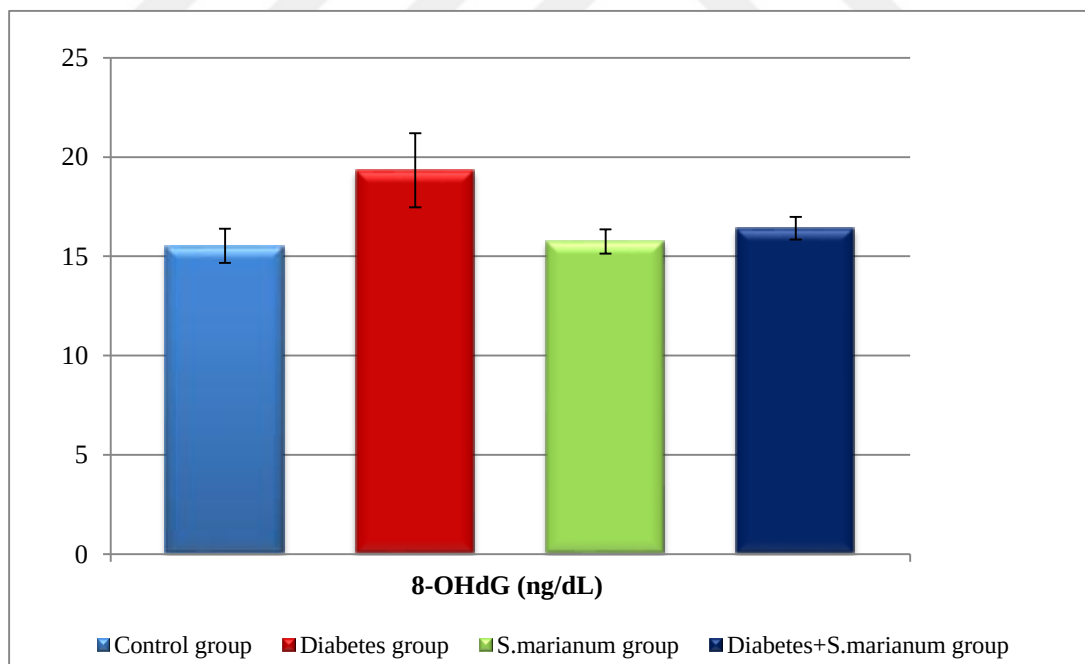


Figure 22. The average of serum 8-OHdG levels (ng/dL) in study groups.

5. DISCUSSION AND CONCLUSION

Diabetes mellitus (DM) is a disease characterized by chronic hyperglycemia. In order to prevent or minimize diabetes and related complications, it is critical that blood sugar levels are kept within certain ranges. To achieve this glycemic goal, adjustments to lifestyle are required but, when not adequate, medical treatments are essential. Medicinal plants have played a crucial role throughout world history in treating and preventing a variety of diseases (Derosa et al., 2015). Recently *S. marianum L.* has been explored for its potential clinical benefits and has been the subject of active research (Abu-Zaiton, 2013). Chemically-active constituent of *S. marianum L.* extract includes a flavolignan known as *silymarin* (Suksomboon et al., 2011).

Silymarin consists of a mixture of flavonolignans from the *S. marianum L.* fruits. It comprises mainly silybin, isosilybin, silydianin, and silychristin (El-Far et al., 2015). DM induced by streptozotocin (STZ) injection in rats. Diabetes in this model likely to be due to the destruction of β –cells of islets of Langerhans as suggested by many sources (Ogden, 2010; ADA, 2016). This proposition is based on the facts that STZ is cytotoxic to pancreatic β -cells, inducing its effect through its structural similarity with glucose, Therefore, STZ binds to glucose receptors on the β -cells in the islets of Langerhans leading to the destruction of the cells (Sajedianfard et al., 2013). DM induced by STZ injection results in the loss of body weight of rats and impairment of glucose oxidation leading also to decreases in insulin biosynthesis and its secretion (Sajedianfard et al., 2014).

Published literature indicates that treatment of D group rats with *S. marianum L.* extract results in a significant decrease ($p < 0.01$) in blood glucose levels and HbA1c% levels indicating, that the plant has anti-diabetic activity. This data is in agreement with others studies such as (El-Far et al., 2015) who reported showing that *silymarin* a significantly reduced the average of fasting blood glucose and HbA1c % in diabetic group treated with silymarin extract (El-Far et al., 2015).

The reaction between hemoglobin and glucose occurs when glucose attaches with the free amino groups to the N-terminals of the beta-chain of hemoglobin

molecules. The reaction is slow, continuous and irreversible. Treatment with *S. marianum L.* extract leads to significant decrease in blood glucose, and this explains the connection with the significant decrease of HbA1c% in this study. This study, therefore, highlights therapeutic effect of *S. marianum L.* extract and its ability to control diabetes (El-Far et al., 2015). *Silymarin* is similar to insulin in having a hypoglycaemic effect (Soto et al., 2003). This latter study, which was confirmed by other investigators, also suggested that the decrease in blood glucose levels in treated diabetic rat with *S. marianum L.* extract is likely to be due to recovery in pancreatic functions as the level of insulin and glucagon increased after the treatment (Soto et al., 2004; Sajedianfard et al., 2014). *Silymarin* with its ability to restore or improve pancreatic function raised expression of PDX-1 protein levels. PDX-1 is a critical regulator of insulin promoter activity and is also believed to be involved in raising level of insulin (Sajedianfard et al., 2014). *Silymarin* can also ameliorate insulin resistance and improve both HbA1c% and FBG significantly (Suksomboon et al., 2011).

S. marianum L. extract is currently used for the treatment of both types of DM, type 1 and type 2. Thus, result of a study carried out by Maghrani and colleagues showed that the *S. marianum L.* extract has a significant hypoglycaemic effect in normal rats and anti-hyperglycaemic activity in diabetic rats (Maghrani et al., 2004). The hypoglycaemic activity of this *S. marianum L.* extract is likely to be due to the suppression of hepatic glucose production and/or an increase in glucose uptake and utilization by peripheral tissues, particularly muscle and adipose tissues. Furthermore, because a state of insulin resistance was shown in recent onset diabetes in diabetic rats, the plant extract may also be normalizing blood glucose levels in diabetic rats through the restoration of normal insulin sensitivity. The plant extracts could also be acting through inhibiting of tubular renal glucose reabsorption (Maghrani et al., 2004). Diabetes is associated with several complications, the main complication of diabetes is decreases with decreasing blood glucose levels (Sajedianfard et al., 2013; El-Far et al., 2015).

This literature also demonstrates that, diabetic rats showed a significant increase ($p<0.01$) in the serum levels of cholesterol, triglycerides and LDL-cholesterol, VLDL with a significant decrease ($p<0.01$) of levels of these lipids in DS group treated with *S.*

marianum L extract. This data is confirmed by other investigator such as studies reported by Sajedianfard and colleagues (Sajedianfard et al., 2014). The elevation in lipid profiles in diabetic rats may be associated with an increase in the rate of lipolysis and a reduction in lipogenesis leading to the release of more fatty acids into the circulation of blood. In addition, insulin deficiency can lead to a reduction in lipoprotein lipase activity and increases in the metabolism of free fatty acids from peripheral fat depots (Ahmed et al., 2001). Diabetes develops disturbances in lipid profile and raises the incidence of atherosclerosis. A variety of irregularities in metabolic and regulatory mechanisms due to insulin insufficiency are responsible for the observed lipids accumulation. Lipid levels in STZ-diabetic rats treated with extract of *S. marianum L*. returned to values closer to control group (Sajedianfard et al., 2014).

The current study has shown that HDL-cholesterol level significantly ($p < 0.01$) increased in D group. These results are not in agreement with other investigations in that it was reported that HDL levels decrease with diabetes, and that reduction is associated with diabetic complications which increases the incidence of atherosclerosis (Sajedianfard et al., 2014). However, the HDL results reported in current study may be due to a technical error, in management of the experimental rats or in preparing the extract from plant. Whilst HDL-cholesterol level increased significantly ($p < 0.01$) increased in D group treated with *S. marianum L*. extract, when compared to normal-control group.

The above finding illustrates that in diabetic animal's treatment with *S. marianum L* extract significantly improves the lipid profile. In diabetic rats, the highest level of glucose and free fatty acids (FFA) leads to the generation of ROS and oxidative stress. These metabolic abnormalities not only promote diabetic complications but also lead to an impaired insulin secretion and cell dysfunction. It was previously observed that STZ-induced diabetic rats develop significant disturbances in cell metabolism in white adipose tissues. These changes are mainly due to insulin deficiency and include a rise in the cAMP content and a rise in the activity of hormone-sensitive lipase in adipocytes. It is known that insulin is a strong anti-lipolytic factor, exerting its effect via phosphorylation of cGMP-inhibited cAMP phosphodiesterase. Activation of this enzyme reduces cAMP in the cell (Sajedianfard et al., 2014).

Silymarin has regulatory action on DNA transcription. As a ribosomal RNA synthesis promoter, induces liver regeneration, and acts as an inhibitor of the transformation of stellate hepatocytes into myofibroblasts. Thus, liver function correction after *silymarin* administration may positively influence glucose and lipid metabolism (Sajedianfard et al., 2014). The mechanism of action of *silymarin* may have an effect on the kinetics of glucose-6 phosphatase gluconeogenesis suppression, raises insulin and improves pancreatic functions. Additionally, *silymarin* reduces hydroxy-3-methyl-glutaryl-CoA reductase activity, the rate-limiting enzyme in cholesterol synthesis (Sajedianfard et al., 2014).

Among other organs, liver functions are important for many vital processes such as metabolism and detoxification of endogenous substances, xenobiotics and drugs. The liver is the first organ which is affected in diabetic patients. Thus diabetes has a role in liver disease. Indicators of liver damage that differentiate from other types of diseases are high levels of AST and ALT enzymes (Abu-Zaiton, 2013; Mehar and Asija, 2015).

The current study has shown that STZ-induced diabetes in rat lead to a significant increased the serum levels of liver enzymes ($p < 0.01$). However, there is a significant decrease ($p < 0.01$) in liver enzymes in group treated with *S. marianum L.* extract. The *S. marianum L.* extract administration decreased the level of liver enzymes AST, ALT, at the same time plasma glucose levels decreased significantly in the group of rats treated with *S. marianum L.* extract. The decrease in the activity of gluconeogenesis leads to the decreased level of glucose in blood. It is hypothesized that a sequential metabolic association between increased glycolysis and reduced gluconeogenesis caused by *S. marianum L.* extract, points to possible biochemical mechanisms through which sugar homeostasis and liver enzymes are maintained. Increased levels of AST and ALT are indicative of cellular damage and functional disorders in liver cell membrane activity (Abu-Zaiton, 2013).

Silibinin, also known as silymarin, has effects on liver carbohydrate metabolism. Thus, silibinin contributes to glycaemic maintenance, such as, gluconeogenesis in the fasting condition, glycogenolysis and glycolysis following food intake (Colturato et al., 2012). *S. marianum L.* with its high polyphenolic content has a hepatoprot-ective effect against pro oxidant products since it removes free radicals. *S. marianum L.* extract also

protects hepatocytes against various other toxins such as ethanol, free fatty acids and carbon tetrachloride (Aslan and Can, 2014).

Non-alcoholic fatty liver disease (NAFLD), is strongly associated with diabetes and insulin resistance. Indeed, diabetes is a strong predictor of cirrhosis among NAFLD patients. In addition, diabetic patients with other liver diseases such as viral hepatitis have a higher risk of developing hepatocellular carcinoma (HCC) than those without diabetes (Gallagher et al., 2016; Wong and Chalasani, 2016). Furthermore, T2D increases the incidence of alcoholic liver disease, NAFLD, autoimmune liver disease and HCC (Wong and Chalasani, 2016).

Data from the current study demonstrated that, D group showed a significant increase ($p < 0.01$) in serum levels of TOS compared to C group but with treatment of *S. marianum* L. extract DS group showed significant decrease ($p < 0.01$) of TOS level in the serum compared to D group. Hyperglycemia, a key clinical manifestation of DM, not only generates more ROS but also weakens antioxidative mechanisms by scavenging enzymes and antioxidant proteins. Hyperglycemia is a link between diabetes and diabetic complications (Xavier et al., 2014). Oxidative is believed to be one of the common pathogenic factors of the onset diabetic complications (Leinonen et al., 1997; Suzuki et al., 1999; Betteridge, 2000; Son et al., 2004; Pan et al., 2007; Pan et al., 2010 Harms-Ringdahl et al., 2012). Diabetic complications observed include nephropathy, neuropathy, cardiomyopathy, vasculopathy, and retinopathy (Kushwaha et al., 2011). In both experimental and clinical studies evidence indicates that oxidative stress has a major role in the pathogenesis of both types of DM. Free radicals are formed in diabetes by glucose oxidation, nonenzymatic glycation of proteins and subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and occurring impairment in antioxidant defense mechanisms can lead to damage to cellular organelles, the production of enzymes. Increased lipid peroxidation and the development of insulin resistance (Maritim et al., 2003). These consequences of oxidative stress can promote the development of complications associated with DM (Suzuki et al., 1999; Shin et al., 2001; Maritim et al., 2003; Milic et al., 2015).

Oxidative stress may be essential for the development of vascular complications of diabetes (Leinonen et al., 1997). Several pathways are caused oxidative stress

associated with acute or chronic hyperglycemia. These include the polyol pathway, prostanoid synthesis, glucose autooxidation and protein glycation by high level of free radical production (Ceriello, 1997; Packer et al., 2000; Maritim et al., 2003). Recently, it was demonstrated that oxidative stress occurs before the manifestation of late diabetic complications consistent with the idea that oxidative stress is an early event in the pathology of diabetes and its complications. Furthermore, many injurious effects of hyperglycemia on endothelial cell functions, such as delayed cell replication, impaired endothelial cell-dependent relaxation, and the activation of nuclear factor kappa -B cell (NF- κ B), are reversed by antioxidants (Packer et al., 2000).

Oxidative stress prevents insulin from crossing the endothelial wall, therefore, the delivery of hormone to tissues will be limited (Bertelsen et al., 2001). Oxidative stress also reduces the CAT, SOD and GPx in liver and raises peroxidation levels. SOD enhances human pancreatic islets survival (Sajedianfard et al., 2013). Oxidative stress can also lead to systemic endothelial cell dysfunction that may directly, or indirectly cause pancreatic β -cell function impairment and glucose intolerance. Pancreatic β -cells are particularly sensitive to ROS because they are low in free-radical destroying antioxidant enzymes such as CAT, GPx and SOD, therefore, oxidative stress can damage mitochondria and reduces insulin secretion. Oxidative stress in muscle and adipose tissues can lead to a state of insulin resistance and, thus, decreasing insulin stimulated glucose uptake by these peripheral tissues and raising glucose level in circulation (Qiu et al., 2011; Sajedianfard et al., 2013).

Red blood cells are accessible to oxidants which accelerate their aging faster than normal cells and increase the breakability index. This cause early removal of red blood cells from the blood stream by phagocytosis. Additionally, oxidant reduce activity of GPx and SOD which in turn leads to changes in red blood cell structure and hemoglobin also reduces oxygen transportation (Sajedianfard et al., 2013). In this respect, the current hypothesis predicts a negative correlation between oxidative stress and the half-life of red blood cells in cycle whereas GPx and SOD enzymes in red blood cells are protective. In settings where oxygen free radicals are not converted to hydrogen peroxide by SOD, and GPx and could not be catabolized form hydrogen peroxide, red blood cells will die very quickly (Sajedianfard et al., 2013). In addition,

Oxidative stress contributes to impaired placental blood flow and leads to hypoxia during pregnancy. Research of pregnancy with complications have indicated that high maternal oxidative stress is one of the risk factors for delayed fetal growth brought about mainly by changes to lipid peroxidation during gestation (Min et al., 2009; Qiu et al., 2011).

This current study is in consistent with other earlier studies. In hyperglycemic conditions, there is an obvious increase in free radicals generated as result of electronic transport chain. This leads glucose to auto-oxidize, a reaction catalyzed in the presence of trace amounts of transition metals generating superoxide, hydroxyl radicals, hydrogen peroxide and reactive ketoaldehydes (Soto et al., 2003; Soto et al., 2004). Reduced glutathione provides one of the more important protection mechanisms against free radicals, such as superoxide ($O_2^{\cdot-}$) and hydroxyl (OH) radicals (Rady et al., 2014). In addition to GSH, there are other defence processes against free radicals, such as the enzymes SOD, GPx and, CAT whose activities contribute to remove superoxide ($O^{\bullet}$) and hydroxyl ($\bullet OH$) radicals (Sajedianfard et al., 2013). There is considerable evidence that hyperglycemia results in the generation of ROS, leading ultimately to increase oxidative stress (El-Wassef et al., 2012).

Finding from many studies *Silymarin*, a flavonoid extracted from *S. marianum*, has been reported to act as an excellent antioxidant, scavenging free radicals (ROS) and inhibiting lipid peroxidation, thereby protecting cells from oxidative stress. *Silymarin* also enhances the pancreatic activity in producing antioxidant enzymes. It also increases the non-enzymatic and enzymatic antioxidant defenses of cells (Soto et al., 2003; Soto et al., 2004; Bhattacharya, 2011; Balszuweit et al., 2013; Aslan and Can, 2014) In addition, Silibinin shows membrane protective properties and it may protect blood components from oxidative damage (Bhattacharya, 2011). *Silymarin*, has demonstrated protective effects against the oxidative peroxidation in several experimental animal models and in human hepatic damage. *Silymarin* induces an increase in the hepatic activity of SOD and GSHPx in rat liver (Soto et al., 2003). Data results from the current study is in agreement with other studies such as (Sajedianfard et al., 2013) regarding the effects of different oral doses of *silymarin* on the oxidative stress indicators SOD, GPx, CAT and MDA in STZ-induced diabetic rats. The results showed that *silymarin* induced

increases in the activity of antioxidant enzymes: GPx, SOD and CAT in red blood cells (Sajedianfard et al., 2013).

The study also showed that, D group demonstrated significant decrease ($p<0.01$) in the serum TAS levels compared to C group, While the TAS level increased significantly ($p<0.01$) in DS group treated with *S. marianum* L extract compared to D group with no treatment.

Generally all serum antioxidants defense levels are evaluated according to TAS status (Kulaksızoglu and Karalezli, 2015). Disruption of the balance between oxidative processes and antioxidative defenses leads to oxidative stress that can damage proteins, lipids, polysaccharides and nucleic acids. This is implicated in diabetes and believed to cause such effect due to either oxidants overproduction, or a decrease in antioxidant defense levels (Cherubini et al., 2005; Juśkiewicz et al., 2008). This imbalance has also been implicated in complications associated with DM (Kulaksızoglu and Karalezli, 2015). In order to prevent the formation of ROS and to limit their damaging effects, cells have developed a set of antioxidant defense mechanisms. These include antioxidant enzymes mainly GPx, SOD and CAT. They also include beta-carotene, tocopherols and ascorbic acid (Kulaksızoglu and Karalezli, 2015).

Data from the literature is in agreement with finding from the current study (Juśkiewicz et al., 2008; Angeline et al., 2015). Thus, when serum levels of TAS were estimated, levels of TAS is effectively decreased in the diabetic groups (Juśkiewicz et al., 2008; Angeline et al., 2015). In study by Seghrouchni and colleagues, oxidative stress was found to be significantly increased in diabetic patients compared with healthy subjects (Seghrouchni et al., 2002). This indicates that, TAS is significantly decreased in diabetic patients

In a study Aslan et al (2014) comparing smokers to nonsmokers, observed that TAS levels significantly decreased in nonsmokers. These investigators attributed the results to the presence of high amounts of free radicals in cigarette smoke leading to the generation of an oxidative stress in the body. Other studies reported that TAS levels in the serum of rabbits with alloxan-induced hyperglycemia decreased by 66% compared

with control rabbits. TAS levels increased above control values in hyperglycemia rabbits treated with oral antidiabetes (Kulaksızoglu and Karalezli, 2015).

Hyperglycemia leads to increased production of ROS and this can induce several DNA lesions. Extracellular chronic hyperglycemia leads to increased oxidative stress due to raised ROS generation, which can also cause DNA lesions (Xavier et al., 2014). ROS have been suggested to play a crucial role in carcinogenesis, mutagenesis and the aging processes. ROS has the ability to react with different cellular components such as proteins, lipids and nucleic acids, and rising chemical modifications (Shin et al., 2001; Maritim et al., 2003; Pan et al., 2007; Harms-Ringdahl et al., 2012). Such chemical modification of proteins and lipids by ROS induce cellular dysfunction. Indeed, it is suggested that these events lead the pathogenesis of macrovascular and microvascular complications in diabetic patient. ROS also causes base modifications and strand breaks in DNA *in vitro*, and probably the occurrence of mtDNA deletions *in vivo* (Suzuki et al., 1999; Simone et al., 2008). Mitochondria and nucleus, two major targets of oxidative stress, contain a variety of DNA repair enzymes to repair oxidant-induced DNA modifications. Damage mostly occurs when the endogenous antioxidant network and DNA repair systems are overwhelmed (Simone et al., 2008).

A significant amount of ROS is created during mitochondrial oxidative phosphorylation and when DNA is exposed to ROS, guanine residues are oxidized to 8-OHdG. The mtDNA is known to accumulate more 8-OHdG relative to nuclear DNA, also Lipid peroxidation in mtDNA increases 8-OHdG formation, therefore, mtDNA is a critical cellular target of ROS (Suzuki et al., 1999; Simone et al., 2008).

Oxidatively-damaged DNA may involve breakage in single and double-strand, base modifications, fragmentation of deoxyribose, formation of DNA–protein cross-links as well as a basic site. Furthermore, ROS can induce base changes including oxidation of guanine residues to 8-OHdG, an oxidized nucleoside of DNA, and this has been considered as oxidative DNA damage biomarker *in vivo*. In humans, higher levels of 8-OHdG are observed in mononuclear cells from diabetic patients (Lee et al., 2005; Pan et al., 2007; Kushwaha et al., 2011; Xavier et al., 2014). 8-OHdG increases in plasma with aging, during the developing of tumors caused by ROS, during radiotherapy and in smokers (Nishikawa et al., 2003). Plasma levels of 8-OHdG were

also found to be increased in an animal model of ischemic stroke, with significant association with brain content of 8-OHdG (Cherubini et al., 2005).

CKD is characterized by an increase in oxidative stress and inflammation which may serve to promote additional damage to the kidneys (Tucker et al., 2015). Urinary 8-OHdG is suggested to be a new putative biomarker of the total systemic oxidative stress *in vivo* (Leinonen et al., 1997). 8-OHdG can be detected in tissues, serum, urine and other biomaterials (Kasai et al., 1986). 8-OHdG passes freely into the urine through glomerular filtration and serves as an index of whole body oxidative stress (Leinonen et al., 1997).

At present considered the 8-OHdG level in urine is a biomarker of the total systemic oxidative stress *in vivo*. Urinary excretion of 8-OHdG has also been shown to be higher in both type 1 and type 2 diabetic patients compared to nondiabetic cases (Shin et al., 2001). Poor glycaemia control is associated with a high urinary excretion of 8-OHdG. Oxidative DNA damage has been shown to be related to the peroxidation of membrane fatty acids and low antioxidant status, both present in diabetes (Leinonen et al., 1997). Raised urinary 8-OHdG, is associated with increased risks of cancer, atherosclerosis, autoimmune disorders, and diabetes (Qiu et al., 2011). In (Mamur et al., 2016) study results indicate that primary DNA damage is raised in CKD patients undergoing hemodialysis, compared to controls. The most common cause of CKD is DM, which is the primary etiology or cause of end-stage renal disease. One of the complications of diabetes is atherosclerosis, which is associated with DNA damage. Recent studies revealed elevated DNA damage with significant positive correlation with triglycerides and LDL, cholesterol, and DNA damage was present in atherosclerotic plaques and in circulating cells of patients with atherosclerosis (El-Wassef et al., 2012). In men, diabetes induces sperm immobility and sperm DNA damage (Kilarkaje et al., 2014). In pregnant women, raised urine 8- OHdG concentrations determined in early pregnancy is associated with reduced infant birthweight and with shortened duration of gestation (Min et al., 2009). 8-OHdG is also associated with increased risk of GDM (Qiu et al., 2011).

Data from the current study showed that 8-OHdG increased significantly ($p < 0.05$) in D group compared with the C group. Whilst the DS group treated with S.

marianum L. extract for 14 days at a dose of 200mg /kg, the level of 8-OHdG decreased, but not significantly ($p>0.05$) compared with the D group without treatment of *S. marianum L.* extract and C group. The study also demonstrated that a 14-days treatment was enough to achieve normal blood glucose levels but not enough to lead to a significant decrease in DNA damage in the diabetic group treated with *S. marianum L.* extract. The study also revealed a significant positive correlation between DNA damage and blood level of glucose and HbA1c%. This data is in agreement with studies by El-Wassef and colleagues in which the number of DNA strand breaks was significantly higher in diabetic patients compared with controls (El-Wassef et al., 2012). The number of DNA strand breaks correlated positively with fasting blood glucose and HbA1c levels. Hyperglycemia causes glucose auto-oxidation, glycation of proteins, activation of polyol metabolism and subsequent formation of ROS. It has also been demonstrated that hyperglycemia is associated with an increased production of free radicals that contribute to more DNA damage (Goodarzi et al., 2010). Such changes might contribute to accelerated aging and atherogenesis in diabetes and to the microangiopathic complications of the disease (Dandona et al., 1996).

Data from the study showing a significant positive correlation between urinary 8-OHdG, a biomarker of oxidative DNA damage, and fasting blood glucose and HbA1c is in agreement with previous study on this issue (Goodarzi et al., 2010). A study by Lorenzi et al. (1986) reported that a significant positive study reported that hyperglycemia causes glycosylation of DNA and decreases the DNA repair process in cultured human endothelial cells. In addition, Dandona and colleagues reported that IDDM and NIDDM patients had significantly higher median concentrations of 8-OHdG in their mononuclear cells than their controls (Dandona et al., 1996). Other investigator reported that the total amounts of urinary 8-OHdG excretion were significantly greater in STZ induced diabetic rats than in control (Kakimoto et al., 2002). A further study by Nishikawa and colleagues revealed positive correlation between urine 8-OHdG and HbA1c (Nishikawa et al., 2003). In addition, these investigators revealed higher levels of 8-OHdG in urine in patients with albuminuria and retinopathy and indicated that 8-OHdG in urine is a useful marker of early type 2 diabetic complications, in which high oxidative stress play an important role in the progression of diabetic complications. Furthermore, urine 8-OHdG associated with diabetic nephropathy retinopathy was

observed. In (Tatsch et al., 2012) study showed the association between type 2 diabetes and DNA damage. Believed generally believed that over production of oxidants with a decrease in the level of defenses in type 2 diabetes causes oxidative damage to lipids, proteins and DNA. Oxidative stress also significantly affected DNA and generated various base modifications (Tatsch et al., 2012).

Polyphenol fraction from the seeds of *S. marianum* L., modulate UVA-induced oxidative damage to human keratinocytes. UVA can indirectly damage DNA structure, and suppress DNA repair as well as it weakening the immune system .in a study by Svobodová and colleagues it was reported that *S. marianum* was able to reduce UVA-irradiated skin cells damage The protective effects of *S. marianum* may be interpreted by its ability to eliminate, or reduce UVA-induced radical generation. The protective activity of *S. marianum* is likely to be due to its flavonolignans, mainly Silybin, which acts against various ROS and its generation, as shown *in vitro* and *in vivo* (Svobodová et al., 2007a)

The study also showed that post-treatment with silybin and 2, 3-dehydrosilybin which are *S. marianum* extract component led to effective elimination of UVA-induced DNA single strand breaks near to the level of non-irradiated cells. In a review article Nichols and Katiyar stated that polyphenols are one of the silymarin components which have the ability to protect the skin from cancers and that the compounds also have significant anti-inflammatory, antioxidant and anti-DNA damaging effects (Nichols and Katiyar, 2010).

Dihydroquercetin (taxifolin) of *S. marianum* have anti-oxidant activities acting suppressing the production of superoxide anions, protecting mitochondria from peroxyl radical damage and inhibiting the activation NADPH-dependent cytochrome P450 reductase induced by microsomal lipid peroxidation (Weidmann, 2012). Dihydroquercetin *S. marianum* component decreases total liver cholesterol, also significantly reduces serum and liver thiobarbituric acid reactive substance (TBARS). TBARS are an index of lipid peroxidation and oxidative stress (Svobodová et al., 2007a; Weidmann 2012). The potential *S. marianum* to increase activities of endogenous antioxidant enzymes is also established. *S. marianum* application led to significant protection of ATP depletion that is essential for cell energy metabolism since

the caspase-3 activation is linked to ROS overproduction, treatment with Silybin and 2,3-dehydrosilybin of *S. marianum* substantially decreased UVA-elevated activation of caspases-3. Their effects may be again explained by their ability to eliminate/alleviate UVA-induced ROS generation (Svobodová et al., 2007b).

A significant reduction in the expression of the cytoplasmic P53 and CD68 was observed after *silymarin* treatment. P53 tumor suppressor protein that acts as a transcription factor that regulates the transcription rate of several genes involved in cell cycle regulation, apoptosis and DNA repair. When the cells are exposed to the external damage, P53 negatively delays cell cycle progression and leads to apoptosis, if the damage cannot be repaired. P53 acts as a protector of genomic activity either by inducing cell cycle arrest (at G1 and/or G2 phase) or programmed cell death (apoptosis). Expression of CD68 at the site of laser injury is consistent with the wound-healing process as a response to local damage (Tousson et al., 2013).

Moreover, (Yue et al. 2015), studied that the effect of several plants including Lycopene, a lipophilic antioxidant found mainly in tomatoes and in other vegetables and fruits, can protect mitochondrial DNA against oxidative damage. The protective effect of lycopene on mitochondrial DNA was associated with a reduction in mitochondrial ROS production decreased 8-hydroxyguanine (8-OHdG) level (Yue et al., 2015). Antioxidants got from nature assists in neutralization of ROS and significantly decrease the chance of diabetic complications progression. In (Rastogi et al., 2010) study showed *Nigella Sativa L* showed significant free radical scavenging and protection against DNA damage in cell free systems (Rastogi et al., 2010). A variety of nutritionally important vitamins, supplements and some constituents of natural food sources, like cappers, broccoli, tomatoes, berries, grapes, spinach, carrots, nuts, etc. naturally decrease the injury induced by oxidative stress in DM (Nawaz Khan et al., 2015).

In conclusion, in this study *S. marianum L* extract demonstrated remarkable anti-diabetic activity through decreasing both glucose and glycated hemoglobin levels, also *S. marianum L* extract demonstrated its ability to decrease diabetic dyslipidemia through decreasing level of total cholesterol, triglyceride LDL, VLDL. *S. marianum L* extract functioning as a free radical scavenging function due to the flavonoids, flavonolignans and other polyphenolic components in its structure. Silymarin, a

flavonoid extracted from *S. marianum* has been reported to act as an excellent antioxidant, scavenging free radicals, and inhibiting lipid peroxidation there by protecting cells against oxidative stress. Silymarin is increasing the pancreatic activity of antioxidant enzymes, it is increases the non-enzymatic and enzymatic antioxidant defense systems of cells which is showed in this study via decreasing TOS and increasing TAS level. Finally, the plant caused slightly decrease in the level of 8-OHdG but not significant

This study demonstrated that a 14-days treatment is not enough to lead to a significant decrease in oxidative DNA damage in diabetic experimental animal treated with *S. marianum L* extract. A number of literatures, publications and research is done on *S. marianum L.* plant as hepato-protecting drug but the research as anti-diabetic effect of this plant is limited and not much explained. We suggest that a huge research is required to check the anti-diabetic effect of this plant in the future, but with longer time of treatment of this plant.

SUMMARY

SHINKI GAM, The effect of the *Silybum marianum* extract in experimental diabetic rats on the serum 8-hydroxy-2'-deoxyguanosine, total oxidant and antioxidant capacity. Yuzuncu Yil University, Health Science Institute, Department of Basic Pharmacy-Biochemistry, Master Science Thesis, Van, 2016. The aim of this study was to find out the effect of *Silybum marianum* L extract (*S. marianum*) on the 8-hydroxy-2'-deoxyguanosine (8-OHdG), total oxidant (TOS), and antioxidant capacity (TAS) activity. In addition, some other biochemical parameters analyzing such as glucose HbA1c lipoprotein, ALT, AST were performed. In this purpose (28) experimental male rats were prepared in the animal house divided into four groups: first group given normal standard diet and determined as control group (C), second group is diabetic (D) non *S. marianum* extract treated, third group healthy with *S. marianum* treated (S) and forth group diabetic with *S. marianum* treated (DS) Diabetes induced by injecting (45 mg/kg) Streptozotocin intraperitoneally in the D group and DS group. The rats in the S group, and DS. group were treated for 14 days with *S. marianum* L extract dissolved in olive oil 200 mg/kg/day, C group in addition to stander diet oil olive were given. Intragastric gavage was used after this period the parameters such as, HbA1c in whole blood, and glucose HDL, LDL, VLDL, cholesterol, triglyceride, ALT, AST TOS, TAS, 8-OHdG in the serum were measured. Glucose levels and (%HbA1c) were significantly increased ($p<0.01$) in the D group, decreased significantly. ($p<0.01$) in DS group. The levels of total cholesterol, triglyceride, LDL, VLDL were significantly increased ($p<0.01$) in the D group, decreased significantly ($p<0.01$) in DS group. ALT, and AST levels increased significantly in the D group, decreased significantly ($p<0.01$) in the DS group. TOS levels increased significantly in the D group ($p<0.01$), decreased significantly ($p<0.01$) in DS group. TAS level decreased significantly in the D group ($p<0.01$), increased significantly ($p<0.01$) in DS group. The 8-OHdG activity increased significantly ($p<0.05$) in the D group, decreased but not significantly ($p>0.05$) in DS group. In conclusion, in this study *S. marianum* L extract demonstrated remarkable anti-diabetic activity, also demonstrated its ability to decrease diabetic dyslipidemia. This plant protecting cells against oxidative stress, increasing the non-enzymatic and enzymatic antioxidant defense systems of cells. Finally, *S. marianum* L extract affected on 8-OHdG but not significant.

Key Words: Diabetes, *S. marianum* extract, lipoprotein, Liver enzyme TAS, TOS, 8-OHdG activity.

ÖZET

SHINKI GAM, Deneysel Diyabetli Ratlarda *Silybum marianum* Ekstresinin Serum 8-Hidroksi-2'-Deoksiguanozin, Total Oksidan ve Antioksidan Kapasiteleri Üzerine Etkisi. Yüzüncü Yıl Üniversitesi, Sağlık Bilimleri Enstitüsü, Temel Eczacılık Bilimleri Anabilim Dalı, Biyokimya Bilim Dalı, Yüksek Lisans Tezi, Van-2016. Bu çalışmada, *Silybum marianum* L. (*S. marianum* L.) ekstresinin deneysel diyabetli sıçanlarda; antidiyabetik ve serumda 8-Hidroksi-2'-deoksiguanozin, total oksidan (TOS) ve antioksidan (TAS) kapasiteleri üzerine etkisinin araştırılması amaçlandı. Bu amaçla, kan glukoz, HbA1c, serum lipoprotein düzeyleri ve karaciğer enzim ALT, AST aktivite analizleri yapıldı. Çalışmada 28 adet erkek sıçan yer aldı ve sıçanlar dört gruba ayrıldı. Birinci grup; normal diyet verilen sağlıklı ve kontrol grup (C), ikinci grup; diyabetli grup (D), üçüncü grup; *S. marianum* L. ekstresi uygulanan grup (S), dördüncü grup; *S. marianum* L. ekstresi uygulanan diyabetli grup (DS) olarak isimlendirildi. Deneysel diyabet oluşumu amacıyla; D ve DS gruplarındaki sıçanlara; streptozotosin (STZ) (45 mg/kg) periton içine enjekte edildi. S ve DS grubundaki sıçanlar, 14 gün süreyle 200 mg/kg/gün zeytin yağı içinde çözündürülmüş, *S. marianum* L. ekstresi ile tedavi edildi. C ve D gruplarına standart diyet ek, zeytin yağı intragastrik gavaj yoluyla verildi. Tam kanda HbA1c ve serumda glukoz, HDL-kolesterol, LDL-kolesterol, VLDL-kolesterol, kolesterol, trigliserit, ALT, AST, TOS, TAS, 8-OHdG ölçüldü. Serum glukoz ve % HbA1c seviyeleri C grubu ile karşılaştırıldığında, D grubunda önemli ölçüde arttığı tespit edildi ($p<0.01$). Glukoz ve HbA1c% seviyeleri; D grubuna kıyasla DS grubunda önemli ölçüde azaldı ($p<0.01$). Total kolesterol, trigliserit, LDL-kolesterol, VLDL-kolesterol seviyeleri C grubu ile karşılaştırıldığında D grubunda önemli ölçüde arttı ($p<0.01$). Bu parametrelerin değerleri D grubuna kıyasla DS grubunda önemli ölçüde azaldı ($p<0.01$). ALT ve AST aktiviteleri, D grubunda önemli ölçüde artmış, DS grubunda ise azalmıştır ($p<0.01$). TOS seviyesinde, D grubuna kıyasla DS grubunda önemli ölçüde azaldığı ($p<0.01$) tespit edildi, C grubuyla kıyaslandığında D grubunda önemli ölçüde arttı ($p<0.01$). TAS seviyesi C grubuyla kıyaslandığında D grubunda önemli ölçüde azaldı ($p<0.01$), fakat D grubuna kıyasla DS grubunda önemli ölçüde arttı ($p<0.01$). DS grubunda 8-OHdG düzeyi, D grubuyla karşılaştırıldığında önemli bir azalma görülmedi ($p>0.05$), C grubuna kıyasla D grubunda önemli ölçüde arttığı tespit edildi ($p<0.05$). Sonuç olarak, bu çalışma *S. marianum* L. ekstresinin antidiyabetik etkisini glukoz ve glikolize hemoglobin miktarını düşürme yoluyla gösterdiği tespit edildi. Ayrıca, total kolesterol, trigliserit, LDL-kolesterol, VLDL--kolesterol seviyelerini düşürmesi ile diyabetik dislipidemiye düşürebileceği gösterildi. Çalışmada, *S. marianum* L ekstresinin, TAS seviyesinin azaltılması ve TOS seviyesinin artırılması yoluyla hücreleri oksidatif strese karşı koruyucu olduğu, hücrelerin enzimatik ve enzimatik olmayan savunma mekanizmalarını arttırdığı tespit edildi. *S. marianum* L ekstresi 8-OHdG seviyesinde anlamlı olmayan hafif bir azalmaya sebep oldu.

Anahtar Kelimeler: Diyabet, *S. marianum* ekstraktı, Lipoprotein, Karaciğer enzimleri, TAS, TOS, 8-OHdG aktivitesi

REFERENCES

- Abu-Zaiton AS (2013). Evaluating the effect of *Silybum marianum* extract on blood glucose, liver and kidney functions in diabetic rats. *Adv Stud Biol*, 5, 10, 447-454.
- Ahmad A, Moinuddin A, Ahsan A, Goel A (2016). Study of electrophysiological changes in sensory nerves among diabetic smokers. *J Clin Diagn Res*, 10, 1, 9-11.
- Ahmed I, Lakhani MS, Gillett M, John A, Raza H (2001). Hypotriglyceridemic and hypochol-esterolemic effects of anti-diabetic Momordica charantia (karela) fruit extract in streptozotocine-induced diabetic rats. *Diabet Res Clin Pract*, 51, 3, 155-161.
- Ajiboye JA, Akintunde JK, Oladejo OS, Sabiu SA (2015). Chemoprevention of silymarin and vitamin C on isoniazid-induced hepatotoxicity in experimental rat model. *J Toxins*, 2, 1, 3.
- Akhavan P, Aghili R, Malek M, Ebrahim Valojerdi A, Khamseh ME (2016). Hypoglycemia: Adverse cardiovascular outcomes in non-critically III people with type 2 diabetes. *Arch Iran Med*, 19, 2, 82-86.
- Akkaya H, Yilmaz O (2012). Antioxidant capacity and radical scavenging activity of *Silybum marianum* and *Ceratonia siliqua*. *Ekoloji*, 21, 82, 9-16.
- Al-Daghri N M, Mohammed AK, Al-Attas OS, Amer OE, Clerici M, Alenad A and Alokail MS (2016). SNPs in FNDC5 (irisin) are associated with obesity and modulation of glucose and lipid metabolism in Saudi subjects. *Lipids Health Dis*, 15, 54.
- American Diabetes Association (ADA) (2015). Standards of medical care in diabetes. *Diabetes Care*, 38, 1, 10, 11.
- American Diabetes Association (ADA) (2016). Standards of medical care in diabetes. *Diabetes Care*, 39, 1, 13-22.
- Angeline T, Caroline JC, Rani JJA, Asirvatham JA, Isabel W (2015). Analysis of DNA damage, plasma lipid peroxidation, and antioxidant status in type 2 diabetes mellitus patients among a south Indian population. *Int J Diabetes Dev Ctries*, 35, 3, 491-495.
- Anonym 1 (2016). DNA helix. [Shttps://www.google.com.tr/search?q=structure&biw=1366&bih=645&tbm=isch&imgil=G3BfnKIS-tMW](https://www.google.com.tr/search?q=structure&biw=1366&bih=645&tbm=isch&imgil=G3BfnKIS-tMW), 26/8/2016.
- Anonym 2 (2016). S. marianum plan. [Shttps://www.google.com.tr/search?q=milk+thistle+dna+damage+articles&biw=8/8/2016](https://www.google.com.tr/search?q=milk+thistle+dna+damage+articles&biw=8/8/2016).
- Anderson JC (2015). Measuring breath acetone for monitoring fat loss. *Obesity (Silver Spring)*, 23, 12, 2327-2334.
- Auberval N, Dal S, Bietiger W, Seyfritz E, Peluso J, Muller C, Zhao M, Marchioni E, Pinget M, Jeandidier N, Maillard E, Schini-Kerth V, Sigrist S (2015). Oxidative stress type influences the properties of antioxidants containing polyphenols in RINm5F beta cells. *Evid Based Compl Alternat Med*, 2015, 859048. 1-11.

Aslan A, Can Mİ (2014). Milk thistle impedes the development of carbon tetrachloride-induced liver damage in rats through suppression of BCL-2 and regulating caspase pathway. *Life Sci*, 117, 1, 13-18.

Aslan R, Kutlu R, Civi S, Tasyurek E (2014). The correlation of the total antioxidant status (TAS), total oxidant status (TOS) and paraoxonase activity (PON₁) with smoking. *Clin Biochem*, 47, 6, 393-397.

Barathmanikanth S, Kalishwaralal K, Sriram M, Pandian SR, Youn HS, Eom S, Gurunathan S (2010). Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. *J Nanobiotech*, 8, 16.

Balszuweit F, John H, Schmidt A, Kehe K, Thiermann H, Steinritz D (2013). *Silibinin* as a potential therapeutic for sulfur mustard injuries. *Chem Biol Interact*, 206, 3, 496-504.

Ballotari P, Roncaglia F, Ranieri SC, Greci M, Manicardi V, Rossi PG (2016). Diagnostic values of glycated haemoglobin and diagnosis of diabetes: Results of a cross-sectional survey among general practitioners in the province of Reggio Emilia, Italy. *J Clin Trans. Endocrinol*, 3, 21-25.

Bansal AK, Bilaspuri GS (2010). Impacts of oxidative stress and antioxidants on semen functions. *Vet Med Int*, 686137, 7.

Betteridge DJ (2000). What is oxidative stress? *Metabolism*, 49, 2, 1, 3-8.

Bertelsen M, Anggard EE, Carrier MJ (2001). Oxidative stress impairs insulin internalization in endothelial cells in vitro. *Diabetol*, 44, 605-613.

Bhattacharya S (2011). Phytotherapeutic properties of milk thistle seeds: An overview. *Adv Pharm Edu Res*, 1, 69-79.

Borzi V, Frasson, S, Gussoni G, Lillo MD, Gerloni R, Augello GE, Gulli G, Ceriello A, Solerte B, Bonizzoni E, Fontanella A (2016). Risk factors for hypoglycemia in patients with type 2 diabetes, hospitalized in internal medicine wards: Findings from the FADOI-DIAMOND study. *Diabetes Res Clinic Pract*, 115, 24-30.

Calani L, Brighenti F, Bruni R, Del Rio D (2012). Absorption and metabolism of milk thistle flavanolignans in humans. *Phytomedicine*. 20, 1, 40-46.

Campbell SA, Hoffman BG (2016). Chromatin regulators in pancreas development and diabetes. *Trends Endocrinol Metab*, 27, 3, 142-152.

Capobianco E, Fornes D, Linenberg I, Powell TL, Jansson T, Jawerbaum A (2016). A novel rat model of gestational diabetes induced by intrauterine programming is associated with alterations in placental signaling and fetal overgrowth. *Mol Cell Endocrinology*, 422, 221-232.

Cavaretta M (2015). Therapeutic review: Milk thistle. *J Exotic Pet Med*, 24, 4, 470–472.

- Ceriello A (1997). Acute hyperglycaemia and oxidative stress generation. *Diab Med*, 14, 3, 45-49.
- Cheng YY, Leu HB, Chen TJ, Chen CL, Kuo CH, Lee SD, Kao CL (2014). Metformin-inclusive therapy reduces the risk of stroke in patients with diabetes: A 4-year follow-up study. *J Stroke Cerebrovasc Dis*, 23, 2, 99-105.
- Cherubini A, Ruggiero C, Polidori MC, Mecocci P (2005). Potential markers of oxidative stress in stroke. *Free Radic Biol Med*, 39, 7, 841-852.
- Cooke MS, Evans MD, Burd RM, Patel K, Barnard A, Lunec J, Hutchinson PE (2001). Induction and excretion of ultraviolet-induced 8-oxo-2'-deoxyguanosine and thymine dimers in vivo: implications for PUVA. *J Invest Dermatol*, 116, 2, 281-285.
- Cooke MS, Olinski R, Loft S (2008). European standards committee on urinary (DNA) lesion analysis measurement and meaning of oxidatively modified DNA lesions in urine. *Cancer Epidemiol Biomarkers Prev*, 17, 1, 3-14.
- Colturato CP, Constantin RP, Maeda AS Jr, Constantin RP, Yamamoto NS, Bracht A, Ishii-Iwamoto EL, Constantin J (2012). Metabolic effects of silibinin in the rat liver. *Chem Biol Interact*, 195, 2, 119-132.
- Cryer PE (2007). Hypoglycemia, functional brain failure, and brain death. *J Clin Invest*, 117, 4, 868-870.
- Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, Nicotera T (1996). Oxidative damage to DNA in diabetes mellitus. *Lancet*, 347, 444-445.
- Derosa G, D'Angelo A, Maffioli P (2015). The role of a fixed Berberis aristata/Silybum marianum combination in the treatment of type 1 diabetes mellitus. *Clin Nutr*, 15, 1-5.
- Desse TA, Eshetie TC, Gudina EK (2015). Predictors and treatment outcome of hyperglycemic emergencies at Jimma University Specialized Hospital, southwest Ethiopia. *BMC Res Notes*, 8, 553, 1-8.
- Dixit S, Maiya A, Shasthry BA, Kumaran DS, Guddattu V (2015). Postural sway in diabetic peripheral neuropathy among Indian elderly. *Ind J Med Res*, 142, 6, 713-720.
- El-Far M, Negm A, Wahdan M (2015). Promising biopharmaceutical use of *Silymarin* and *Silybin* as antidiabetic natural agents in streptozotocin-induced diabetic rats: First comparative assessment. *World J Pharm Pharmaceut Scien*, 4, 2, 27-28.
- El-Wassef M, El-Saeed GSM, El-Tokhy SE, Raslan HM, Tawfeek S, Siam I, Salem SI (2012). Oxidative DNA damage in patients with type 2 diabetes mellitus. *Diabetol Croat*, 41, 4, 121-127.
- Forlenza GP, Buckingham B, Maahs DM (2015). Progress in diabetes technology: Developments in insulin pumps, continuous glucose monitors, and progress towards the artificial pancreas. *J Pediatr*, 13-20.

Fowler MJ (2008). Microvascular and macrovascular complications of diabetes. *Clin Diabetes*, 26, 2, 77-82.

Fraga CG, Shigenaga MK, Park JW, Degan P, Ames BN (1990). Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc Natl Acad Sci USA*, 87, 12, 4533-4537.

Gallagher EJ, LeRoith D, Stasinopoulos M, Zelenko Z, Shiloach J (2016). Polyol accumulation in muscle and liver in a mouse model of type 2 diabetes. *J Diabet Compl*, 30, 6, 999-1007.

Gingras V, Rabasa-Lhoret R, Messier V, Ladouceur M, Legault L, Haidar A (2015). Efficacy of dual-hormone artificial pancreas to alleviate the carbohydrate-counting burden of type 1 diabetes: A randomized crossover trial. *Diabetes Metab*, 42, 1, 47-54.

Ghule EA, Jadhav SS, Bodhankar SL (2012). Effect of ethanolic extract of seeds of *Linum usitatissimum* (Linn.) in hyperglycaemia associated ROS production in PBMNCs and pancreatic tissue of alloxan induced diabetic rats. *Asia Pacif J Tropical Dis*, 2, 5, 405-410.

Goodarzi MT, Navidi AA, Rezaei M, Babahmadi-Rezaei H (2010). Oxidative damage to DNA and lipids: Correlation with protein glycation in patients with type 1 diabetes. *Clin Lab Anal*, 24, 2, 72-76.

Greenberg MM (2004). In vitro and in vivo effects of oxidative damage to deoxyguanosine. *Biochem Soc Trans*, 32, 1, 46-50.

Halliwell B, Aruoma OI (1991). DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett*, 281, 1, 2, 9-19.

Harms-Ringdahl M, Jenssen D, Haghdoust S (2012). Tomato juice intake suppressed serum concentration of 8-oxodG after extensive physical activity. *Nutr J*, 11, 29.

Hinokio Y, Suzuki S, Hirai M, Chiba M, Hirai A, Toyota T (1999). Oxidative DNA damage in diabetes mellitus: Its association with diabetic complications. *Diabetologia*, 42, 8, 995-998.

Hsieh HM, Lin TH, Lee IC, Huang CJ, Shin SJ, Chiu HC (2016). The association between participation in a pay-for-performance program and macrovascular complications in patients with type 2 diabetes in Taiwan: A nationwide population-based cohort study. *Prev Med*, 85, 53-59.

Huynh K, Bernardo BC, McMullen JR, Ritchie RH (2014). Diabetic cardiomyopathy: Mechanisms and new treatment strategies targeting antioxidant signaling pathways. *Pharmacol Ther*, 142, 3, 375-415.

Huang JP, Huang SS, Deng JY, Chang CC, Day YJ, Hung LM (2010). Insulin and resveratrol act synergistically, preventing cardiac dysfunction in diabetes, but the advantage of resveratrol in diabetics with acute heart attack is antagonized by insulin. *Free Radic Biol Med*, 49, 11, 1710-1721.

- Huseini HF, Larijani B, Heshmat R, Fakhrzadeh H, Radjabipour B, Toliat T, Raza M (2006). The efficacy of *Silybum marianum* (L.) Gaertn. (*Silymarin*) in the treatment of type II diabetes: A randomized, double-blind, placebo-controlled, clinical trial. *Phytother Res*, 20, 12, 1036-1039.
- Hwang JW, Pyun SB, Kwon HK (2016). Relationship of vascular factors on electro physiologic severity of diabetic neuropathy. *Ann Rehabil Med*, 40, 1, 56-65.
- Johnson BL, Blackhurst DW, Latham BB, Cull DL, Bour ES, Oliver TL, Williams B, Taylor SM, Scott JD (2013). Bariatric surgery is associated with a reduction in major macrovascular and microvascular complications in moderately to severely obese patients with type 2 diabetes mellitus. *J Am Coll Surg*, 216, 4, 545-556.
- Joshi SR, Parikh RM, Das AK (2007). Insulin-history, biochemistry, physiology and pharmacology. *J Assoc Physicians India*, 55, 19-25.
- Johansen JS, Harris AK, Rychly DJ, Ergul A (2005). Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovasc Diabetol*, 4, 5, 1-11.
- Juśkiewicz J, Zduńczyk Z, Jurgoński A, Brzuzan Ł, Godycka-Kłos I, Zary-Sikorska E (2008). Extract of green tea leaves partially attenuates streptozotocin-induced changes in antioxidant status and gastrointestinal functioning in rats. *Nutr Res*, 28, 5, 343-349.
- Kaatabi H, Bamosa AO, Badar A, Al-Elq A, Abou-Hozafa B, Lebda F, Al-Khadra A, Al-Almaie S (2015). *Nigella sativa* improves glycemic control and ameliorates oxidative stress in patients with type 2 diabetes mellitus: placebo controlled participant blinded clinical trial. *PLoS One*, 10, 2, 1-15.
- Kai M (2016). Roles of RNA-binding proteins in DNA damage response. *Int J Mol Sci*, 17, 310, 1-9.
- Kakimoto M, Inoguchi T, Sonta T, Yu HY, Imamura M, Etoh T, Hashimoto T, Nawata H (2002). Accumulation of 8-hydroxy-2'-deoxyguanosine and mitochondrial DNA deletion in kidney of diabetic rats. *Diabet*, 51, 5, 1588-1595.
- Kamiya H, Suzuki A, Yamaguchi Y, Handa H, Harashima H (2009). Incorporation of 8-hydroxyguanosine (8-oxo-7, 8-dihydroguanosine) 5'-triphosphate by bacterial and human RNA polymerases. *Free Radic Biol Med*, 46, 12, 1703-1707.
- Kanauchi M, Nishioka H, Hashimoto T (2002). Oxidative DNA damage and tubulointerstitial injury in diabetic nephropathy. *Nephron*, 91, 2, 327-329.
- Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H (1986). Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis*, 7, 11, 1849-1851.
- Kasai H (1997). Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat Res*, 387, 3, 147-163.

- Kaur AK, Wahi AK, Kumar B, Bhandari A, Prasad N (2011). Milk thistle (*Silybum marianum*). *IJPRD*, 3, 2, 1-10.
- Kelly MC, Whitaker G, White B, Smyth MR (2007). Nickel (II)-catalysed oxidative guanine and DNA damage beyond 8-oxoguanine. *Free Radic Biol Med*, 42, 11, 1680-1689.
- Khan I, Yousif AM, Johnson SK, Gamlath S (2015). Acute effect of sorghum flour-containing pasta on plasma total polyphenols, antioxidant capacity and oxidative stress markers in healthy subjects: A randomised controlled trial. *Clin Nutr*, 34, 3, 415-421.
- Kilarkaje N, Al-Hussaini A, Al-Bader M (2014). Diabetes-induced DNA damage and apoptosis are associated with poly (ADP ribose) polymerase 1 inhibition in the rat testis. *Eur J Clin Pharmacol*, 15, 737, 29-40.
- Knak Jensen SJ (2003). Oxidative stress and free radicals. *J Mol Struct (Theochem)*, 666, 667, 387-392.
- Koulis C, Watson AM, Gray SP, Jandeleit-Dahm KA (2015). Linking RAGE and nox in diabetic micro and macrovascular complications. *Diabetes Metab*, 41, 4, 272-281.
- Kumari S, Rastogi RP, Singh KL, Singh SP and Sinha RP (2008). DNA damage: Detection strategies. *EXCLI J*, 7, 44-62.
- Kushwaha S, Vikram A, Trivedi PP, Jena GB (2011). Alkaline, endo III and FPG modified comet assay as biomarkers for the detection of oxidative DNA damage in rats with experimentally induced diabetes. *Muta Res*, 726, 2, 242-250.
- Kulaksızoglu S, Karalezli A (2015). Aqueous humour and serum levels of nitric oxide, malondialdehyde and total antioxidant status in patients with type 2 diabetes with proliferative diabetic retinopathy and nondiabetic senile cataracts. *Can J Diabetes*, 40, 2, 115-119.
- Lee J, Lee M, Kim JU, Song KI, Choi YS, Cheong SS (2005). Carvedilol reduces plasma 8-hydroxy-2'-deoxyguanosine in mild to moderate hypertension: A pilot study. *Hypertension*, 45, 5, 986-990.
- Lei C, Wu B, Liu M, Chen Y (2014). Association between hemoglobin A1c levels and clinical outcome in ischemic stroke patients with or without diabetes. *J Clinical Neuroscience*, 498-503.
- Liu XX, Liu KY, Li P, Han S, Peng XD, Shen L (2014). Adiponectin is expressed in the pancreas of high-fat-diet-fed mice and protects pancreatic endothelial function during the development of type 2 diabetes. *Diabetes Metab*, 40, 5, 363-372.
- Leinonen J, Lehtimäki T, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, Laippala P, Rantalaiho V, Wirta O, Pasternack A, Alho H (1997). New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. *FEBS Lett*, 417, 1, 150-152.

- Lombardo M, Parravano M, Serrao S, Ziccardi L, Giannini D, Lombardo G (2016). Investigation of adaptive optics imaging biomarkers for detecting pathological changes of the cone mosaic in patients with type 1 diabetes mellitus. *PLoS One*, 11, 3, e0151380.
- Lorenzi M, Montisano DF, Toledo S, Barrieux A (1986). High glucose induces DNA damage in cultured human endothelial cells. *J Clin Invest*, 77, 1, 322-325.
- Luepker RV, Murray DM, Jacobs DR, Mittelmark MB, Bracht N, Carlaw R, Crow R, Elmer P, Finnegan J, Folsom AR, Grimm R, Hannan PJ, Jeffrey R, Lando R, McGovern P, Mullis R, Peny CL, Pechacek T, Pirie P, Spralka JM, Weisbrod R, Blackblum H (1994). Community education for cardiovascular disease prevention: Risk factor changes in the Minnesota Heart Health Program. *Am J Public Health*, 84, 9, 1383-1393.
- Maghrani M, Zeggwagh NA, Lemhadri A, El Amraoui M, Michel JB, Eddouks M (2004). Study of the hypoglycaemic activity of *Fraxinus excelsior* and *Silybum marianum* in an animal model of type 1 diabetes mellitus. *J Ethnopharmacol*, 91, 2-3, 309-316.
- Mahmoudi M, Mercer J, Bennett M (2006). DNA damage and repair in atherosclerosis. *Cardiovasc Res*, 71, 2, 259-268.
- Mamur S, Unal F, Altok K, Deger SM, Yuzbasioglu D (2016). DNA damage in hemodialysis patients with chronic kidney disease; a test of the role of diabetes mellitus; a comet assay investigation. *Mutat Res*, 800, 801, 22-27.
- Maritim AC, Sanders RA, Watkins JB (2003). Diabetes, oxidative stress, and antioxidants: A review. *J Biochem Mol Toxicol*, 17, 1, 24-38.
- Mellado-Gil JM, Jiménez-Moreno CM, Martín-Montalvo A, Álvarez-Mercado AI, Fuente-Martín E, Cobo-Vuilleumier N, Lorenzo PI, Bru-Tari E, De Gracia Herrera-Gómez I, López-Noriega L, Pérez-Florido J, Santoyo-López J, Spyralis A, Meda P, Boehm BO, Quesada I, Gauthier BR (2016). PAX4 preserves endoplasmic reticulum integrity preventing beta cell degeneration in a mouse model of type 1 diabetes mellitus. *Diabetologia*, 59, 4, 755-765.
- Mehar G, Asija R (2015). Relation of liver diseases in type II diabetes patients: An overview. *J Drug Discov Therapeutics*, 3, 27, 10-14.
- Merecz A, Markiewicz L, Sliwinska A, Kosmalski M, Kasznicki J, Drzewoski J, Majsterek I (2015). Analysis of oxidative DNA damage and its repair in Polish patients with diabetes mellitus type 2: Role in pathogenesis of diabetic neuropathy. *Adv Med Sci*, 60, 2, 220-230.
- Milic M, Frustaci A, Del Bufalo A, Sánchez-Alarcón J, Valencia-Quintana R, Russo P, Bonassi S (2015). DNA damage in non-communicable diseases: A clinical and epidemiological perspective. *Mutat Res*, 776, 118-127.

- Min J, Park B, Kim YJ, Lee H, Ha E, Park H (2009). Effect of oxidative stress on birth sizes: consideration of window from mid pregnancy to delivery. *Placenta*, 30, 5, 418-423.
- Murphy NM, McCarthy FP, Khashan AS, Myers JE, Simpson NA, Kearney PM, Greene RA, Poston L, Kenny LC (2016). Compliance with National Institute of Health and care excellence risk-based screening for gestational diabetes mellitus in nulliparous women. *Eur J Obstet Gynec Reprod Biol*, 199, 60-65.
- Nawaz Khan A, Ali Khan R, Ahmad M, Mushtaq N (2015). Role of antioxidant in oxidative stress and diabetes mellitus. *J Pharmac Phytochem*, 3, 6, 217-220.
- Neufang M (2011). Coma diabeticum. *Doctor Consult – J. Wissen für Klin Prax*, 2, 217-220.
- Nichols JA, Katiyar SK (2010). Skin photoprotection by natural polyphenols: Anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch Derma Res*, 302, 2, 71-83.
- Nishimura S (2011). 8-Hydroxyguanine: A base for discovery. *DNA Rep (Amst)*, 10, 11, 1078-1083.
- Nishikawa T, Sasahara T, Kiritoshi S, Sonoda K, Senokuchi T, Matsuo T, Kukidome D, Wake N, Matsumura T, Miyamura N, Sakakida M, Kishikawa H, Araki E (2003). Evaluation of urinary 8-hydroxydeoxy-guanosine as a novel biomarker of macrovascular complications in type 2 diabetes. *Diabetes Care*, 26, 5, 1507-1512.
- Ogden A (2010). Diabetes A to Z, 6th ed. by the American Diabetes Association, Virginia, 171-172.
- Olamoyegun M, Ibraheem W, Iwuala S, Audu M, Kolawole B (2015). Burden and pattern of micro vascular complications in type 2 diabetes in a tertiary health institution in Nigeria. *Afr Health Sci*, 15, 4, 1136-1141.
- Olagbuji BN, Atiba AS, Olofinbiyi BA, Akintayo AA, Awoleke JO, Ade-Ojo IP, Fasubaa OB; Gestational Diabetes Study Group-Nigeria (2015). Prevalence of and risk factors for gestational diabetes using 1999, 2013 WHO and IADPSG criteria upon implementation of a universal one-step screening and diagnostic strategy in a sub-Saharan African population. *Eur J Obstet Gynecol Reprod Biol*, 189, 27-32.
- Packer L, Rosen P, King GL, Tritschler HJ, Azzi A (2000). Antioxidant in diabetes management, USA, Marcel Dekker, Chapter 13, 185.
- Pan HZ, Chang D, Feng LG, Xu FJ, Kuang HY, Lu MJ (2007). Oxidative damage to DNA and its relationship with diabetic complications. *Biomed Environ Sci*, 20, 2, 160-163.
- Pan HZ, Zhang L, Guo MY, Sui H, Li H, Wu WH, Qu NQ, Liang MH, Chang D (2010). The oxidative stress status in diabetes mellitus and diabetic nephropathy. *Acta Diabetol*, 47, 1, 71-76.

- Patel DK, Kumar R, Laloo D, Hemalatha S (2012). Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects. *Asian Pac J Trop Dis*, 239-250.
- Petersen KS, Smith C (2016). Ageing-associated oxidative stress and inflammation are alleviated by products from grapes. *Oxid Med Cell Longev*, 6236309.
- Pourvali K, Abbasi M, Mottaghi A (2016). Role of superoxide dismutase 2 gene ala16 val polymorphism and total antioxidant capacity in diabetes and its complications. *Avicenna J Med Biotech*, 8, 2, 48-56.
- Qari F (2015). Clinical characteristics of patients with diabetic ketoacidosis at the Intensive Care Unit of a University Hospital. *Pak J Med Sci*, 31, 6, 1463-1466.
- Qiu C, Hevner K, Abetew D, Enquobahrie DA, Williams MA (2011). Oxidative DNA damage in early pregnancy and risk of gestational diabetes mellitus: A pilot study. *Clin Biochem*, 44, 10-11, 804-808.
- Quilichini E, Haumaitre C (2015). Implication of epigenetics in pancreas development and disease. *Best Pract Res Clin Endocrinol Metab*, 29, 6, 883-898.
- Radomska-Leśniewska DM, Hevelke A, Skopiński P, Bałan B, Józwiak J, Rokicki D, Skopińska-Różewska E, Białoszevska A (2016). Reactive oxygen species and synthetic antioxidants as angiogenesis modulators: Clinic implication. *Pharmacol Rep*, 68, 2, 462-471.
- Rady MR, Matter MA, Ghareeb HA, Hanafy MS, Saker MM, Eid SA, Hammada FM, Shams I, Imbaby SI, Naglaa H, Nazief NH (2014). In vitro cultures of *Silybum marianum* and *Silymarin* accumulation. *Genet Eng Biotechnol J*, 12, 1, 75-79.
- Rahmannezhad G, Mashayekhi FJ, Goodarzi MT, Rezvanfar MR, Sadeghi A (2016). Association between vitamin D receptor ApaI and TaqI gene polymorphisms and gestational diabetes mellitus in an Iranian pregnant women population. *Gen*, 581, 1, 43-47.
- Ramachandran S, Naveen KR, Baskaran Rajinikanth B, Akbar M, Rajasekaran A (2012). Antidiabetic, antihyperlipidemic and in vivo antioxidant potential of aqueous extract of *Anogeissus latifolia* bark in type 2 diabetic rats. *Asia Pacific J Tropic Dis*, S596-S602.
- Ramasamy K, Agarwal R (2008). Multitargeted therapy of cancer by *Silymarin*. *Cancer Lett*, 269, 2, 352-362.
- Rastogi L, Feroz S, Pandey BN, Jagtap A, Mishra KP (2010). Protection against radiation-induced oxidative damage by an ethanolic extract of *Nigella sativa* L. *Int J Radiat Biol*, 86, 9, 719-731.
- Reifegerste D, Hartleib S (2016). Hypoglycemia-related information seeking among informal caregivers of type 2 diabetes patients: Implications for health education. *J Clin Transl Endocrinol*, 4, 7-12.

Ridha TM, Moog CH (2015). Model free control for type-1 diabetes: A fasting-phase study. *IFAC-Papers Online*, 48-20, 076-081.

Robertson RP, Zhang HJ, Pyzdrowski KL, Walseth TF (1992). Preservation of insulin mRNA levels and insulin secretion in HIT cells by avoidance of chronic exposure to high glucose concentrations. *J Clin Invest*, 90, 2, 320-325.

Rodríguez AJ, Nunes Vdos S, Mastronardi CA, Neeman T, Paz-Filho GJ (2016). Association between circulating adipocytokine concentrations and microvascular complications in patients with type 2 diabetes mellitus: A systematic review and meta-analysis of controlled cross-sectional studies. *J Diabetes Complications*, 30, 2, 357-367.

Rolo AP, Palmeira CM (2006). Diabetes and mitochondrial function: Role of hyperglycemia and oxidative stress. *Toxicol Appl Pharmacol*, 212, 2, 167-178.

Safi SZ, Batumalaie K, Qvist R, Mohd Yusof K, Ismail IS (2016). Gelam honey attenuates the oxidative stress-induced inflammatory pathways in pancreatic hamster cells. *Evid Based Compl Altern Med*, 5843615, 1-13.

Sajedianfard J, Behrooz Z, Nazifi S, Rajaian H (2013). Effect of different oral doses of hydroalcoholic extract of *Silymarin* on the blood oxidative stress indicators in streptozotocin induced diabetic rats. *Int J Pept Res Ther*, 19, 1, 93-98.

Sajedianfard J, Behrooz Z, Nazifi S (2014). The effects of a hydroalcoholic extract of *Silymarin* on serum lipids profiles in streptozotocine induced diabetic rats. *Comp Clin Pathol*, 23, 3, 779-784.

Salardi S, Porta M, Maltoni G, Cerutti F, Rovere S, Iafusco D, Tumini S, Cauvin V, Zucchini S, Cadario F, D'Annunzio G, Toni S, Salvatori A, Zedda MA, Schiaffini R (2016). Diabetes study group of the Italian Society of Pediatric Endocrinology and Diabetology (ISPED) ketoacidosis at diagnosis in childhood-onset diabetes and the risk of retinopathy 20 years later. *J Diabetes Complications*, 30, 1, 55-60.

Samson WK, Stein LM, Elrick M, Salvatori A, Kolar G, Corbett JA, Yosten GL (2016). Hypoglycemia unawareness prevention: Targeting glucagon production. *Physiol Behav*, 162, 30, 147-150.

Sanocka D, Kurpisz M (2004). Reactive oxygen species and sperm cells. *Reprod Biol Endocrinol*, 2, 12, 1-7.

Savage DB, Petersen KF, Shulman GI (2005). Mechanisms of insulin resistance in humans and possible links with inflammation. *Hypertension*, 45, 5, 828-833.

Seghrouchni I, Draï J, Bannier E, Rivière J, Calmard P, Garcia I, Orgiazzi J, Revol A (2002). Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency. *Clin Chim Acta*, 321, 1, 2, 89-96.

Shakeel AH, Yar AK (2014). Effect of *Milk thistle (Silybum marianum)* plant parts (seeds and leaves) to control the alloxan induced diabetes in rabbits. *Global J Res. Med. Plants Indigen Med*, 3, 1, 1-7.

- Shin CS, Moon BS, Park KS, Kim SY, Park SJ, Chung MH, Lee HK (2001). Serum 8-hydroxy-guanine levels are increased in diabetic patients. *Diabetes Care*, 24, 4, 733-737.
- Shinde SN, Dhadke VN, Suryakar AN (2010). Evaluation of oxidative stress in Type 2 diabetes mellitus and follow-up along with vitamin E supplementation. *Ind J Clin Biochem*, 21, 1, 74-77.
- Shou J, Zhou L, Zhu S, Zhang X (2015). Diabetes is an independent risk factor for stroke recurrence in stroke patients: A meta-analysis. *J Stroke Cerebrovasc Dis*, 24, 9, 1961-1968.
- Sikka SC, Rajasekaran M, Hellstrom WJ (1995). Role of oxidative stress and antioxidants in male infertility. *J Androl*, 16, 6, 464-468.
- Simone S, Gorin Y, Velagapudi C, Abboud HE, Habib SL (2008). Mechanism of oxidative DNA damage in diabetes: tuberin inactivation and downregulation of DNA repair enzyme 8-oxo-7, 8-dihydro-2'-deoxyguanosine-DNA glycosylase. *Diabetes*, 57, 10, 2626-2636.
- Son SM, Whalin MK, Harrison DG, Taylor WR, Griendling KK (2004). Oxidative stress and diabetic vascular complications. *Curr Diab Rep*, 4, 4, 247-252.
- Soto C, Recoba R, Barrón H, Alvarez C, Favari L (2003). *Silymarin* increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. *Comp Biochem Physiol C Toxicol Pharmacol*, 136, 3, 205-212.
- Soto C, Mena R, Luna J, Cerbón M, Larrieta E, Vital P, Uría E, Sánchez M, Recoba R, Barrón H, Favari L, Lara A (2004). *Silymarin* induces recovery of pancreatic function after alloxan damage in rats. *Life Sci*, 75, 18, 2167-2180.
- Styskal J, Van Remmen H, Richardson A, Salmon AB (2012). Oxidative stress and diabetes: What can we learn about insulin resistance from antioxidant mutant mouse models? *Free Radic Biol Med*, 52, 1, 46-58.
- Sugiyama T, Metoki H, Hamada H, Nishigori H, Saito M, Yaegashi N, Kusaka H, Kawano R, Ichihara K, Yasuhi I, Hiramatsu Y, Sagawa N (2014). A retrospective multi-institutional study of treatment for mild gestational diabetes in Japan. *Diabetes Res Clin Pract*, 103, 3, 412-418.
- Suksomboon N, Poolsup N, Boonkaew S, Suthisisang CC (2011). Meta-analysis of the effect of herbal supplement on glycemic control in type 2 diabetes. *J Ethnopharmacol* 137, 3, 1328-1333.
- Sun Y, Zhao H (2016). The effectiveness of lifestyle intervention in early pregnancy to prevent gestational diabetes mellitus in Chinese overweight and obese women: A quasi-experimental study. *Appl Nurs Res*, 30, 125-130.
- Suzuki S, Hinokio Y, Komatu K, Ohtomo M, Onoda M, Hirai S, Hirai M, Hirai A, Chiba M, Kasuga S, Akai H, Toyota T (1999). Oxidative damage to mitochondrial

DNA and its relationship to diabetic complications. *Diabetes Res Clin Pract*, 45, 2-3,161-168.

Svobodová A, Zdarilová A, Walterová D, Vostálová J (2007a). Flavonolignans from *Silybum marianum* moderate UVA-induced oxidative damage to HaCaT keratinocytes. *J Dermatol Sci*, 48, 3, 213-224.

Svobodová A, Zdarilová A, Malisková J, Mikulková H, Walterová D, Vostálová J (2007b). Attenuation of UVA-induced damage to human keratinocytes by *Silymarin*. *J Dermatol Sci*, 46, 1, 21-30.

Takakura S, Toyoshi T, Hayashizaki Y, Takasu T (2016). Effect of ipragliflozin, an SGLT2 inhibitor, on progression of diabetic microvascular complications in spontaneously diabetic *Torii fatty rats*. *Life Sci*, 147, 125-131.

Tatsch E, Bochi GV, Piva SJ, De Carvalho JA, Kober H, Torbitz VD, Duarte T, Signor C, Coelho AC, Duarte MM, Montagner GF, Da Cruz IB, Moresco RN (2012). Association between DNA strand breakage and oxidative, inflammatory and endothelial biomarkers in type 2 diabetes. *Mutat Res*, 732, 1, 16-20.

Toorang F, Djazayeri A, Djalali M (2016). Effects of Omega-3 fatty acids supplement on antioxidant enzymes activity in type 2 diabetic patients. *Iran J Publ Health*, 45, 3, 340-345.

Toklu HZ, Tunali-Akbay T, Erkanli G, Yüksel M, Ercan F, Sener G (2007). *Silymarin*, the antioxidant component of *Silybum marianum*, protects against burn-induced oxidative skin injury. *Burns*, 33, 7, 908-916.

Tousson E, Beltagy DM, Gazia MA, Al-Behbehani B (2013). Expressions of P53 and CD68 in mouse liver with *Schistosoma mansoni* infection and the protective role of *silymarin*. *Toxicol Ind Health*, 29, 8, 761-770.

Tucker PS, Scanlan AT, Dalbo VJ (2015). Chronic kidney disease influences multiple systems: Describing the relationship between oxidative stress, inflammation, kidney damage, and concomitant disease. *Oxid Med Cell Longev*, 806358, 1-8.

Tyrberg B, Anachkov KA, Dib SA, Wang-Rodriguez J, Yoon KH, Levine F (2002). Islet expression of the DNA repair enzyme 8-oxoguanosine DNA glycosylase (Ogg1) in human type 2 diabetes. *BMC Endocr Disord*, 2, 1, 2.

Ullah A, Khan A, Khan I (2015). Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharm J*, 1319, 164, 76-86.

Unal D, Kara A, Aksak S, Altunkaynak ZB, Yıldırım S (2012). Insulin hormone: Mechanism and effects on the body and relationship with central nervous system. *Dic Med J*, 39, 2, 310-315.

Valavanidis A, Vlachogianni T, Fiotakis C (2009). 8-hydroxy-2'-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*, 2, 120-139.

- Vaurs C, Brun JF, Bertrand M, Burcelin R, du Rieu MC, Anduze Y, Hanaire H, Ritz P (2016). Post-prandial hypoglycemia results from a non-glucose-dependent inappropriate insulin secretion in Roux-en-Y gastric bypassed patients. *Metabolism*, 65, 3, 18-26.
- Wang X, Tang L, Gao L, Yang Y, Cao D, Li Y (2016). Myopia and diabetic retinopathy: A systematic review and meta-analysis. *Diabetes Res Clin Pract*, 111, 1-9.
- Watson JD, Baker TA, Bell SP, Gann A, Levine M, Losick R (2004). Molecular Biology of the Gene. Fifth Edition, San Francisco, Chapter 6, 97.
- Weidmann AE (2012). Dihydroquercetin: More than just an impurity? *Eur J Phar*, 684, 1, 3, 19-26.
- Wilcox G (2005) Insulin and insulin resistance. *Clin Biochem Rev*, 26, 2, 19-39.
- Wojtunik-Kulesza KA, Oniszczyk A, Oniszczyk T, Waksmundzka-Hajnos M (2016). The influence of common free radicals and antioxidants on development of Alzheimer's disease. *Biomed Pharmacother*, 78, 39-49.
- Wong VW, Chalasani N (2016). Not routine screening, but vigilance for chronic liver disease in patients with type 2 diabetes. *J Hepatol*, 64, 6, 1211-1213.
- Xavier DJ, Takahashi P, Manoel-Caetano FS, Foss-Freitas MC, Foss MC, Donadi EA, Passos GA, Sakamoto-Hojo ET (2014). One-week intervention period led to improvements in glycemic control and reduction in DNA damage levels in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract*, 105, 3, 356-363.
- Yin DH, Liang XC, Zhao LI, Zhang H, Sun Q, Wang PY, Sun LQ (2015). Jinmaitong decreases sciatic nerve DNA oxidative damage and apoptosis in a streptozotocin-induced diabetic rat model. *Exp Ther Med*, 10, 2, 778-786.
- Yue R, Xia X, Jiang J, Yang D, Han Y, Chen X, Cai Y, Li L, Wang WE Zeng C (2015). Mitochondrial DNA oxidative damage contributes to cardiomyocyte ischemia/reperfusion-injury in rats: cardio protective role of lycopene. *Cell Physiol*. 230, 9, 2128-2141.
- Zalewska A, Knaś M, Maciejczyk M, Waszkiewicz N, Klimiuk A, Choromańska M, Matczuk J, Waszkiel D, Car H (2015). Antioxidant profile, carbonyl and lipid oxidation markers in the parotid and submandibular glands of rats in different periods of streptozotocin induced diabetes. *Arch Oral Biol*, 60, 9, 1375-1386.
- Zemestani M, Rafrat M, Asghari-Jafarabadi M (2015). Chamomile tea improves glycemic indices and antioxidants status in patients with type 2 diabetes mellitus. *Nutrition*, 32, 1, 66-72.
- Zephy D, Ahmad J (2015). Type 2 diabetes mellitus: Role of melatonin and oxidative stress. *Diabetes Metab Syndr*, 9, 2, 127-131.
- Ziqiang X, Yunqiang H, Hongxing F, Jinjun W, Yong C (2016). Islet transplantation restores the damage of glomerulus filtration membrane in a rat model of streptozotocin-induced diabetic nephropathy. *J Pak Med Assoc*, 66, 3, 296-301.

CURRICULUM VITAE

Gulala Ahmed Mohammed, I am from Erbil city in the North of Iraq, I was born on April 1, 1964, in Slemaniya city. I finished Primary, Secondary and High Schools in Slemaniya, after that I went to Baghdad city to begin my study at the University of Baghdad-College of Science/Chemistry during the years (1985-1989), I was awarded a Bachelor's degree in Chemistry. While I got my Bachelor degree in Baghdad University I worked as a Teacher (High Schools) at the Ministry of education (1990-1996), later I moved to Health sector (1996) Ministry of Health I worked as Chemist in Laboratory Biochemistry department in Rizgary Hospital in Erbil. After that I worked as Head of biochemistry department, and Lab. manager deputy and Quality control manager in Laboratory /Medya clinical center Private Clinic at Erbil city. During my work Also I graduated college of Language /English in college of Sallahadin in Erbil city (2000-2005). Finally, I came to the Republic of Turkey to begin the postgraduate admission process at the Yuzuncu Yil University-Institute of Health Science, Department of Basic Pharmaceutical Science / Biochemistry Science during the years (2014-2016).

ATTACHMENT

Attachment 1. Acceptance letter from experimental animal ethics committee

Evrak Tarih ve Sayısı: 01/02/2016-E.5533

T.C. YÜZÜNCÜ YIL ÜNİVERSİTESİ HAYVAN DENEYLERİ YEREL ETİK KURULU

ARAŞTIRMA BAŞVURU ONAY BELGESİ

Araştırmanın Adı	Deneysel Diyabetli Sıçanlarda <i>Silybum marianum</i> Ekstresinin Serum 8-Hidroksi-2'-Deoksiguanozin, Total Oksidan ve Antioksidan Kapasiteleri Üzerine Etkisi
Araştırmanın Yürütücüsü	Doç. Dr. Tahir KAHRAMAN
Yardımcı Araştırmacılar	Chemist Gulnara Ahmed Muhammed SHINKI
Kurumu	Eczacılık Fakültesi
Araştırmanın Tahmini Süresi	10 Ay
Kullanılacak Hayvan Türü ve Sayısı	Sıçan 28 Adet
Destekleyecek Kuruluş (lar)	YYÜ. Bilimsel Araştırma Projeleri Başkanlığı
Başvuru Tarihi	04.01.2016

KARAR BİLGİLERİ	Karar No:2016/01 Tarih:28.01.2016 Yüzüncü Yıl Üniversitesi Eczacılık Fakültesi Öğretim Üyesi/Öğrencisi Doç. Dr. Tahir KAHRAMAN sorumluluğunda yürütülmesi planlanan ve yukarıda başvuru bilgileri verilen Yüksek Lisans projesi gerekçe, amaç ve yöntemler dikkate alınarak ilgil başvuru belgeleri incelendi. Çalışmanın etik açıdan uygun olduğuna, projenin aşağıdaki hususlar dikkate alınarak yürütülmesine ve proje yürütücüsüne iletilmesine oy birliği/_oy çokluğu ile karar verildi. 1) Projede herhangi bir değişiklik gerektiğinde komiteye yazılı olarak onay alınması. 2) Projede çalışacağı bildirilen şartlarda değişiklik olduğunda komiteye yazılı olarak onay alınması. 3) Deneysel hayvanların üzerinde yapılacak girişimler başlangıç ve bitiş tarihlerinin bildirilmesi. 4) Çalışma sırasında meydana gelen her türlü olumsuzluk bildirilmesi. 5) Çalışma tamamlandığında sonuç raporunun gönderilmesi.
------------------------	---

ETİK KURUL ÜYELERİ	
BASKAN	
Prof. Dr. Semiha DEDE	
ÜYELER	
Prof. Dr. Duran BOLAT	Prof. Dr. Sıddık KESKİN
Prof. Dr. Fazıl ŞEN	Doç. Dr. M. Fatih GARÇA
Doç. Dr. Şenol GÜZEL	Doç. Dr. Naim ÖZDAL
Doç. Dr. Mustafa DÜKMÜŞ	Doç. Dr. Abdullahi AKSAKAL
Yrd. Doç. Dr. Ferda KARAKUŞ	Yrd. Doç. Dr. Fatih KAZANCI
Vet. Hek. Yrd. Doç. Dr. Yıldırım BAŞBUĞAN	Zir. Mith. Kenan YEDİRİMOĞLU
Vet. Hek. İsmail Hakkı BEHÇET	

*Bu form YÜHADYEK tarafından doldurulacaktır.

Bu belge 5070 sayılı Elektronik İmza Kanununun 5. Maddesi gereğince güvenli elektronik imza ile imzalanmıştır.

Attachment 2. The acceptance letter for results of the thesis by experimental animal committee in Yuzuncu Yil University



**T.C.
YÜZÜNCÜ YIL ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
ARAŞTIRMA KESİN SONUÇ ONAY BELGESİ**

**YUZUNCU YIL UNIVERSITY (TURKEY)
ANIMAL RESEARCHES LOCAL ETHIC COMMITTEE
RESEARCH FINAL REPORT APPROVAL CERTIFICATE**

Araştırmanın Adı	Deneyisel Diyabetli Sıçanlarda <i>Silybum marianum</i> Ekstresinin Serum 8-Hidroksi-2'-Deoksiguanozin, Total Oksidan ve Antioksidan Kapasiteleri Üzerine Etkisi	
Title of the Research	Effect of the <i>Silybum marianum</i> extract in experimental diabetic rats on the sera 8-hydroxy-2'-deoxyguanosine, total oxidant and antioxidant capacity	
Araştırmacı(lar) Investigator(s)	Yürütücü / Chief investigator : Doç. Dr. Tahir KAHRAMAN Yardımcı Araştırmacı(lar) / Co-investigator(s): Gulala Ahmed MOHAMMED SHINKI	
Araştırmanın Başlama Tarihi / Research Starting Date:	28.01.2016	
Araştırmanın Bitiş Tarihi / Research Completion Date:	22.08.2016	
Proje Süresi / Total Time of Project:	10 ay	
Proje No / Project Number:	TYL-2016-5107	
Araştırmayı Destekleyen Kuruluş (varsa) / Funding institution(s) (if available):	YYÜ BAPB	
Destek Şekli ve Miktarı / Type and amount of funding:	Tez Projesi (Yüksek Lisans), 3.739.00.-TL	
Karar: Yukarıda bilgileri verilen araştırma projesinin kesin sonuç raporu Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun 25 / 08 / 2016 tarih ve 2016/08 sayılı kararı ile kabul edilmiştir. Decision: Final report of the research project detailed above was approved by Yuzuncu Yil University Animal Researches Local Ethic Committee in the session held on 25 / 08 / 2016 (decision number 2016/08).		
	BAŞKAN/CHAIR Prof. Dr. Seniha DEDE ÜYE	
ÜYE	ÜYE	ÜYE
Prof. Dr. Fazıl ŞEN ÜYE	 Prof. Dr. Sıddık KESKİN ÜYE	Prof. Dr. Suphi DENİZ ÜYE
 Doç. Dr. N. Tuğba BİNGÖL ÜYE	 Doç. Dr. Atila DURMUŞ ÜYE	Doç. Dr. Abdulkaki AKSAKAL ÜYE
Doç. Dr. Nalan İZDAL ÜYE	Yrd. Doç. Dr. Özer ALKAN ÜYE	Yrd. Doç. Dr. Feride KARAKUŞ ÜYE
Yrd. Doç. Dr. Yıldırım BAŞBUĞAN	 Vet. Hek. İsmail Hakkı BEHÇET	 Zir. Msc. Kerem YILDIRIMOĞLU