REPUBLIC OF TURKEY

VAN YUZUNCU YIL UNIVERSITY

INSTITUE OF HEALTH SCIENCE

ANTIOXIDANT AND ANTIHYPERLIPIDEMIC EFFECT OF *SOLANUM NIGRUM* FRUIT EXTRACT IN EXPERIMENTAL DIABETES MODEL

Mohammed NoorAddin Fethullah

DEPARTMENT OF BIOCHEMISTRY

(VETERINARY PROGRAM)

MASTER THESIS

SUPERVISOR

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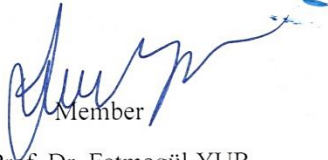SECOND SUPERVISOR

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Finally I would like to dedicate this thesis to my only son **Anas**, I also would like to use this opportunity to apologize from you to leaving you alone while I was busy completing this master, my dear son. I'm wishing that one day you realize that my absence was to create a better life for you.

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ABBREVIATIONS

DM	**:**	Diabetes Mellitus
T1DM	**:**	Type 1 diabetes mellitus
T2DM	**:**	Type 2 diabetes mellitus
GDM	**:**	Gestational diabetes mellitus
HDL	**:**	High-density lipoprotein
LDL	**:**	Low-density lipoprotein
VLDL	**:**	Very-low-density lipoprotein
TC	**:**	Total Cholesterol
TG	**:**	Triglyceride
TAC	**:**	Total antioxidant capacity
TAS	**:**	Total antioxidant status
TOS	**:**	Total oxidant status
DPP - 4	**:**	Dipeptidyl Peptidase-4
SGLT2	**:**	Sodium - glucose co - transporter 2
GLP - 1	**:**	Glucagon-like peptide-1
ROS	**:**	Reactive oxygen species
RNS	**:**	Reactive Nitrogen species
RCS	**:**	Reactive chlorine species
MAPK	**:**	Mitogen activated protein kinase
ERK	**:**	Extracellular- signal-regulated kinase
SOD	**:**	Superoxide dismutase
GSH–Px	**:**	Glutathione peroxidase
E. coli	**:**	*Escherichia coli*
AgNPs	**:**	Silver nanoparticles
STZ	**:**	Streptozocin
ATP	**:**	Adenosine triphosphate
NADP	**:**	Nicotinamide adenine dinucleotide phosphate
H_2O_2	**:**	Hydrogen peroxide
WHO	**:**	World Health Organization

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic non-communicable disease; it is globally considered as the fifth cause of death and it has attained worldwide epidemic proportions. As of 2015, more than 415 million adults were investigated to have DM, and this number is predicted to elevate to 642 million by 2040 (Domingueti et al., 2016; Unnikrishnan et al., 2016). DM is a group of metabolic disorder of carbohydrate, protein and fat which can be recognized by chronic hyperglycemia (the elevation of glucose level in blood) following defects in secretion and/or action of insulin, a protein (hormone)is produced in β-cells of pancreas (Patel et al., 2012). Hyperglycemia is also followed by the generation of reactive species (ROS) which subsequently leads to lipid peroxidation and membrane damage. The inhibition of peroxidation chain reaction through antioxidants protect β-cells from oxidation and consequently plays important roles in regulating related-metabolic activities to diabetes. Such antioxidants including phenolic compounds (tannins, flavonoids and stilbenes) and vitamin C and E can naturally be found from plant extracts preserving functions of β-cells and also preventing diabetes induced formation of ROS (Patel et al., 2011a; Patel et al., 2011b).

In addition, extracts of several herbal medicines have been used and recommended to have potential therapeutic effects on diabetes and its complications (Tiwari et al., 2002; Mukherjee et al., 2006).Extracts from *Solanum nigrum Linn* (European black nightshade) which belongs to *Solanum* genus and Solanaceae family (Knapp, 2002) has been reported to exhibit anti-tumor activity against different types of cancers (Xiang et al., 2016) and significant antidiabetic activities against diabetes (Poongothai et al., 2010).

Due to its antipyretic and diuretic activities, this plant is extensively used as Chinese folk medicine. Recent studies revealed that extracts of *S. nigrum* exhibits antioxidant (Aly and Shallan, 2011), hepatoprotective (Hsu et al., 2009), antihyperlipidemic and antidiabetic (Hou et al., 2013; Sohrabipour et al., 2013) and anti-inflammatory activities (Wang et al., 2017).

In regards to the above statements, biological and therapeutic activities of this plant can considerably be varied according to where is grown and its cultivars are being grown (retrieved from Poongothai et al., 2010). Therefore, biochemical activities of strains or accessions of this plant can be varied according to their origins and their locations are being grown. The present study is carried out to evaluate antioxidant and antihyperlipidemic activities of the extract of *S. nigrum Linn* grown in northern Iraq on the rate of diabetes of rats.

We can say that our first goal is evaluate whether antioxidant and antihyperlipidemic effect of *Solanum nigrum* fruit extract on experimental diabetic rats.

2. GENERAL INFORMATION

2.1. What is Diabetes Mellitus (DM)

The term *diabetes* is a Greek word which means "to run through" or "asiphon" that describes the excessive urination. Moreover, the term *mellitus* is originated from the Latin and Greek ancestry for word "honey," that was latterly added to the name of this disorder, the taste of diabetic urine was known to be sweet. This condition has been used as an indication to recognize this disease by ancient traditional medicinal systems such as Egyptian medicine or Ayurveda in India (Scobie 2006; Anjana et al., 2011).

DM can be characterized by increased concentrations of plasma glucose following significant deficiency of insulin, resistance of insulin, or both, leading to metabolic disorders and glucose intolerance, this metabolic abnormality is characterized by hyperglycemia (Iso et al., 2006). This condition is associated with the increase of metabolic disturbance of carbohydrate, fat, and protein enhancing the production of free radicals that follow by oxidative stress, renal failure, neurodegeneration, cardiovascular abnormalities and immune dysfunction (Hung et al., 2012).

The general symptoms of DM are recognised by thirst, loss of weight, blurring of vision and polyuria. Ketoacidosis (a serious complication of DM that presents when the body produces blood acids at high levels) or a non-ketotic hyperosmolar state (a serious condition occurs as a result of extremely high levels of blood sugar in the body) is a valuable complication of severe diabetic progression, might result in stupor, and the absence of effective treatment may increase the risk of coma and eventually death (Sen et al., 2016).

Over the past decade, the global diabetic burden it has been evidenced to increase sharply as a consequence of the evolving diabetes pandemic. DM is becoming one of the leading health challenges worldwide which affect individuals of all ages, namely children, young, adults, and pregnant women. DM and its complications as a result of DM impose quite a remarkable burden on individuals and also on healthcare system (World Health Organization, 1999).

2.2. Classification of DM

World Health Organization (WHO) in 1980 published the classification of DM and it subsequently revised by 1985. The first report of WHO stated two major classes of DM including insulin-dependent diabetes mellitus (IDDM) diabetes and non-insulin-dependent diabetes mellitus (NIDDM) or type1 and type 2 diabetes subsequently. By 1985, along with the classes IDDM and NIDDM, the Expert Committee of WHO named another class and is called "malnutrition- related DM". Based on the both reports of 1980 and 1985, WHO is also characterized another classes of diabetes, namely other types, gestational diabetes mellitus (GDM) and impaired glucose tolerance (IGT). The 1895's classification was globally accepted and is also used internationally (World Health Organization, 2006; American Diabetes Association 2013). Currently, according to etiology, DM is classified into four types as follow (DeFronzo et al., 2015; Sen et al., 2016):

1. Type 1 diabetes mellitus (IDDM) or (T1DM)

2. Type 2 diabetes mellitus (NIDDM) or (T2DM)

3. Other specific types including Genetic defects of β-cell function, Genetic defects in insulin action, Diseases of the exocrine pancreas, Endocrinopathies, Drug- or chemical-induced, Infections, Uncommon forms of immune-mediated diabetes, Other genetic syndromes sometimes associated with diabetes

4. Gestational diabetes mellitus (GDM)

Moreover, in 1999, the WHO revealed an approach that clearly separated the related-criteria to etiology from the degree of insulin deficiency or insulin action, and then defined each patient based on these two sets of criteria (Figure 1). The disease process might present but may not be far enough progressed to lead to hyperglycemia. The etiological classification can be possible as the defect or process that might cause diabetes and can be diagnosed at any stage in the developmental stages of diabetes, even at normoglycemia stage (American Diabetes Association, 2014).

Stages Types	Normoglycemia Normal glucose tolerance	Hyperglycemia			
		IGT and/or IFG	Diabetes Mellitus		
			Not insulin requiring	Insulin: for control	Insulin: for survival
Type 1 • Autoimmune • Idiopathic					
Type 2 • Predom. • insulin resistance • Predom. insulin secretory defects					

Other specific types
• Genetic defects of
  β-cell function
• Genetic defects of
  insulin action
• Diseases of exocrine
  pancreas
• Endocrinopathies
• Drug or chemical induced
• Others					

Gestational hyperglycemia					

Figure 1. Disorders of glycemia: etiologic types and clinical stages. Retrieved from (DeFronzo et al., 2015).

2.2.1. Type 1 diabetes mellitus (T1DM)

T1DM was also called IDDM. Earlier it was also called as "juvenile onset diabetes," it is mostly afflicts people in puberty or youngsters. However, some latent forms can appear later in life. This type of DM results from complete deficiency of insulin following the destruction of pancreatic β cell. According to recent epidemiological studies, the frequency of T1DM is increased among children and young aged individuals (Sen et al., 2016).

In developed countries, T1DM occupies about 5–10 % of all DM, and this range is even higher in developing countries. This type of DM is further classified to type 1A (also known as immune-mediated Diabetes) and type 1B or idiopathic diabetes (Sperling, 2003; Mohan, 2005; Davis, 2006; Scobie, 2007; Tripathi, 2013). Ozougwu et al., (2013) revealed that the beginning of T1DM refers to the end-stage β cell's destruction. Though the precise etiology and pathogenesis of this type of DM is still

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unknown, genetic factor and environmental factors including infection and disease is well acknowledged to have effective roles on T1DM.

2.2.2. Type 2 diabetes mellitus (T2DM)

T2DM is exemplified through resistance of tissue toward either the insulin action or relative decrease in the secretion of insulin. This type of DM is associated with obesity and often occurs later in life (usually after an age of 40). T2DM can be asymptomatic (remains undetected) for several years, which might subsequently causes different diabetic complications. Individuals suffering from this type of DM are not reliant on exogenous source of insulin. T1DM represents about 90–95 % of total DM cases. Ketoacidosis is less likely but possible in this form of diabetes (Mohan 2003; Davis 2006; Scobie 2006; Tripathi 2013; DeFronzo et al., 2015).

As stated by Sen et al. (2016), there are three major problems related to T2DM including the diminished secretion of insulin, impaired action of insulin and increased production of hepatic glucose. Pathogenesis of T2DM can be associated with the involvement of numerous factors, in which disturbance of the cross talk between the pancreas, liver, adipose tissue, skeletal muscle and probably central nervous system might be in charge of the fluctuation of glucose homeostasis in T2DM.

A comprehensive comparison between T1DM and T2DM, representing their symptoms, risk factors, drugs and type of population affected are shown in (Table 1).

Table 1. Comparisons between T1DM and T2DM (Sen et al., 2016).

Feature	T1DM	T2DM
General	Absolute deficiency of insulin	Either insulin resistance or decrease in producing insulin
Type of individuals affected	In general, people aged from puberty to youngster (<35 years).	In general, people aged from middle to old (>35 years).
Incidence	represents about 5–10 % of total DM cases	Represents about 90–95 % of total DM cases
β-cell	Reduced	Variable
Antibodies to β-cells	Yes, about 90–95 %	No
Inflammatory cell in islet	Might initially present	Absent
Identical twins concordance	About 30–50 %	About 90–95 %
Genetic predisposition	Low	High
Family history of diabetes	Uncommon	Common
Metabolic ketoacidosis	Often or frequent	It is rare except in African-Americans
Habitus of body	Can be normal or wasted	Might be obese
Obesity at onset	Uncommon	Common
Sign and symptoms	Sudden and dramatic dry mouth and thirst, persistent urination, strong tiredness, constant hunger, hyperglycemia, blurry in vision, frequent infections, weight loss and slow-healing wounds	Polyuria, polyphagia, polydipsia, glycosuria, hyperglycemia, recurrent infections, dry/itchy skin and sexual dysfunction
Risk factor	Incompatibility in blood group, maternal viral infections during pregnancy, early exposure to components of cow's milk, weight and growth and lifestyle,	Diet, obesity, unhealthy lifestyle, no physical activity, smoking, family history, abnormal lipid profile, insulin resistance, intrauterine environment, high intake of alcohol, hypertension, IFG/IGT, family history of vascular diseases
Drugs	Requires to take insulin	Oral hypoglycemic medicines, to some extent requires to take insulin

2.3. Complications of DM

As stated previously, symptoms of marked hyperglycemia including polyuria, polydipsia, and weight loss, occasionally with polyphagia as well as blurred vision. Growth abnormalities and susceptibility to certain infections might also leads to chronic hyperglycemia. Long-term complications of diabetes may lead to renal failure and peripheral neuropathy, gastrointestinal, and sexual dysfunction (American Diabetes Association, 2014).

In addition, high rates of morbidity and mortality are observed in DM carriers. For example, the relative risk of death as a result of vascular complications is three folds higher in individuals with DM in comparison with normal people (Haffner et al., 1998); cardiovascular diseases are being responsible for almost 80% of deaths in DM carriers (Schaan et al., 2004).

In a study to demonstrate the effect of DM in inducing bone fragility, Napoli et al. (2017), proposed that the fragility fractures are recognized to strongly associate with complications of both T1DM and T2DM. They also stated that the previous studies have shown a significant interaction between levels of glucose and bone metabolism, which subsequently opened-up new scientific scenarios in terms of explaining the increased fracture risk in patients with DM.

Bandeira et al. (2013), stated that there is a direct link between complications of DM and chronic hyperglycemia. Thus, oxidative stress has been proposed to be a key mechanism reinforcing this link. In a review on chronic complications of DM by Lofty et al. (2017), they concluded that chronic complications of DM are largely consequences of DM-induced hyperglycaemia on cellular and molecular impairment of vascular and neural structure and function. They also stated that the DM-induced hyperglycaemia might induce oxidative stress which is a major player in the development of long-term complications of DM.

In general, complications of DM can be classified into two main groups (Wallace, 1999; Mohan, 2002; Asmat et al., 2016)

a. Metabolic acute complications: This group is usually short term including hypoglycemia, ketoacidosis and hyperosmolar non-ketonic coma.

b. Systemic late complications: This group is often long term chronic sort of complications which include diabetic nephropathy, diabetic neuro- and retinopathy, microangiopathy, atherosclerosis and infections.

2.4. Prevention and Treatment of Diabetes

The strongest evidence for diabetes prevention comes from the Diabetes Prevention Program including life style intervention is regarded to be the strongest evidence to reduce risks of DM. In this context, the initial focus is on reducing total dietary fat. In this case, patients with DM can be individualized under medical nutrition therapy which is an effective way in lowering A1C in patients diagnosed with prediabetes (American Diabetes Association, 2017). It has been well documented that fatty acids play important roles in regulating blood glucose and the pathogenesis of diabetes. Significant changes in circulating free fatty acids might stimulate insulin secretion through the direct action on the β-cells of the pancreatic islet (McGarry et al., 2002). As shown in (Figure 2), change of life style and dietary fat consumption is very important. For example, despite chronic exposure to free fatty acid might collectively contributes to deregulation of blood glucose, particular types of free fatty acid can explicitly affect secretion and sensitivity of insulin by binding to extracellular membrane receptors (Neuman et al., 2017).

Physical activity is another way to prevent and/or reduce complications of DM, for example, walking and brisk for 150/week showed useful effects in individuals with prediabetes (Knowler et al., 2002). Furthermore, moderate intensity physical activity has been demonstrated effective roles in improving insulin sensitivity and reducing abdominal fat in children and young adults (Fedewa et al., 2014). The preventative influences of exercise seem to extend to the prevention of gestational diabetes mellitus (GDM) (Russo et al., 2015).
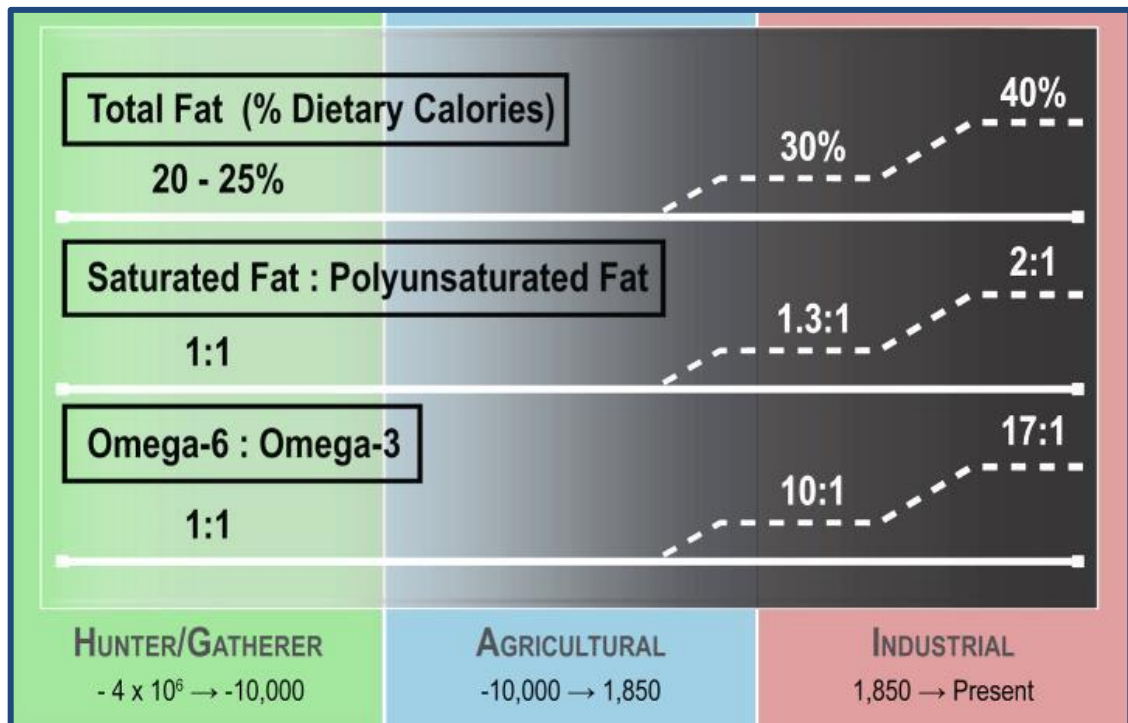
Figure 2. Changes in dietary fat composition over human existence (Neuman et al., 2017).

Investing a good glucose control is the aim of any antidiabetic treatment and is of value to reduce the risk of complications. Despite patients T1DM necessitate immediate insulin after the detection, several oral hypoglycemic agents including sulfonylureas, biguanides, thiazolidinediones, meglitinides, and α-glucosidase inhibitors are being used to treat T2DM along with insulin. Moreover, in last few years, numerous new oral hypoglycemic agents such as Dipeptidyl Peptidase-4 (DPP - 4) inhibitors, Sodium - glucose co - transporter 2 (SGLT2) inhibitors as well as injectable Glucagon-like peptide-1 (GLP - 1) analog, amylin analog are being used as antidiabetic drugs in terms of treating individuals with T2DM, which further offers an opportunity to treat and/or manage DM and its complications in a valuable way (Sen et al., 2016).

Despite advancements in understanding the therapeutic approaches, scientists harbor a highly amount of treatment difficulty with less effective and/or significant side effects of therapeutic strategies. Therefore, Xiang et al. (2016) demonstrated that looking for the novel therapeutic approaches considering several combinations of

medications in terms of enhancing the therapeutic efficacy are urgently desired. So, natural phytochemicals and extracts from medicinal plants have gained incredible recognition as ideal approaches in the prevention, treatment and control of several diseases (Munari et al., 2014).

As the human body is continuously exposed to several types of agents, such agents might result in the production of reactive species called as free radicals which further leads to oxidation of cellular machinery. Any impairment between free radicals and antioxidants of the body results in oxidative stress and subsequently the development of pathological condition among which one is DM (Asmat et al., 2016).

2.5. Free Radicals and Oxidative Stress

2.5.1. Free radicals

Free radicals can be defined as reactive chemical entities; they are generally short lived, unstable, and very much reactive species. These species contain one or more unpaired electrons. By passing such unpaired electrons, the free radicals induce damage to cells, this damage results in oxidation of cell components and molecules (Bansal and Bilaspuri, 2011). Asmat et al. (2016), demonstrated three types of free radicals, namely Reactive oxygen species (ROS), Reactive Nitrogen species (RNS), and Reactive chlorine species (RCS).

Free radicals play significant roles in origin and evolution of life. These are further important to activate numerous signaling pathways inside the cell. For example, the Mitogen activated protein kinase (MAPK) and extracellular- signal-regulated kinase (ERK) pathways which alter gene expression and in combination with superoxide dismutase might initiate cell death (Cho and Wolkenhauer, 2003). In addition, RNS generated involves in pathways that act as mediators of immunity. These are also regulates leukocyte adhesion, thrombosis, angiogenesis and vascular tone. In a similar way, ROS is involved in regulating gene transcription and cell signaling against biotic and a biotic stresses (Fang et al., 2002).

Furthermore, both endogenous and exogenous substances in cells and its surroundings contribute in the production of free radicals. They can be produced as a result from non-enzymatic reactions of organic compounds with oxygen (Pham-Huy et al., 2008). Mitochondrion is the place of this process which occurs by oxidative phosphorylation. To scavenge the deleterious impacts of such free radicals, the body possesses various mechanisms to neutralize elevated amounts of those products by producing antioxidants. As shown in (Figure 2), these antioxidants are involved in protecting the cells against toxic effects of free radicals and which consequently prevents the body from diseases (Asmat et al., 2016).
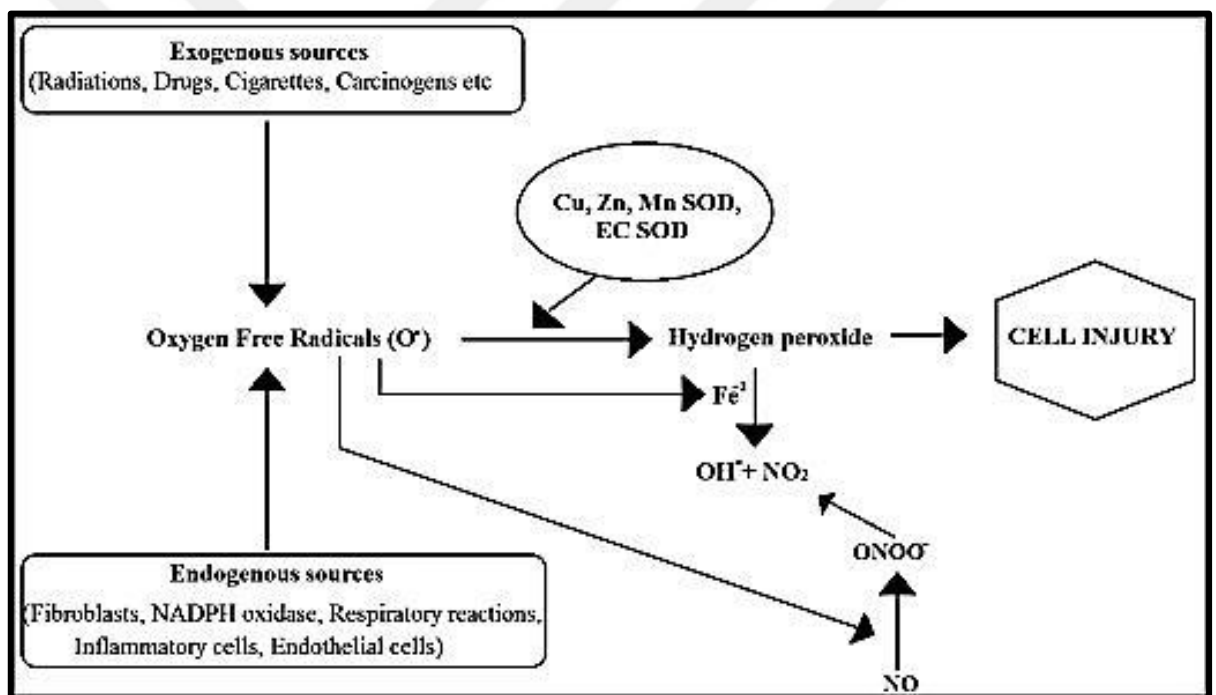
Figure 3. The contributions of exogenous and endogenous sources in the induction of cell injury by free radicals (Asmat et al., (2016).

2.5.2. Oxidative stress and antioxidants

Oxygen is universally the major factor making the life finite. It is considered to be one of the important ions of aerobic life. However, this ion may occasionally be a killer of cells when it involves in generating reactive species causing necrosis and ultimately the cell death. Moreover, RNS and RCS also lead to oxidation that interferes

with the normal physiological activities inside the cell and the intracellular (Weseler and Bast, 2010). Sies (1985), defined that oxidative stress is any disturbance of antioxidants and pro-oxidants balances in favour of the later because of various factors like aging, toxicity, drug action and inflammation or addiction, or both. Generally, it excess formation and/or insufficient removal of powerful reactive molecules including RNS and ROS (Johansen et al., 2005). Oxygen is a highly reactive component that is capable to become part of significantly harmful and damageable molecules (Free Radicals). Asmat et al. (2016), concluded that oxidative stress attacks healthy cells making them to lose their structures and their functional activities as well. Nodaway, pathogenesis of almost 50 diseases in various parts of the body has been implicated by oxidative stress (Table 2).

Table 2. Harmful effects of oxidative stress that induced organ damage, retrieved from (Nosratola et al., 2003; Asmat et al., 2016).

Body part	Pathogenesis
Lungs	Asthma and chronic bronchitis
Kidneys	Glomerulonephritis and chronic renal failure
Joints	Arthritis and rheumatism
Brain	Depression, stroke, Alzheimer's disease, Parkinson's disease and memory loss
Eyes	Cataract and retinal diseases
Fetus	Preeclampsia and IU growth restriction
Heart vessels	Hypertension, ischemia, arteriosclerosis, cardiomyopathy and heart failure
Multi-organs	Cancer, aging, inflammation infection and diabetes,

Damages to DNA, proteins, and other macromolecules caused by oxidation are implicated in the pathogenesis several of diseases, most notably associated with cancer and heart disease (Halliwell, 1994). Therefore, the term antioxidant might refer to any substance whose availability, even in minute concentration either delay or inhibits the oxidation of a substrate. Several antioxidant defense players such as endogenous

(internally synthesized) or exogenous (consumed) species or molecules can be named as biomarkers of oxidative stress. Moreover, based on their mechanism of action, antioxidants are mainly classified as either chain breaking antioxidants or preventive antioxidants (Somogyi et al., 2007). There are several types of biological antioxidants such as Glutathione (oxidized/reduced), Vitamin C and E, cystine, etc., these components can naturally be present and they effectively involve numerous biological pathways (Khanna, 2000).

2.6. Oxidative Stress in DM

It is well known that oxidative stress is a valuable player in the development of vascular complications in DM particularly T2DM (Pham-Huy, 2008). Elevations of levels of ROS in diabetes might lead to reduce in destruction or/and increase in the production though catalase (CAT–non-enzymatic/enzymatic), superoxide dismutase (SOD) and glutathione peroxidase (GSH–Px) antioxidants. In addition, the variation in the levels of such enzymes causes tissues susceptible to oxidative stress, which further, complications of DM will be developed (Lipinski, 2001). Based on a previous study by Pham-Huy (2008), diabetic mortalities might notably be explained by an increase in vascular diseases rather than hyperglycemia.

2.7. Antioxidants

Developing a series defense mechanism in organisms due to the exposure to free radicals from a variety of sources has been extensively studied and confirmed (Boveris, 1997). Defense mechanisms to combat free radical-induced oxidative stress might involve (1) preventative mechanisms, (2) physical defenses, (2) repair mechanisms, and (4) antioxidant defenses. Defenses from enzymatic antioxidant include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). In contrast, non-enzymatic antioxidants associated with carotenoids, flavonoids, Vitamin C, Vitamin E, glutathione (GSH), and other antioxidants. Moreover, both activities and the intracellular concentrations of such antioxidants are in balance under normal conditions. This balance is of value in surviving organisms and keeping their healthy manner (Valko et al., 2007).

Various amounts of antioxidants were established to counteract the accumulation of reactive oxygen species (ROS) accumulation in tissues that further inhibit free-radical cascade (Foyer et al., 1994; Knight, 2000). Antioxidants can be provided from diet including plants and vegetables which they neutralize ROS in the body. Respiratory chain enzymatic reactions, processes of phagocytosis and prostaglandin synthesis, are the main internal sources of ROS. Intensification of producing ROS might occur during increased physical activity, inflammation, tissue ischemia and reperfusion, and certain mental states including stress and depression (Singh et al., 2004; Harasym and Oledzki, 2014).

In addition, the effective prevention from the excessive quantities of oxygen-free radicals which penetrates the human system sourced from the environment, the necessity in providing appropriate amounts of antioxidants from foods to human body is very important. Such exogenous antioxidants from food promote activities of endogenous antioxidants defending against excessive numbers of ROS (Stahl and Sies, 2005; Pandey et al., 2009). As studied by Knight (2000), and Jakus (2000), uncontrolled and long-term oxidative stress effectively contributes in initiation and progression of cardiovascular disease (ischemic heart disease and chronic heart failure) hyperlipidemia and diabetes (insulin resistance). However, through the enhanced survival of tumour cells, antioxidant therapy might significantly stimulate growth of tumours during the progression stage of cancer (Valko et al., 2004). As stated by Harasym and Oledzki (2014), the antioxidant status is clinically investigated according to various measurements such as total antioxidant capacity (TAC) and total antioxidant status (TAS).

2.7.1. Total antioxidant status (TAS)

Levels of antioxidant enzymes are usually determined together as the total antioxidant status (TAS). In regards to their inverse associations with various chronic diseases, TAS in cells and extracellular fluids have been interestingly used in current studies as biomarkers or intermediate end points in terms of assessing risk of development of chronic diseases such as cancers and cardiovascular diseases (Diplock, 2000; Mayne, 2003; Collins, 2005). Haldar et al. (2007), stated that decreasing risks of

such diseases are associated with increased intakes of rich-antioxidants fruits and vegetables. Moreover, reports on the antioxidants status and antioxidant enzymes in patients with DM observe somewhat contradictory, as both increases and decreases have been reported in antioxidant activity. Generally, TAS has been reported to be incredibly lower in patients with proliferative diabetic retinopathy than in diabetics who have no developed retinopathy (Victor et al., 2014).

2.7.2. Total oxidant status (TOS)

Various systemic and ocular diseases including uveitis, glaucoma, and retinopathy of prematurity and macular degeneration have been pathophysiologically implicated by oxidative stress. Common procedures that have been used to determine the predicted oxidative stress are indicated by measuring total antioxidant status (TAS) and total oxidant status (TOS), measuring the later one indicates oxidative stress in biological samples (Erel, 2004; Ferreira et al., 2004). In a study by Toprak et al. (2014), measuring oxidative stress in patients with keratoconus, they determined serum TOS and TAS in terms of establishing the effect of systemic oxidative stress of patients with keratoconus on the pathogenesis of keratoconus.

A recent study to determine total oxidant/antioxidant status in sera of Esophageal cancer patients, Huang et al. (2017) found that TOS and oxidative stress index of serum are potential indicators to diagnose biomarkers that can subsequently be used to identify cases of Esophageal Cancer. Moreover, Wu et al. (2017), studied significance of serum TOS and TAS as oxidative stress parameters in individuals with colorectal cancer. They demonstrated that the TOS level was significantly increased and contrarily the TAS level was significantly decreased in the colorectal cancer group in comparison to those in the healthy control group.

In relevant study examining the possible effects of resveratrol on levels of blood glucose, and other oxidative markers in T2DM rats, results of this study indicated that TOS level in diabetic rats were significantly increased, and rats treated with resveratrol were observed decreased level of TOS which is used as an oxidative stress biomarker. Collectively, above studies demonstrate circulated relations between oxidative stress,

exogenous and endogenous antioxidants and total antioxidant status, confirming that such parameters are considerable factors affecting individuals with DM (Victor et al., 2014), (Figure 4).
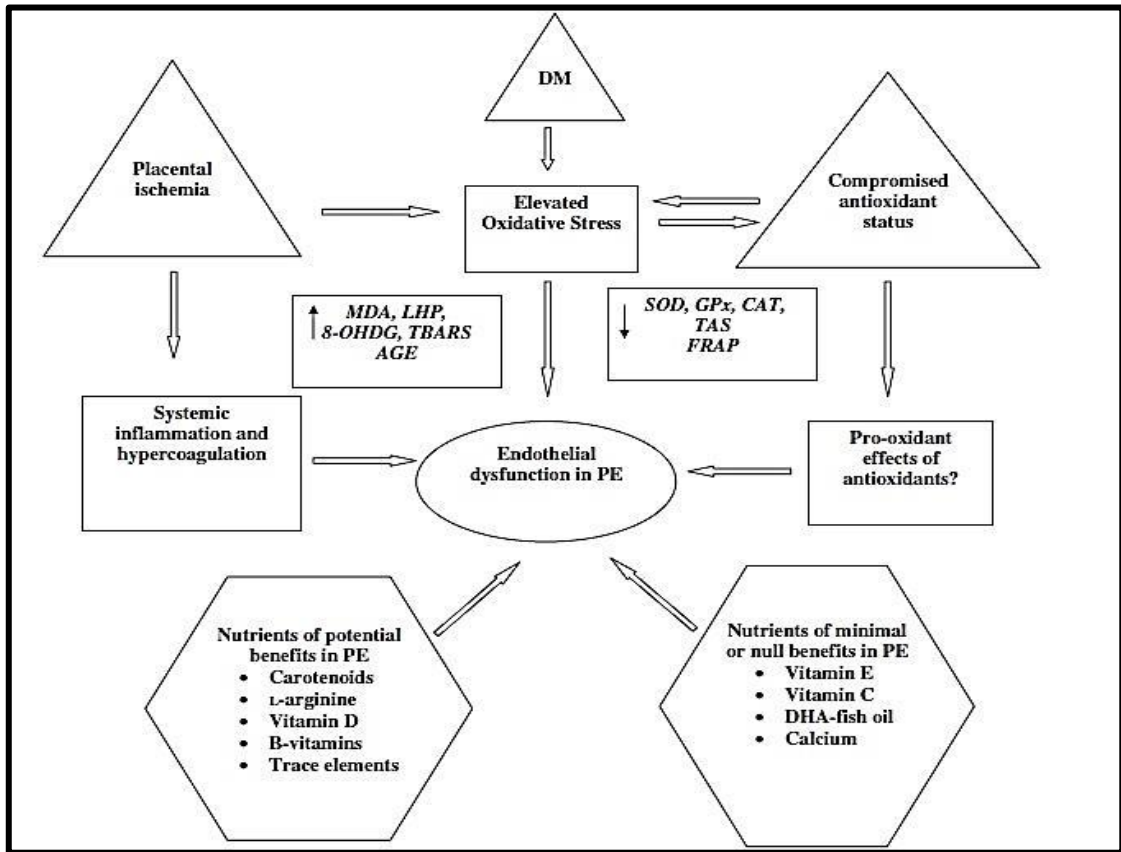
Figure 4. A summary of pathways represent associations between oxidative stress, DM and preeclampsia (a pregnancy-specific hypertensive disorder), (Victor et al., 2014).

PE= preeclampsia; MDA= malondialdehyde; TAS= total antioxidant status; LHP= lipid hydroperoxides; TBARS= thiobarbituric acid reactive substances; AGE= advanced glycation end products; SOD= superoxide dismutase; GPx= glutathione peroxidase; CAT= catalase; FRAP= ferric reducing ability of plasma; 8-OHDG= 8-hydroxydeoxyguanosine; DHA= docosahexaenoic acid

2.8. Cholesterol

Cholesterol is the most abundant steroid, it is the most important component in the human body, and it serves as an effective component of plasma membrane in all animal cells (Figure 5). Due to the significant correlation between high levels of serum cholesterol with various diseases, the determination of cholesterol serum levels becomes

a valuable biomarker. It is in a dynamic state in the body and it is constantly circulates in the blood (Bettelheim et al., 2013).
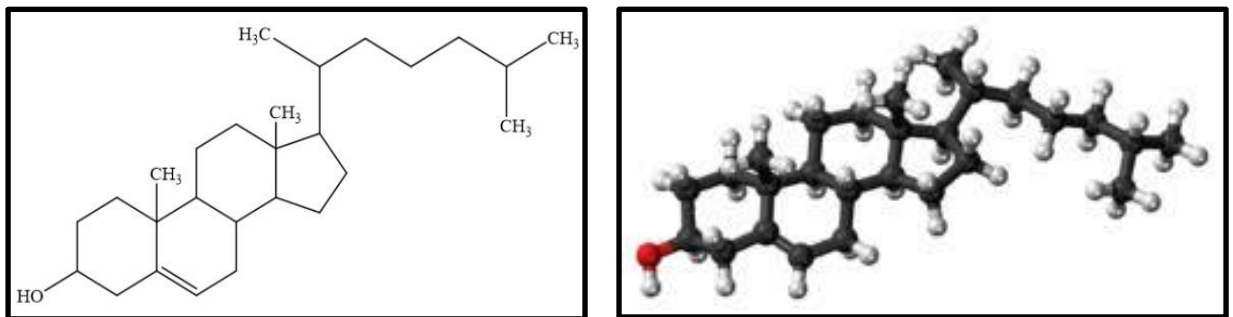
Figure 5. Chemical structure of Cholesterol

Cholesterol is usually transported by lipoproteins that they contain a core of hydrophobic lipid molecules. In general, four kinds of lipoproteins are classified including

1. High-density lipoprotein (HDL), it is also called good cholesterol which consists of about 33% and 30% of protein and cholesterol respectively,
2. Low-density lipoprotein (LDL), it is also called bad cholesterol which consists of about 25% and 50% of protein and cholesterol respectively,
3. Very-low-density lipoprotein (VLDL), it mostly is produced by liver and carries triglycerides
4. Chylomicrons, it is mostly produced in the intestines and carries dietary lipids (Bettelheim et al., 2013)

A low level of HDL cholesterol is a T2DM risk factor which precedes the onset of the disease. HDL content and various lipoprotein particles are gained attention to scientists as new markers of established risk factors form DM patients (Sparks et al., 2012; Mackey et al., 2015). Moreover, based on previous studies, combined six lipoprotein parameters including particles of VLDL, small LDL, HDL, HDL particle size and smaller mean LDL and larger mean VLDL size are independently associated with incident diabetes (Shalaurova et al., 2014; Mackey et al., 2015; Brahimaj et al., 2017).

As stated previously, diabetic dyslipemia can be characterized through the determination of hypertriglyceridemia. HDL cholesterol levels and hyperapolipoprotein B (apo B) are quantitative alteration characteristics to DM. In addition, the qualitative patterns of both HDL and LDL are regarded to alter in diabetes (Witztum, 1997). HDL atheroprotective functions such as reverse cholesterol transport and also antioxidant properties can be impaired in individuals with T2DM (Kontush, 2010). Such patients furtherreflect a highprevalence of small particles of dense LDL and increasingits modification (Scheffer et al., 2003). Patients with both T1DM and T2DM observed increased level of a minor plasma LDL sub-fraction,which is called electronegative LDL LDL (-). These patients demonstrated decreased proportion of LDL (-) as result of glycaemic optimization (Sanchez-Quesada et al., 2005). In regards to diabetic subjects, there is no difference between LDL (-) and native LDL in its either glycation or oxidative level, but they differ in other physicochemical characteristics (Benitez et al., 2006).

In a recent study by Estruch et al. (2017), to determine the inflammatory effect of LDL and HDL in T2DM. They found that patients with T2DM with poor glycaemic control observe increased proportion of LDL (-) and plasma inflammatory markers. They also foundvery low ability of HDL to inhibit LDL (-) -induced cytokine in patients with T2DM. They concluded that an imbalance in the pro- and anti-inflammatory are associated with lipoproteins characteristics from T2DM patients.

2.9. *Solanum nigrum* **L.**

The black nightshade (*Solanum nigrum* L.) is well-known as being a noxious weed species. It belongs to *Solanum* genus and Solanaceae family. Despite its reputation as a poisonous plant, black nightshade has globally been grown as a pot-herb and the berries (fruits) are used in baking pies (Edmonds and Chweya 1997; Mabberley 1997).

Although *Solanum species* are mainly similar in gross vegetative appearance, variable vegetative and reproductive structure within a species can be found under different environmental conditions. These variables are resulted in genetic variations

within a species leading to the recognition of numerous geographical ecotypes and consequently determining various types and concentrations of chemical components in the extracts of such ecotypes (Defelic, 2003).

2.9.1. Description of *Solanum nigrum* L.

As shown in figure 6, this plant is an erect to spreading from 0.15 to 1 m in tall; it is branched, and annual to short-lived perennial herb, recognized with mainly fibrous roots possessing a shallow tap root. Stems are generally round to angular covered with curved multicellular hairs and often tipped with a glandular head. Leaves are simple, their sizes ranging from 2 to 8 cm in long and 1 to 5.5 cm in width. Inflorescences of this plant are simple; flowers are white-coloured, star-shaped and clustered of 2 to 10 flowers. Fruit are usually ovoid and recognized with dull purple to black or yellowish-green berries. Berries are ranged from 6 to 10 mm. Seeds are usually tan, obovate, flat in cross-section, and their sizes ranged from 1.7 to 2.4 mm long (Knapp, 2002; Defelic, 2003).

Edmonds and Chweya (1997), stated that despite South America is thought to be the centre of origin of *Solanum*; the native of black nightshade is Eurasia and might have been originated in the Middle East, India and/or Africa. Black nightshade have also been recorded in Britain indicating establishment of this plant dated back to Neolithic man or earlier (Salisbury 1961). Black nightshade has been introduced in North America, New Zealand, and Australia by European emigrants (Defelic, 2003).
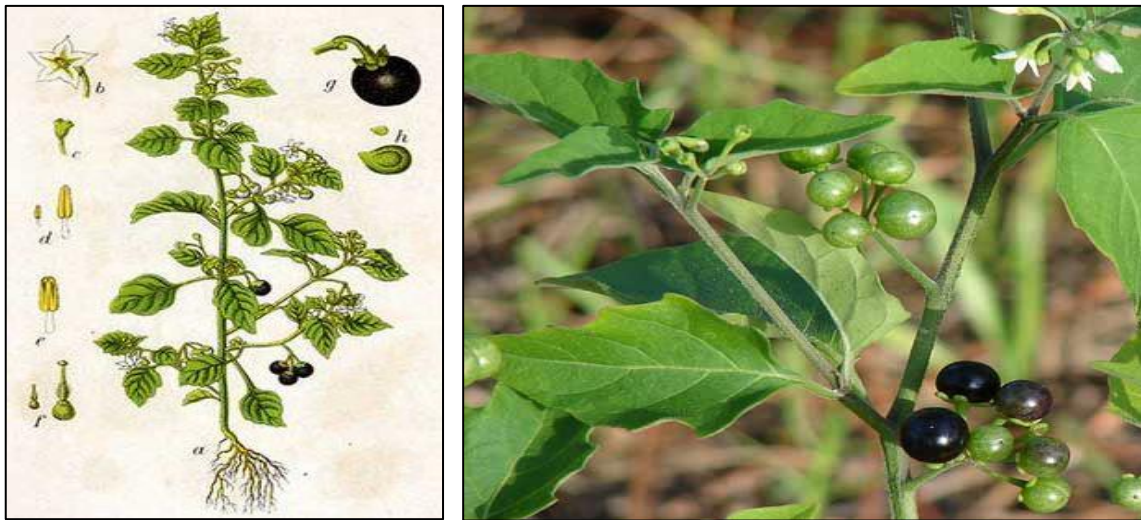
Figure 6. Represents parts of black nightshade, *Solanum nigrum* L. (Nyeem et al., 2017)

2.9.2. Chemical constituents of *Solanum nigrum*

Several compounds have been isolated from different fractions of this plant which have shown pharmacological relevance to the observed effects of whole plant preparation of *S. nigrum.*A chemical study on this plant reported the variability of the concentration of organic acids between seedlings and the mature plants.In this study, they identified that its major organic acids includedacetic acid, tartaric acid, malic acid and citric acid (Sun et al., 2006). Moreover, solanine whichis a glycoalkaloid compound was found in most parts of *Solanum nigrum* and highest level of this compound was found in unripe berries. However, the berries becomeleast toxic part of the plant that might occasionally be eaten without ill effects (Cooper and Johnson, 1984). In addition, Bhat et al. (2008), also demonstrated that the salinity dependent production of a structurally similar steroidal alkaloid, solasodine. Despite previous studies indicated highest level of steroidalalkaloid solasodine in roots, Eltayeb et al. (1997), reported that this constitute was given highest levels in the leaves.

During leaf development, the absolute amount of alkaloid perleaf can be increased. Although small unripe fruits of *Solanum nigrum* were observed high concentration of solasodine, its concentration and absolute amount per fruit were

decreased with fruit maturation. Hu et al. (1999), isolated three anti-neoplastic steroidal glycosides; beta 2-solamargine, solamargine and degalactotigonin. A chemical analysis on seeds of *Solanum nigrum* determined the presence several minerals as this order Mg>K>Ca>Fe>Na>Mn>Zn in theleaves and Mg>K>Fe>Ca>Na>Mn>Zn. Moreover, levels of phosphorus and sulphur were 75.22 and 8.55mg/100 g in the leaves and 62.50 and 14.48, g/100g inthe seeds. Vitamin content indicate the order of magnitudeas Vit C>Vit B,>Folic acid>Vit E>Vit A in both theleaves and seeds. Phytochemical analysis revealed highoxalate, phenol, but low sterol content in the studied plant materials. Cyanide levels were higher in the leaves compared to the seeds (Atanu et al., 2011).

2.9.3. Antimicrobial activity of *Solanum nigrum*

The leaves extracts of *Solanum nigrum* L. have been used to investigate antibacterial studies. Antimicrobial activities of methanol and aqueous extracts from all the plant showed significant effect against bacterial growth. A clear inhibition zone eliminates growth of microorganisms were observed after the addition of the methanol extracts of *S. nigrum* (Britto et al., 2011). Extracts from this plant against *Escherichia coli* (*E. coli*) observed maximum inhibition zone (30.1 mm) in comparison with control drug penicillin (Venkatesan and Karrunakaran, 2010). In an *in vitro* study, different extracts from seed, leaf and roots of *Solanum nigrum* using six solvents were carried out against pathogenic bacterial growth including *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, klebsiella pneumonia E.coli, Proteous vulgaris*, *and Pseudomonas putrida*. Seed extracts from the organic (ethanol, methanol, ethyl acetate, diethyl ether, chloroform and hexane) solvent observed stronger inhibitory effect against most respective pathogenic bacteria (Parameswari et al., 2012).

Moderate antibacterial activities against both gram-positive and gram-negative bacteria were demonstrated when such bacteria treated with ethanolic extracts of dried fruits of *Solanum nigrum* L. (Karmakar et al., 2010). Different concentrations 10μg, 50μg and 100 μg of plant extracts of *S. nigrum* were used against *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa*. Recorded data were compared to the zones of inhibitions of standard control streptomycin. The highest antimicrobial activities were observed with methanolic extracts rather than ethanolic

extracts, both treatments showed higher antimicrobial activitiesin comparison to the control (Parameswari et al., 2012; Nyeem et al., 2017*).*

In addition, Zubair et al. (2011) studied antimicrobial potential of *Solanum nigrum* using different extracts and fractions of leaves;they evaluated methanolic extract and different fractions including n-butanol, ethyl acetate,chloroform and n-hexane. In this study, the disc diffusion method was used to determine antimicrobial activitiesof four bacteria*Pasturella multocida, Escherichia coli, Staphylococcus aureus*and *Bacillus subtilis,*and three fungalstrains, namely *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus solani*. Results indicated that leafextract and fractionswere mildly effective as an antibacterialagent. While such extracts was poorly recognized against fungal activity.

A comprehensive study by Kumar et al. (2016), using crude extracts of leaves,fruits and stems of *Solanum nigrum* L. against activities of different pathogenic bacteria, namely Staphylococcus aureus, *Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli, Enterobacter aerogens*and*Salmonella typhimurium* using five different solvents including hexane, chloroform, acetone, ethanol and water.Results showed active role of such extracts against all respective pathogenic bacteria. However,the maximum inhibition zones were recorded when the medium supplemented with aqueous extract of leaves, hexane extract of stems and acetone extract of fruits against *Pseudomonas aeroginosa*. They finally concluded broad antimicrobial activities of this plant against various pathogenic bacteria.They also suggested the use of particular components of this medicinal plant in order to find novel antibacterial compounds that cause several diseases.

A recent study by Jinu et al. (2017), describes the efficient antibacterial and antibiofilm effect of aqueous leaf extracts of *Solanum nigrum* against multidrug resistant human pathogens using bioengineering and characterization of silver nanoparticles (AgNPs) method. They observed the effective role of using bioengineered AgNPs as nano-drug in treating multidrug resistant bacterial infections. In addition, another recent study on Adriamycin resistance by *Solanum nigrum* was studied by Jagadeeshan et al. (2017), their cytotoxicity assay observed that the methanolic glycosidic extract fraction of *Solanum nigrum* was directly correlated with surviving

cell population. They also found that the extract from this plantpossessed apoptotic patterns by mediating cell growth suppression suggesting that unripe fruit of the respective plantmight be used as achemosensitizing agent in treating Adriamycin resistant cancers.

Moreover*, Salmonella typhi* and *Staphylococcus aureus*were exposed to Phyto-Assisted synthesis of silver nanoparticles usingaqueous leaf extractof *Solanum nigrum* (Kumar et al., 2016). Results from this study showed significant antibacterial effect of nanoparticle of the extract of this medicinal plant against diseases that causes virulent bacterial strains including *Salmonella typhi* and *Staphylococcus aureus*. In an antimicrobial study by Opande et al. (2017), *Fusarium oxysporum* and *Pseudomonassyringae* was exposed to crude leaf extract of *Solanum nigrum.* Results showed antimicrobial properties of this plant against both respective microorganisms. They also determined flavanoids, tannin, terpenoids, phenol,saponin and steroidal compounds in the leaf extract. They finally suggested further studies to identify active ingredients and their concentrations against microbial growth.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Experimental Animals

28 male rats weighing 200-250 g were obtained from the Experimental Medical Applicated and Research Center of Yuzuncu Yil University, Medical Faculty. Subjects randomly composed of seven rats each; control (K), Diabetes Mellitus without SN (D), Diabetes Mellitus with SN group given (DSN) and SN given (SN)

The following materials were used to investigate the current study

3.1.2. İnstruments

a. Total antioxidant statues kit (Rel assay, Turkey)
b. Total oxidant statues kit (Rel assay, Turkey)
c. Total cholesterol kit (Roche, Germany)
d. Triglycerides kit (Roche, Germany)
e. Blood glucose kit (Roche, Germany)
f. HDL kit (Roche, Germany)
g. LDL kit (Roche, Germany)
h. VLDL kit (Roche, Germany)

3.1.3. Chemicals

a. Alfamin (Ketamine HCl)
b. Distilled water
c. Sodium strait buffer 4% (Merc, Turkey)
d. Streptozotocin (Sigma, USA)

3.2. Methods

3.2.1. Preparation of plant extract

Plant samples of *Solanum nigrum* were thoroughly washed under tap water, and then dried in the shade. The dried samples were finely pulverized to a powder sample.

100 g of powder was suspended in 250 ml of water for two hours and then heated to 60-65 ° C for 30 minutes. The extract was collected by the separation process and this process was repeated three times. The collected extracts were put together and passed through a thin cotton swab. The filtrate was evaporated at 40-50 ° C in rot vapor under reduced pressure. The obtained dark semi-solid material (yield 14%) was maintained at 0-4 ° C until using. A known amount of residual extract was then suspended in distilled water and administered to animals via oral gut intubation (Ahmed et al., 2014).

3.2.2. Experimental design

Diabetes-induced D and DSN group rats were administered intraperitoneally (i.p.) by dissolving 45 mg / kg single dose streptozocin (STZ) (Sigma, USA) in citrate buffer at pH 4.5 (Bloch and Vardi, 2005). The same amount of saline was injected into the control group. D and DSN group, 72 hours after injection of STZ, blood glucose levels were determined by means of Plus MED Accuro biosensor screener and striplines in the blood samples taken from the tail of the rats. Blood sugar levels higer than or equal to 270 mg / dl were included in the study. The rats in the SN and D+SN groups were orally administered with 250 mg / kg / day gavage of SN extract dissolved in distilled water every day (Ahmed et al., 2014).

Control Group: Seven randomly selected rats were divided into control groups.

Group 1: Seven rata STZ solutions in this group were given 45 mg / kg IP route. (D)

Group 2: Seven rats in this group were dissolved in water and the SN solution was administered orally for 25 days at 250 mg / kg / day. (SN)

Group 3: Seven rats in this group were treated with STZ solution 45 mg / kg ip, followed by 72 hours after the glucose measurement was performed. Water was added to the rinsed aqueous solution at a dose of 250 mg / kg / day for 28 days. (D+SN)

3.2.3. Collection of samples

Blood samples were drawn from the left ventricle of the hearts of the animals to the glazed glass serum tubes under ketamine and rompun anesthesia after a twenty eight

day trial. The blood samples were centrifuged at 3000 rpm for 10 minutes at + 4 ° C. TAS, TOS and biochemical parameter analyzes were obtained in these samples.

3.2.4. Preparation of samples

Blood samples were carefully collected from the heart. About 5 mL blood samples from each rat were withdrawn into vacutainer tubes with gel and centrifuged to (3000 rpm in 4oC) 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 ºC prior to analysis.

Biochemical parameters including (glucose, high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol, triglyceride, VLDL-cholesterol were determined using kits and biochemical auto analyzers (Architect Plus, ci16200, by Abbott Company, USA) and (Cobas C311, Roche- Germany).

3.3. Biochemical Analysis

3.3.1. Measurement of biochemical serum parameters

Blood glucose level determination

Blood glucose levels were determined by using automatic biochemical analyzer (Architect Plus, ci16200, by Abbott Company, USA).

Principle of the test: The principle of determination of blood glucose is based on the enzymatic method. Hexokinase has role in catalyzing glucose to glucose 6-phasphate when ATP is used in this process. Glucose 6-phosphates undergo the process of oxidation and converted to gluconate 6 phosphates when the NADP is used in this process. The rate of NADPH produced is directly proportional to the level of glucose in the sample.

Total cholesterol level determination in serum

The level of total cholesterol in the serum samples was determined using automatic biochemical analyzer (ARCHITECT PLUS, ci16200, by Abbott Company, USA).

Principle of the test: Homogenous enzymatic colorimetric assay was used in the cholesterol test. Cholesterol esters are hydrolyzed into cholesterol and free fatty acids by cholesterol esterase enzyme. Then the cholesterol oxidized to cholest-4 ene-3-one and hydrogen peroxides. The hydrogen peroxide combines with hydroxybenzoic acid and 4-aminoantipyrine to form a chromophore (Quinoneimine dye). Finally colorimetric method was used to determine the severity of color which is directly proportional to the level of cholesterol.

Triglycerides level determination in serum

The level of triglycerides in the serum samples was determined using automatic biochemical analyzer (Architect Plus, ci16200, by Abbott Company, USA).

Principle of the test: The enzymatic colorimetric method was used in the detection of triglyceride. Firstly, triglycerides were hydrolyzed by lipoprotein lipase to yield glycerol. Glycerols undergo several reactions using several enzymes to form Quinoneimmune red dyestuff which can be determined by colorimetric method and its intensity is directly proportional to triglycerides level.

HDL-cholesterol level determination in serum

The level of HDL-cholesterol in the serum samples was determined using automatic biochemical analyzer (Architect Plus, ci16200, by Abbott Company, USA).

Principle of the test: Homogenous enzymatic colorimetric test was used and first step is elimination of all other lipoproteins by cholesterol esterase, then, cholesterol oxidase oxides cholesterols to cholestenone and hydrogen peroxides. Then hydrogen peroxides are converted by the help of peroxidase to purple-blue pigments which can be determined and hence the level of HDL can be measured.

VLDL-cholesterol level determination in serum

The VLDL levels were calculated from the triglyceride levels by the biochemical analyzer as follow:

VLDL = triglyceride level /5

LDL- cholesterol level determination in serum

The LDL-cholesterol levels were estimated by calculation according the following formula:

LDL = total cholesterol −HDL cholesterol – VLDL (El-Far et al., 2016).

3.3.2. Oxidative stress paramateres

Determination of total antioxidant status (TAS) in serum

Total antioxidant stauts (TAS) assay kit (Rel assay diagnostcs, Turkey) were used to determine TOS (Jansen and Ruskovska, 2013).

Principle: The presence of antioxidants in the sample reduce dark blue-green colored 2,2'-azino-bis (3-ethylbenzotiazoline-6-sulphonic acid) (ABTS) radical to a colorless form of reduced ABTS.

Procedure: The level of total antioxidant status (TAS) from blood samples was measuredspectrophotometrically (Eliza) at 660 nm.A stable antioxidantstandard solution of Vitamin E analog which is traditionally known as Trolox Equivalent was used to calibrate this method. Levels of TAS were expressed in mmol/L. The assay was carried out in microtiter plates

Determination of total oxidant status (TOS) in serum

Total oxidant stauts (TOS) assay kit (Rel assay diagnostcs, Turkey) were used to determine TOS (Jansen and Ruskovska, 2013).

Principle of the test:it is based on the oxidization of the ferrous ion–chelator complex to ferric ion in the respective.Enhacer molecules prolongs the oxidation reaction, such molecules occure abundantly in the reaction medium. In an acidic medium ferric ion with chrmogen makes a coloured complex.

Procedure: The colour intensity was spectrophotometrically (Eliza) measured at 530 nm, determining total amount of oxidants. Hydrogen peroxide was used to calibrate this assay and the determined results were expressed in μmol H_2O_2 Equiv./L. The assay was carried out in microtiter plates. The variation of intra-assay of TOS assay was 3.6%, in whichmeasured with samples as two quality control samples.

3.4. Statistical Analysis

The results obtained in the study were assessed using (SPSS 22.0). Multiple comparisons to compare the data of groups 1-3, Raw values from all analyzes were presented as the mean ± standard error of respective groups ($p < 0.05$)

4. RESULTS

The present study was investigated to study the effect of *Solanum nigrum* extract on glucose level, total cholesterol, triglyceride, HDL, LDL, TAS and TOS on blood samples of rats. The levels of those parameters belonging to the experimental groups are shown in Table 3.

Table 3. The level of serum biochemical parameters in experimental groups.

Parameters	Control (C)	Diabetes (D)	*Solanum nigrum* (SN)	Diabetes + *Solanum nigrum* (EX). D+SN
Glucose	131 ± 9.8 a	663 ± 21.8 c	196.14 ± 12.1a	484.8 ± 40.0 b
T.Cholesterol	51.50 ±1.9 a	59.40 ± 3.6 a	53.28 ± 2.5 a	60.06 ± 3.6 a
Triglyceride	54.66 ± 7.1 a	73.57 ± 12.1 b	41.42 ± 3.6 a	59.66 ± 7.8ab
HDL. Chol.	38.6 ± 2.6 a	41.62 ± 3.1 a	42.48 ± 1.4a	45.81 ± 3.5 a
LDL. Chol.	4.1 ± 0.2 a	4.1 ± 0.8 a	6.0 ± 1.1 a	5.7 ± 0.07 a
VLDL. Chol.	10.93± 1.42a	14.71 ±2.42 b	8.28 ± 0.72 a	11.93 ± 1.56 ab
TAS	1.28 ± 0.17a	1.85 ± 0.15b	1.27 ± 0.10 a	1.54 ± 0.07 ab
TOS	3.87 ± 0.34a	6.30 ± 1.41 b	4.87 ± 0.80 a	4.14 ± 0.34 a

*Different letters between the lines is statistically significant ($p<0.05$).
X=mean, SE=Standard error.

Glucose levels

Results from table 1 and figure 7 represent significant increasing of serum glucose level ($p<0.05$) in diabetes group (D) ($663 ± 21.8$ mg/dL) in comparison with control group (C) ($131 ± 9.8$ mg/dL). However, there were no significant differences ($p<0.05$) in glucose level between C group ($131 ± 9.8$ mg/dl) and *Solanumnigrum* extract group (SN) ($196.14 ± 12.1$ mg/dL).

Moreover, in regards to glucose level, results from table 1 demonstrated that (D+SN) group ($484.8 ± 40.0$ mg/dL) was significantly higher than results from all of C ($131 ± 9.8$mg/dl), and SN groups ($196.14 ± 12.1$ mg/dL) and lower than D groups ($663 ± 21.8$mg/dl).

Figure 7. The level of serum Glucose in experimental groups.
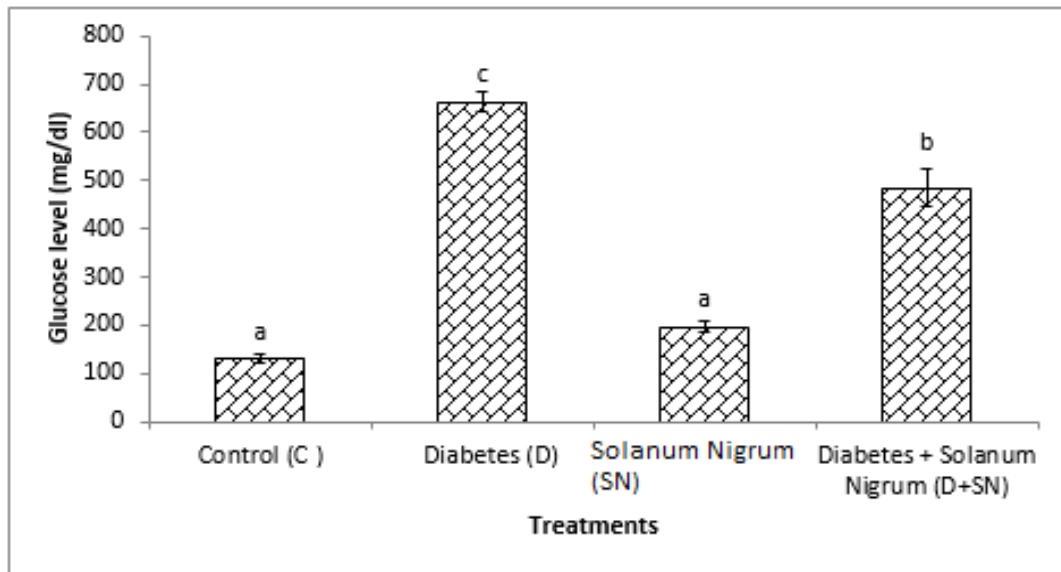
Cholesterol levels

In return to table 1, results showed that there were no significant differences ($p < 0.05$) in cholesterol levels between C group (51.50 ±1.9 mg/dL) and SN group (53.28 ± 2.mg/dL), D group (59.40 ± 3.6 mg/dl) and D+SN group (60.06 ± 3.6 mg/dl) (Table 1 and Figure 8).
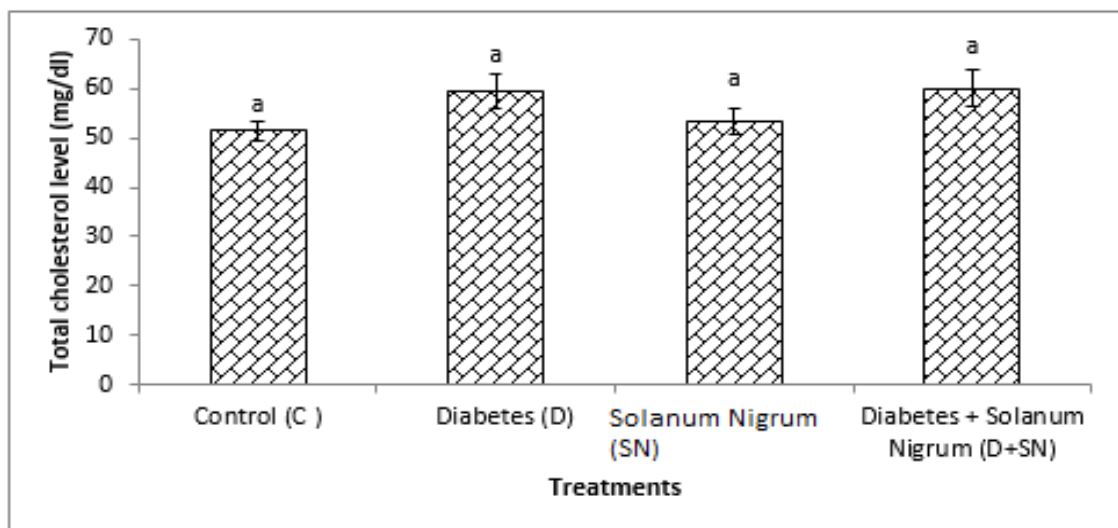

Figure 8. The level of serum Total Cholesterol in experimental groups.

Triglyceride levels

Diabetes caused significant increase ($p<0.05$) in Triglycerides (Tg) level in D group (73.57 ± 12 mg/dL) in comparison to both C and SN group (54.66 ± 7.1 and 41.42 ± 3.6 and mg/dL respectively). In contrast, no significant differences were found between D group and D+SN group (59.66 ± 7.8). Furthermore, triglyceride (Tg) levels showed no significant differences ($p<0.05$) between SN, D+SN and C group (Table 1 and Figure 9).
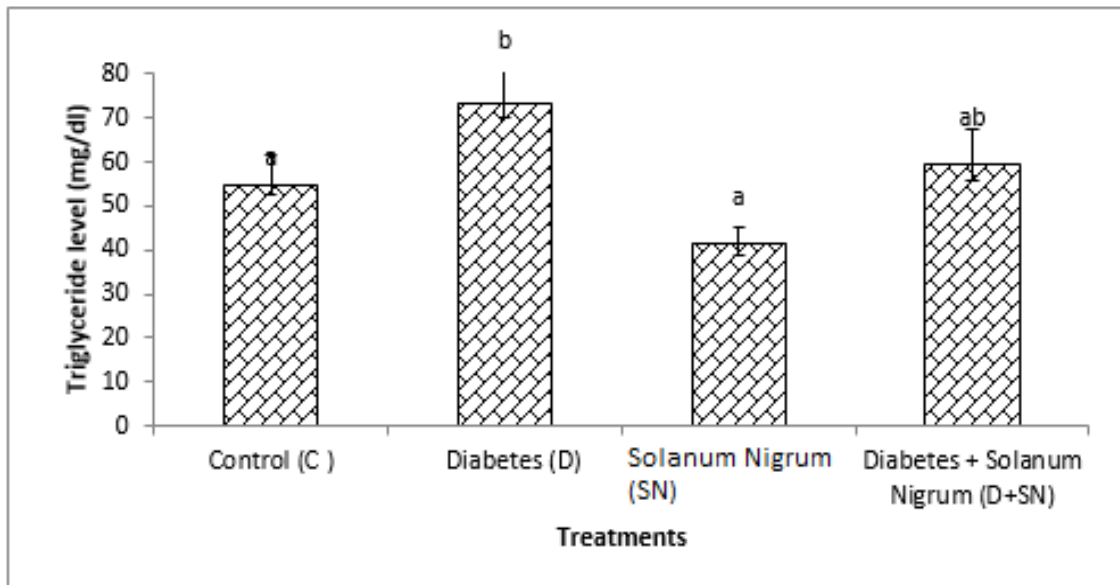
Figure 9. The level of serum Triglyceride in experimental groups.

HDL-cholesterol

Despite recording higher levels of HDL-cholesterol in D, SN and D+SN groups in comparison to control (C group), these levels showed no significant differences ($p<0.05$) in C group (38.6 ± 2.6 mg/dL) when compared to SN group (42.48 ± 1.4 mg/dL), D group (41.62 ± 3.1 mg/dl) and D+SNgroup (45.81 ± 3.5 mg/dl) (Table 1 and Figure 10).

Figure 10. The level of serum HDL Cholesterol in experimental groups.

LDL-cholesterol levels

LDL-cholesterol levels demonstrated no significant difference ($p<0.05$) in C group(4.1 ± 0.2 mg/dL) compared to D group (4.1 ± 0.8 mg/dl), SN group (6.0 ± 1.1 mg/dL) and D+SNgroup (5.7 ± 0.07 mg/dl) (Table 1 and Figure 11).

Figure 11. The level of serum LDL Cholesterol in experimental groups.

VLDL-cholesterol levels

Results from table 1 and figure S6 observe that the diabetes resulted in significant increase ($p < 0.05$) in VLDL-cholesterol levels in D group (14.71 mg/dl) in comparison with control C (10.93 mg/dl). Whereas, SN group observed lower level (8.28) of VLDL-cholesterol in comparison to control C group, this change was not significant. Similarly, the level of VLDL-cholesterol in D+SN (11.93 mg/dl) was not significantly higher in comparison with control (Table 1 and Figure 12).
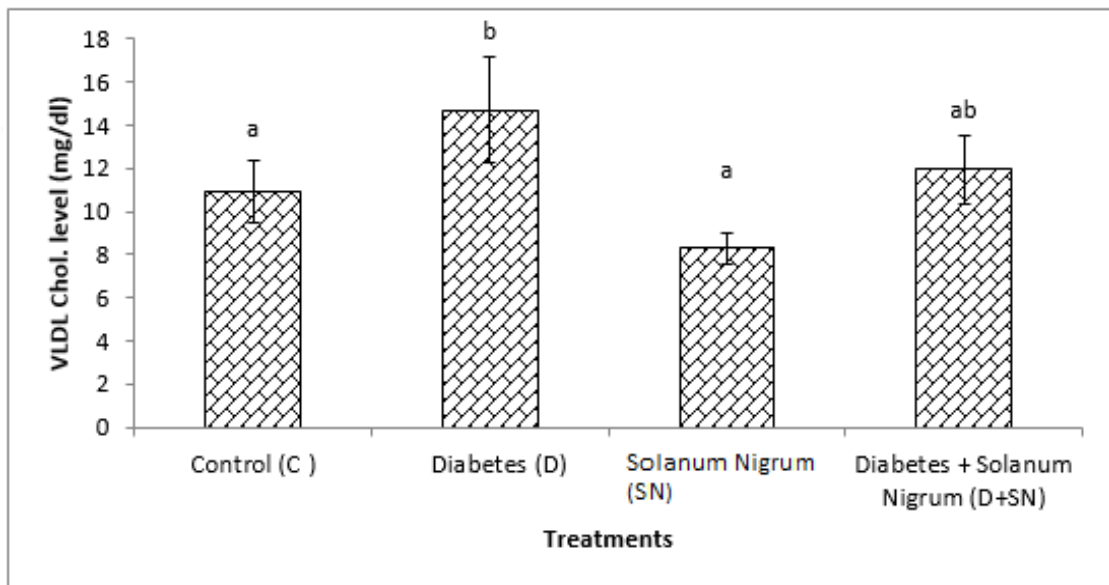
Figure 12. The level of serum VLDL Cholesterol in experimental groups.

Oxidative Stress Analysis

As a consequence of diabetes, results showed significant increase ($p < 0.05$) in TAS level in D group ($1.85 \pm 0.15.7$ mmol Trolox Eq/L) compared to control group (C) (1.28 ± 0.17 mmol Trolox Eq./L). However, results determined from respective samples observed no significant differences between D group and D+SN group (1.54 ± 0.07 mmol Trolox Eq./L). Moreover, TAS levels showed no significant difference ($p < 0.05$) in both SN (1.27 ± 0.10 mmol Trolox Eq./L) and D+SN (1.54 ± 0.07 mmol Trolox Eq./L) groups in comparison to control, when SN administrated to diabetes rats (Table 1 and Figure 13).

35

Diabetes resulted in significant increase ($p < 0.05$) in total oxidant status (TOS) in D group (6.30 ± 1.41 µmol H_2O_2 Equiv./L) in comparison with control C (3.87 ± 0.34 µmol H_2O_2 Equiv./L), SN (4.87 ± 0.80 µmol H_2O_2 Equiv./L) group and D+SN (4.14 ± 0.34 µmol H_2O_2 Equiv./L) groups. In contrary, there were no significant differences ($p < 0.05$) between all of C, SN D+SN groups (Table 1 and Figure 14).
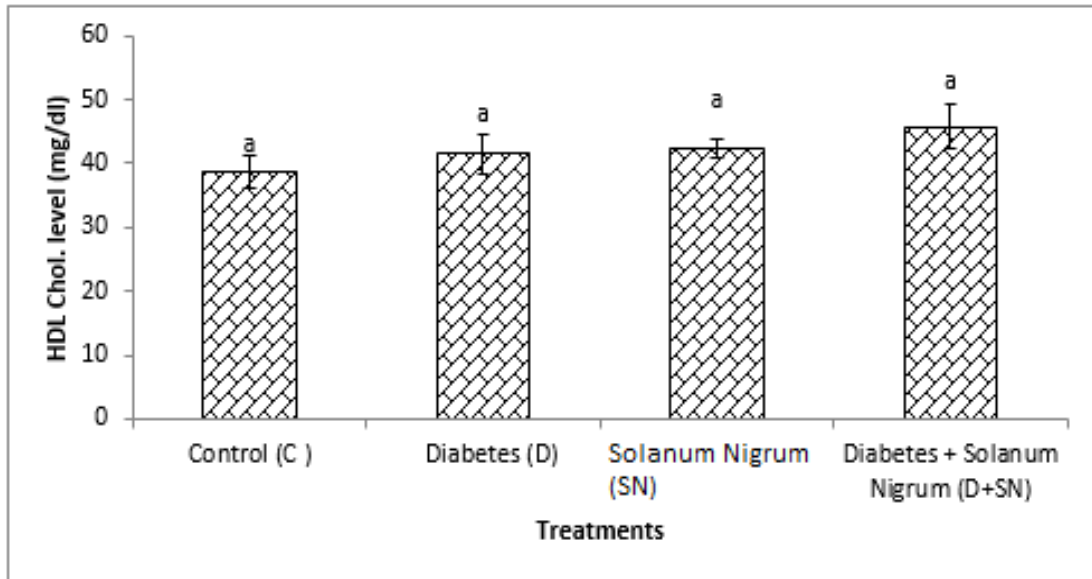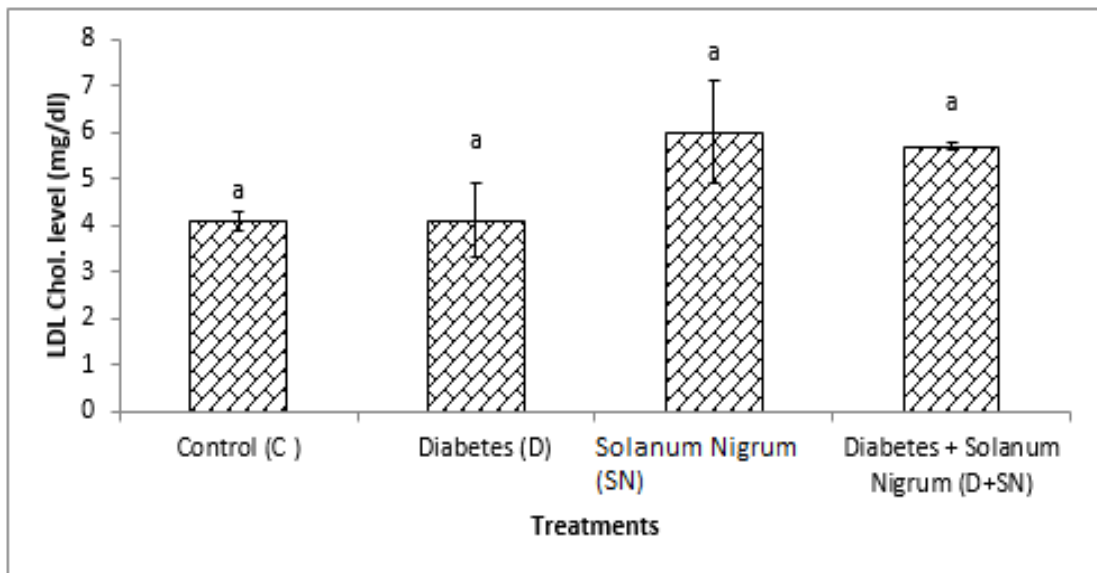

Figure 13. The level of serum TAS in experimental groups.

Figure 14. The level of serum TOS in experimental groups.

5. DISCUSSION AND CONCLUSION

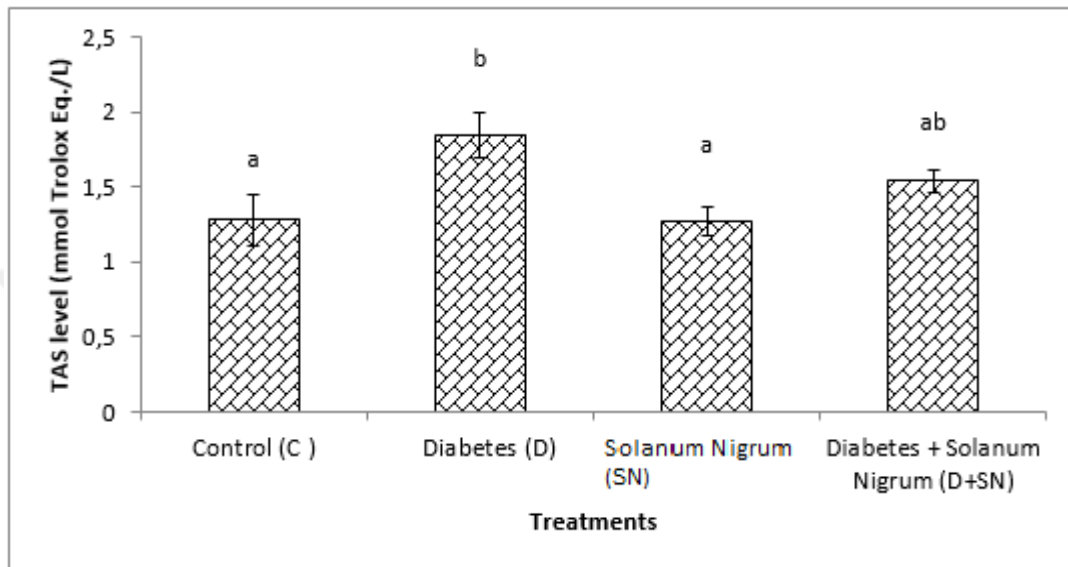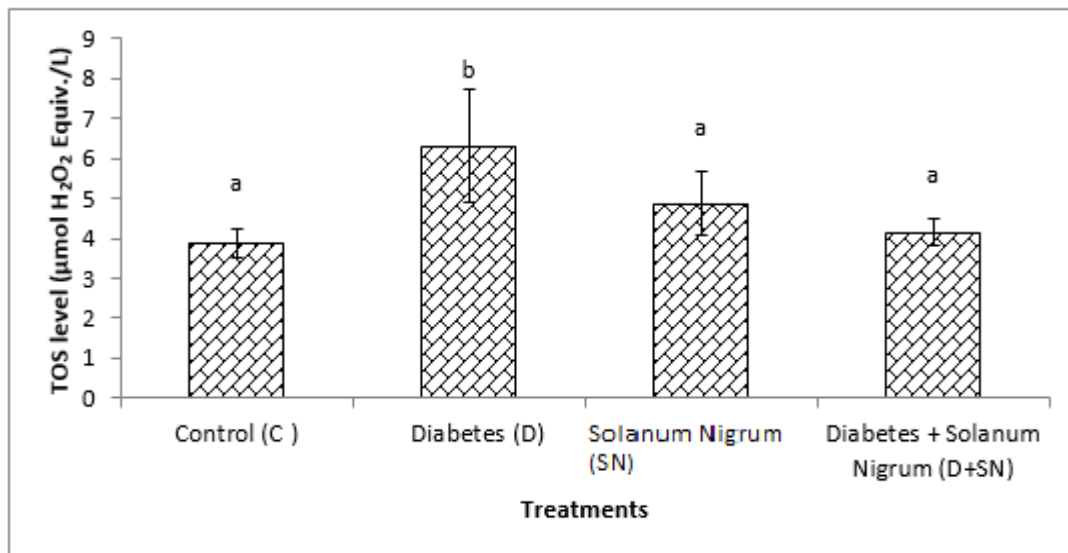The present study was carried out to examine the effect of *Solanum nigrum* extract (SN) on levels of glucose, total oxidant and antioxidant capacity on experimental diabetic rats. This study also investigated possible protective activity of this plant to enhance oxidative stress and DM damage.

As previously described, high concentrations of plasma glucose leads to metabolic disorders and glucose intolerance, this metabolic abnormality is characterized by hyperglycemia (Iso et al., 2006). This condition is associated with the increase of metabolic disturbance of carbohydrate, fat, and protein enhancing the production of free radicals that follow by oxidative stress, renal failure, neurodegeneration, cardiovascular abnormalities and immune dysfunction (Hung et al., 2012).

As shown in table 1 and figure 7, glucose level in diabetes group (D) was significantly increased in comparison with control group (C). This result agrees with previous studies by Iso et al. (2006) and Hung et al. (2012) which they stated that the concentrations of plasma glucose are increased in DM patients following significant deficiency in insulin. However, results showed no significant differences in glucose level between control group and *Solanum nigrum* extract group (SN). In this context, extracts of *Solanum nigrum* had no significant effect in decreasing glucose level in normal rats.

However, an interesting result was found when diabetic group was treated with extracts of *Solanum nigrum*; in this case the glucose level in diabetic group (D) was 663 ± 21.8 mg/dl. However, this level was significantly decreased to 484.8 ± 40.0 mg/dl in D+SN group. This result shows the effective role of the *Solanum nigrum* extract in reducing glucose levels. This result agrees with findings by Poongothai et al. (2010) which they determined significant reduction in glucose level after administration of the aqueous extract of *Solanum nigrum* to induced diabetic rats. In addition, a relevant study considering antidiabetic activity of *Solanum nigrum* in Alloxan Induced diabetic rats by Umamageswari et al. (2017) indicated that aqueous extract of berries of this plant at 200 mg/kg/day observed significant reduction in blood glucose by 7 days, they also found highly significant reduction in blood glucose when rats exposed to 400

mg/kg/day of this extract. They finally concluded effective roles of berries extracts of *Solanum nigrum* as an antidiabetic activity. Tiwari and Jain (2017) studied hypoglycemic activity of *Solanum nigrum* on alloxan Induced DM in rats. In this study alcoholic extracts of leaves of this plant were used at different doses 50, 100, 200, 400 mg/kg of body weight. Their results indicated significant hypoglycemic role of this plant.

Our results are in line with previous studies regarding antidiabetic activities of *Solanum nigrum*, in this context, aqueous extracts from the respective plant seems to have effective roles in regulating blood glucose levels and possesses therapeutic characteristics against hyperglycemic activities.

Hyperlipidemia can be found as a major risk factor of cardiovascular pathologies. It is confirmed that the *Solanum nigrum* plays effective roles in inhibiting H^+K^+ATPase which might subsequently serve as cardio protective regimen (Atanu et al., 2011). Some studies observed reduced level of the plasma lipoprotein levels (TG, TC and LDL) in mice treated with *Solanum nigrum* extracts. This extract might inhibit the cholestyramine-induced hepatic HMG-Co A reductase activities (Lee et al., 2005). This study was designed to examine the effect of oral administration of the extract of *Solanum nigrum* on cholesterol, triglycerides, LDL, HDL, and VLDL levels in normal and diabetic rats.

Despite Sohrabipour et al. (2014) observed significant decrease in total cholesterol after administration of aqueous extract of *S. nigrum* to normal and diabetic rats, our results demonstrated that *Solanum nigrum* administration has no significant effect on cholesterol. Our result is similar to a study by Lee et al. (2005) on the effect of aqueous extract of *Solanum nigrum* glycoprotein on levels of plasma lipid including total cholesterol in mice. They found that the administration of (10g head body weight g^{-1}) of this plant was observed to be not significant in compare with control. However, they demonstrated that the administration of (20 and 40 g head body weight g^{-1}) were significantly decreased total cholesterol levels. Moreover, a relevant study determining effects of ethanolic extract of fruit of *Solanum nigrum* on serum cholesterol level of the fructose induced hyperlipidaemia and hyperinsulinemia of rats were carried out by Ahir et al. (2008). In this study, the administration of 100 mg/kg to fructose-fed rats was not

significant in comparison to control. However, the administration of 250 mg/kg was significantly reduced the level of serum cholesterol.

In this context, the use of appropriate concentrations might significantly be effective; this statement might be true to our results. Furthermore, as sated by Zubair et al. (2011), the use of proper solvent including ethanol, methanol, hexane and ethyl acetate might play important roles on the phytochemical efficacy of the respective plant. Based on this statement, our results might due to the use of aqueous extract instead of using other solvents.

From a reductionist's point of view, obesity as well as diabetes in mammals follows by an imbalance between the rates of synthesis of fat synthesis and its catabolism. This concept strongly supports studies showing that expansion of fat mass in obese individuals and subsequently diabetes results from elevated levels of triglyceride synthesis and decreased triglyceride breakdown in mammalian tissues. Triglyceride breakdown consists of enzymatic cleavage of triglycerides and the formation of fatty acids and glycerol. This important process is called lipolysis which requires at least three distinct hydrolases (Schweiger et al., 2017). Herbal components have been used as sources of important regulators to such processes. In regards to this statement, the present study determined triglyceride levels of respective rat groups.

Our results showed that despite some reductions in the level of triglyceride in SN group in comparison to control C group, and in D+SN in comparison to D group, this result was not significant. In other word, the administrations of the extract of *Solanum nigrum* to respective rats were not significantly effective against this biochemical parameter in the present study. This result is similar to the finding by Arulmozhi et al. (2010), which they found that the use of the fruit extract of *Solanum nigrum* was not significantly reduced the level of triglyceride when rats treated with 250 mg/kg b.wt of this plant. A similar result was found by Gupta et al. (2009), they demonstrated that despite the reduction of triglyceride level of rats received high cholesterol diet and exposed to saponins of *Solanum nigrum* (100 mg/kg body weight p.o.), this reduction was not significant in comparison to triglyceride level in rats received normal diet.

HDL, LDL and VLDL contents are gained attention to scientists as new markers of established risk factors form DM patients (Sparks et al., 2012; Mackey et al., 2015). Moreover, based on previous studies confirmed that such parameters are independently associated with incident diabetes (Shalaurova et al., 2014; Mackey et al., 2015; Brahimaj et al., 2017).

As stated previously, diabetic dyslipemia can be characterized through the determination of hypertriglyceridemia. HDL cholesterol levels and hyperapolipoprotein B (apoB) are quantitative alteration characteristics to DM. In addition, the qualitative patterns of both HDL and LDL are regarded to alter in diabetes (Witztum, 1997). HDL atheroprotective functions such as reverse cholesterol transport and also antioxidant properties can be impaired in individuals with T2DM (Kontush, 2010). Such patients further reflect a highprevalence of small particles of dense LDL and increasing its modification (Scheffer et al., 2003).

In regards to levels of LDL, HDL and VLDL, despite some changes in levels of such biochemical parameters in SN group in comparison to control C group, and in D+SN in comparison to D group, these results were not significant. In other word, the administrations of the extract of *Solanum nigrum* to respective rats were not significantly effective against those biochemical parameters in the present study.

Some researcerhs assessed antihyperglycaemic and antioxidant effects of leaves extract of *Solanum nigrum* in alloxan induced-diabetic rats, they found that leaves extracts at 100 mg/kg was not significantly reduced the amount of VLDL in comparison to its level in normal rats (Maharana et al., 2011). A relevant study investigating activities of 150 kD a glycoprotein isolated from *Solanum nigrum* plasmic cholesterol patterns in mouse, Lee et al. (2005) studied the effect of aqueous extract of *Solanum nigrum* glycoprotein on levels of plasma lipid including HDL and LDL in mice. They found that the administration of (10, 20 and 40 g head body weight g^{-1}) of this plant on HDL levels was observed to be not significant in compare with control.They also demonstrated that the administration of (10g head body weight g^{-1}) of this plant was not significantly effective on LDL levels in compare with control. However, they observed that the administration of (20 and 40 g head body weight g^{-1}) were significantly decreased LDL-cholesterol levels. These researchers determined that the treatment of

Solanum nigrum glycoprotein has not changed the amount of HDL. However the combined effect of *Solanum nigrum* with Triton WR-1339 was significant on HDL levels in that study.

Similarly, hyperlipidemic rats when treated with 100 and 200 mg/kg body weight of steroidal saponins, isolated from *Solanum nigrum* and *Solanum xanthocarpum* respectively. After 28 days of the treatment, the serum lipid levels including cholesterol, LDL and triglyceride were decreased and the level HDL was elevated by varying extents (Gupta et al., 2009).These results indicate that the extract of the respective plant in combination with either particular chemical compound or other plant extract can be much more effective on lipidemic status of animals. In this context, results from the present study might be due less effective role of the extract of *Solanum nigrum* alone on lipidemic status of restive rats.

However, Meera and Sivaranjani (2017) stated that that the fruit extract of *Solanum nigrum* played important roles in restoring the level of lipid to nearly normal concentration showing anti-hyperlipidemic activity of *Solanum nigrum*. Moreover, *Solanum nigrum* fruit extract was found to significantly elevate the levels of total cholesterol, triglycerides, LDL and VLDL, whereas the HDL level was observed to be reduced in the plasma of rats (Arulmozhi et al., 2010). These results indicate that the extract from a particular part of this might valuably be effective and others might be not. According to those studies, results from the present study considering cholesterol, triglycerides, LDL, VLDL and HDL levels might be due to the use of *Solanum nigrum* whole extract.

A part of our results might be due to use of only one solvent, because the use of appropriate concentrations might significantly be effective. Furthermore, as sated by Zubair et al. (2011), the use of proper solvent including ethanol, methanol, hexane and ethyl acetate might play important roles on the phytochemical efficacy of the respective plant. Based on this statement, our results might due to the use of aqueous extract instead of using other solvents.

Many studies have been shown oxidative stress to have association with pathological states encompassing both communicable and non-communicable diseases.

Consequently, the need for potent antioxidants in our diet and drug supplements becomes very necessary (Atanu et al., 2011). A study utilizing six pretreatment methods before cooking on the peroxidase activity, chlorophyll and antioxidant status of *Solanum nigrum* showed that pretreatment methods have significant effects on the parameters measured. A sharp difference in the carotenoids, phenolic, flavonoids and tannins contents has been reported, indicating the fragility of this antioxidant present in *Solanum nigrum* (Adebooye et al., 2008). *Solanum nigrum* leaves glycoprotein showed a dose-dependent radical scavenging activity on radicals, including 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals, hydroxyl radical (OH), and superoxide anion (O_2^-).

Important oxidative parameters including Total oxidant status (TOS) and all serum antioxidants which are known as total antioxidant status (TAS). These parameters are used to monitor the progression and range of damages that resulted from oxidative stress in rat blood serum (Turkez et al., 2012). In the current study, we analyzed both TAS and TOS levels of serum in diabetic rats and we found significant differences in both parameters when compared to non-diabetic rats.

Despite *Solanum nigrum* acts as an anti-tumor, the glycoprotein of this medicinal might induce apoptosis by the inhibition of NF-B activation, induced by oxidative stress in HT-29 cells (Heo et al., 2004). A 50% of crude ethanolic extract of this plant also possess hydroxyl radical scavenging potential which is suggested to act as cyto-protective mechanism (Kumar et al., 2001; Al-Fatimi et al., 2007). Evaluation of the antioxidant potential of *Solanum nigrum* leaves on the modulation of a 6 h restraint induced oxidative stress, which subsequently suggests that this plant was better as an antioxidant with post-restraint treatment than with pre restraint administration.

Various systemic and ocular diseases including uveitis, glaucoma, and retinopathy of prematurity and macular degeneration have been pathophysiologically implicated by oxidative stress. Common procedures that have been used to determine the predicted oxidative stress are indicated by measuring total antioxidant status (TAS) and total oxidant status (TOS), measuring the later one indicates oxidative stress in biological samples (Erel, 2004; Ferreira et al., 2004).

In the current study, as indications to oxidative stress we analyzed both TAS and TOS levels of serum in diabetic rats, and we found significant differences in both parameters when compared to non-diabetic rats. TAS levels were reduced, while TOS levels were increased in diabetic group. These results suggest an imbalance between antioxidant defense and free radical generation which has an important role in the progression of diabetic complication (Beyazyıldız et al., 2013). Moreover, reports on the antioxidants status and antioxidant enzymes in patients with DM observe somewhat contradictory, as both increases and decreases have been reported in antioxidant activity. Generally, TAS has been reported to be incredibly lower in patients with proliferative diabetic retinopathy than in diabetics who have no developed retinopathy (Victor et al., 2014). In relevant study examining the possible effects of resveratrol on levels of blood glucose, and other oxidative markers in T2DM rats, results of this study indicated that TOS level in diabetic rats were significantly increased, and rats treated with resveratrol were observed decreased level of TOS which is used as an oxidative stress biomarker.

Due to the above investigations, we analyzed levels of both TOS and TAS in respective rats. Our results indicate that *Solanum nigrum* might influence oxidative stress because of the significant reduction in total oxidant capacity (TOS) and valuable increase in antioxidant capacity (TAS) after administration of SN in diabetic rats.

Results from the present study reflect the contribution of *Solanum nigrum* extracts in the regulation of oxidant and antioxidant capacity and subsequently the oxidative stress harmony of diabetic rats. Moreover, the present results suggest that *Solanum nigrum* exerts its chemotherapeutic effects by modulating the antioxidant status during hyperglycemic infection.

In conclusion, the biological effectiveness of this extract can be limited at a particular level of blood glucose and might be effective when glucose level becomes out of normal range which subsequently shows medicinal contribution of *Solanum nigrum* in patients with DM. Thus it might be worthy to conclude the anti- diabetic property of *Solanum nigrum*. This protective role might resulted from antioxidant and detoxifying effects of this plant as consequences of containing steroidal saponins including

nigrumin I and II (Aali et al., 2010). Further studies elucidating mechanisms of action and exploring medicinal value of respective components of this extract can be of value.

The administration of the extract of this plant was not significantly effective on levels cholesterol, Triglyceride, LDL, HDL and VLDL. These results might reflect non antihypercholesterolemia and hypotriglycemia effects of the extract of *Solanum nigrum*. These results might be due to the statement by Poongothai et al. 2010 in which biological and therapeutic activities of this plant can considerably be varied according to where is grown and its cultivars are being cultivated. It is recommended to investigate further studies using different accessions (plants from different places), different plant parts including roots, leaves and berries at different concentrations of such plant against lipoproteins. It will also be valuable to use different solvents such as ethanol, methanol, ethyl acetate, diethyl ether, chloroform (Parameswari et al., 2012), n-butanol and n-hexane (Zubair et al., 2011). Finally, as previous researches revealed that the alkaloidal content of *Solanum nigrum* parts changes during developmental stages (Cooper and Johnson, 1984), we recommend using extracts from this plant at different developmental stages.

Important oxidative parameters including TOS and TAS were used to monitor the progression and range of damage results from oxidative stress in rat blood serum (Turkez et al., 2012). TAS levels were reduced, while TOS levels were increased in diabetic group. These results suggest an imbalance between antioxidant defense and free radical generation which has an important role in the progression of diabetic complication. However, TAS levels were reduced, while TOS levels were increased in diabetic group in response to administrations of *Solanum nigrum* extract. It is concluded that treatment of diabetic rats with *Solanum nigrum* decreased antioxidant factors and supported antioxidant factors. These results might improve reproductive complications as a consequence to diabetes (Beyazyıldız et al., 2013).

Due to the significant effects of our extracts on TAS and TOS, it appears that respective *Solanum nigrum* might associates with pathological states encompassing both communicable and non-communicable diseases. This indicates the need for components of this plant in our diet as potent antioxidants (Atanu et al., 2011). Based on our results, it is suggested that *Solanum nigrum* leaves glycoprotein might contributes in the

regulation of radical scavenging activities including 1, 1-diphenyl-2- picrylhydrazyl radicals, hydroxyl radical, and superoxide anion (Adebooye et al., 2008).

As a result, it was determined that the SN plant was statistically significant on the shaped diabetes and that the experimental diabetes level was lowered but the developing diabetes and the rising sugar could not be reduced to normal levels.

SUMMARY

Fethulla MN. Antioxidant and antihyperlipidemic effect of *Solanum nigrum* fruit extract in experimental diabetes model. Van Yuzuncu Yıl Unıversıty, Institute of Health Science, Department of Biochemistry, Veterinary Program, MSc Thesis, Van, 2017. Diabetes mellitus (DM) is a chronic metabolic non-communicable disease; it is globally considered as the fifth cause of death and it has attained worldwide epidemic proportions. In addition, extracts of several herbal medicines have been used and recommended to have potential therapeutic effects on diabetes and its complications. Extracts from *Solanum nigrum Linn* (European black nightshade) which belongs to *Solanum* genus and Solanaceae family has been reported to exhibit anti-tumor activity against different types of cancers and significant antidiabetic activities against diabetes. In our study, we aimed to investigate the diabetic effects of *Solanum nigrum* extract using the control group (C), diabetes group (D), groups given the Solanum nigrum extract (SN) and (D + SN). Our results observed the biological effectiveness of *Solanum nigrum* extract on glucose levels, significant increasing of serum glucose level group (D) (663 ± 21.8 mg/dL) in comparison with C (131 ± 9.8 mg/dL) were recorded. However, there were no significant difference in glucose level between C group (131 ± 9.8 mg/dl) and SN group (196.14 ± 12.1 mg/dL). Moreover, glucose level of D+SN group (484.8 ± 40.0 mg/dL) was significantly higher than C (131 ± 9.8 mg/dl), D (663 ± 21.8 mg/dl) and SN groups (196.14 ± 12.1 mg/dL). Furthermore, despite noticeable changes in blood levels of Cholesterol, triglyceride, HDL, LDL and VLDL in response to the respective extract, results showed no significant differences in levels of those parameters between all groups. These results might reflect non antihypercholesterolemia and hypotriglycemia effects of the extract of *Solanum nigrum*. These results might be due to the changes of biological and therapeutic activities of this plant according to where is grown and its cultivars are being cultivated. Total antioxidant status (TAS) level in D group (1.85 ± 0.15.7) was significant when compared C group (1.28 ± 0.17). However, significant differences were observed between D group and D+SN group (1.54 ± 0.07).TAS levels showed no significant difference in both SN (1.27 ± 0.10) and D+SN (1.54 ± 0.07) groups in comparison to control. Total oxidant status (TOS) level in D group (6.30 ± 1.41) was given significant differences in comparison with control C (3.87 ± 0.34), SN (4.87 ± 0.80) group and D+SN (4.14 ± 0.34) groups. In contrary, there were no significant differences between all of C, SN D+SN groups. These results suggest an imbalance between antioxidant defense and free radical generation which has an important role in the progression of diabetic complication. The extract of this plant might have therapeutic effect on patients with diabetes. As a result, we can say that the solanum nigrum plant extract is effective on diabetes, but it can not lower the glucose level to normal levels, it needs to be investigated in future studies and its effects at different doses by different extraction methods..

Key words: Hypolipidemic effect, *Solanum nigrum* L., STZ, TAS (Total antioxidant status), TOS (total oxidant status).

ÖZET

Fethulla MN. Deneysel diyabet modelinde *Solanum nigrum* meyve ekstraktının antioksidan ve antihiperlipidemik etkisi. Van Yuzuncu Yıl Üniversitesi, Sağlık Bilimleri Enstitüsü, Biyokimya Anabilim Dalı, Veteriner Program, Yüksek Lisans Tezi, Van, 2017. Diabetes mellitus (DM) kronik metabolik bulaşıcı olmayan bir hastalıktır; küresel olarak beşinci ölüm nedeni olarak görülmektedir ve dünya çapında salgın boyutlara erişmiştir. Buna ek olarak, çeşitli bitkisel özlerin ekstraktları kullanılmış, diyabet ve komplikasyonları üzerinde potansiyel terapötik etkilere sahip olduğu önerilmiştir. Solanum cinsine ve Solanaceae familyasına ait olan Solanum nigrum Linn'den (European black nightshade) elde edilen ekstraktların, farklı kanser tiplerine karşı anti-tümör etkinliği ve diyabete karşı önemli antidiyabetik etkinlik sergilediği rapor edilmiştir. Çalışmamızda kontrol grubu (C), Diyabet (D) grubu, Solanum nigrum ekstraktı verilen gruplar (SN) ve (D + SN) kullanılarak, Solanum nigrum özütünün diyabetik etkilerini araştırmak amaçlandı. Solanum nigrum ekstraktının glikoz düzeyleri üzerindeki biyolojik etkinliğine bakıldığında; kontrol grubu (131 ± 9.8 mg / dL) ile karşılaştırıldığında diyabet grubunun (663 ± 21.8 mg / dL) serum glikoz düzeyini belirgin şekilde arttığı kaydedildi. Bununla birlikte, kontrol grubu (131 ± 9.8 mg / dl) ve SN grubu (196.14 ± 12.1 mg / dL) arasında glikoz düzeyinde anlamlı bir farklılık yoktu. Dahası, D + SN grubunun (484.8 ± 40.0 mg / dL) glukoz düzeyi kontrol (131 ± 9.8 mg / dl) ve SN gruplarına (196.14 ± 12.1 mg / dL) göre anlamlı derecede yüksek, D (663 ± 21.8 mg / dl) grubuna göre anlamlı derecede düşüktü ($p<0.05$). Ayrıca, kolesterol, trigliserit, HDL, LDL ve VLDL'nin ilgili ekstrakta yanıt olarak kan düzeylerinde belirgin değişiklikler olmasına rağmen, sonuçlar tüm gruplar arasında bu parametrelerin seviyelerinde anlamlı bir farklılık göstermedi. Bu sonuçların Solanum nigrum özütünün antihiperololesterolemi ve hipotriglisemi etkilerinden olduğu düşünülmektedir. D grubunda total antioksidan status (TAS) düzeyi ($1.85 \pm 0.15.7$) kontrol grubuyla (1.28 ± 0.17) karşılaştırıldığında anlamlı bulundu ($p<0.05$). Bununla birlikte, D grubu ile D + SN grubu (1.54 ± 0.07) arasında anlamlı fark bulundu ($p<0.05$). TAS düzeyleri, SN (1.27 ± 0.10) ve D + SN (1.54 ± 0.07) gruplarında kontrol grubuna göre anlamlı fark göstermedi. D grubundaki (6.30 ± 1.41) total oksidan status (TOS) düzeyi kontrol (3.87 ± 0.34), SN (4.87 ± 0.80) ve D + SN (4.14 ± 0.34) grubuna göre anlamlı farklılık gösterdi ($p<0.05$). Aksine, tüm kontrol, SN ve D + SN grupları arasında anlamlı bir farklılık yoktu. Sonuç olarak, solanum nigrum bitki ekstresinin, diyabet üzerine etkili olduğu fakat glukoz seviyesini normal düzeylere düşüremediği, ileride yapılacak çalışmalar ile farklı eksraksiyon yöntemleri ile farklı dozlarda etkisinin araştırılması gerektiğini söyleyebiliriz..

Anahtar Kelimeler: Hipolipidemik etki, Solanum nigrum L., STZ, TAS (Total antioksidan status), TOS (total oxidant status).

REFERENCES

Aali NS, Singh K, Khan M, Rani S (2010). Protective effect of ethanolic extract of *Solanum nigrum* on the blood sugar of albino rats. *International Journal of Pharmaceutical Science and Research,* 1, 97-99.

Adebooye OC, Vijayalakshmi R, Singh V (2008). Peroxidase activity, chlorophylls and antioxidant profile of two leaf vegetables (*Solanum nigrum* L. and Amaranthus cruentus L.) under six pretreatment methods before cooking. *International journal of food science & technology,* 43, 173-178.

Ahir K, Patel B, Patel S, Mehta F, Jani D, Shah J (2008). Effects of *Solanum nigrum* fruits on lipid levels and antioxidant defenses in rats with fructose induced hyperlipidemia and hyperinsulinaemia. *Pharmacology online,* 3, 797-807.

Ahmed M, Laing MD, Nsahlai IV (2014). In vivo effect of selected medicinal plants against gastrointestinal nematodes of sheep. *Tropical animal health and production*, 46, 411-417.

Al-Fatimi M, Wurster M, Schröder G, Lindequist U (2007). Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *Journal of Ethnopharmacology,* 111, 657-666.

Aly Y, Shallan M (2011). Antioxidant properties of wild. *Solanum nigrum* ripe fruit. *Planta Medica,* 77, 20.

Anjana R, Ali M, Pradeepa R, Deepa M, Datta M, Unnikrishnan R, Rema M, Mohan V (2011). The need for obtaining accurate nationwide estimates of diabetes prevalence in India-rationale for a national study on diabetes. *The Indian journal of medical research,* 133, 369.

Arulmozhi V, Krishnaveni M, Karthishwaran K, Dhamodharan G, Mirunalini S (2010). Antioxidant and antihyperlipidemic effect of *Solanum nigrum* fruit extract on the experimental model against chronic ethanol toxicity. *Pharmacognosy magazine,* 6, 42.

Asmat U, Abad K, Ismail K (2016). Diabetes mellitus and oxidative stress—a concise review. *Saudi Pharmaceutical Journal*, 24, 547-553.

Association AD (2013). Standards of medical care in diabetes—2013. *Diabetes care,* 36, 11-66.

Association AD (2014). Diagnosis and classification of diabetes mellitus. *Diabetes care,* 37, 81-90.

Association AD (2017). Standards of medical care in diabetes—2017 abridged for primary care providers. *Clinical diabetes*, 35, 5-26.

Atanu F, Ebiloma U, Ajayi E (2011). A review of the pharmacological aspects of *Solanum nigrum* Linn. *Biotechnology and Molecular Biology Reviews,* 6, 1-8.

Bandeira MS, Da Fonseca LJS, da S Guedes G, Rabelo LA, Goulart MO, Vasconcelos SML (2013). Oxidative Stress as An Underlying Contributor in the Development of Chronic Complications in Diabetes Mellitus. *International Journal of Molecular Sciences,* 14, 3265-3284.

Bansal AK, Bilaspuri G (2011). Impacts of oxidative stress and antioxidants on semen functions. *Veterinary Medicine International*, 7.

Benítez S, Pérez A, Sánchez-Quesada JL, Wagner AM, Rigla M, Arcelus R, Jorba O, Ordóñez-Llanos J (2007). Electronegative low-density lipoprotein subfraction from type 2 diabetic subjects is proatherogenic and unrelated to glycemic control. *Diabetes/metabolism research and reviews,* 23, 26-34.

Bettelheim F, Brown W, Campbell M, Farrell S, Torres O (2012). *Introduction to general, organic and biochemistry*. Nelson Education.

Beyazıldız E, Çankaya AB, Ergan E, Anayol MA, Özdamar Y, Sezer S, Tırhış MH, Yılmazbaş P, Öztürk F (2013). Changes of total antioxidant capacity and total oxidant status of aqueous humor in diabetes patients and correlations with diabetic retinopathy. *International journal of ophthalmology,* 6, 531.

Bhat MAMA, Ahmad S, Aslam J, Mujib A (2008). Salinity stress enhances production of solasodine in *Solanum nigrum* L. *Chemical and Pharmaceutical Bulletin,* 56, 17-21.

Bloch K, Vardi P (2005). Toxin-Based Selection of Insulin-Producing Cells With Improved Defense Properties for Islet Cell Transplantation. *Diabetes/Metabolism Research and Reviews*, 21, 253-261.

Britto AJ, Gracelin D, Kumar P (2011). Antimicrobial Activity of a Few Medicinal Plants Against Gram Negative Bacteria. *International Journal of Applied Biology and Pharmaceutical Technology*, 2, 457-461.

Boveris A, Cadenas E (1997). Cellular sources and steady-state levels of reactive oxygen species. *Lung Biology in Health and Disease,* 105, 1-26.

Brahimaj A, Ligthart S, Ikram MA, Hofman A, Franco OH, Sijbrands EJ, Kavousi M, Dehghan A (2017). Serum levels of apolipoproteins and incident type 2 diabetes: a prospective cohort study. *Diabetes care,* 40, 346-351.

Cho KH, Wolkenhauer O (2003). Analysis and modelling of signal transduction pathways in systems biology. Portland Press Limited.

Collins AR (2005). Antioxidant intervention as a route to cancer prevention. *European Journal of Cancer,* 41, 1923-1930.

Cooper MR, Johnson AW (1984). *Poisonous plants in Britain and their effects on animals and man*. HM Stationery Office.

Benítez S, Pérez A, Sánchez-Quesada JL, Wagner AM, Rigla M, Arcelus R, Jorba O, Ordóñez-Llanos J (2007). Electronegative Low-Density Lipoprotein Subfraction From Type 2 Diabetic Subjects is Proatherogenic and Unrelated to Glycemic Control. *Diabetes/Metabolism Research and Reviews, 23*, 26-34.

Defelice MS (2003). The black nightshades, *Solanum nigrum* L. et al.—poison, poultice, and pie. *Weed Technology, 17*, 421-427.

DeFronzo RA, Ferrannini E, Zimmet P, Alberti G (2015). *International Textbook of Diabetes Mellitus, 2 Volume Set*. John Wiley & Sons.

Diplock A (2000). Introduction: markers of oxidative damage and antioxidant modulation. *Free radical research, 33*, 21-26.

Domingueti CP, Dusse LMSA, das Graças Carvalho M, de Sousa LP, Gomes KB, Fernandes AP (2016). Diabetes mellitus: The linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. *Journal of diabetes and its complications, 30*, 738-745.

Edmonds JM Chweya JA (1997). *Black nightshades: Solanum nigrum L. and related species*. Bioversity International.

El-Far M, Negm A, El-Azim A, Wahdan M (2015). Antioxidant therapeutic actions of medicinal phytochemicals, silymarin and silibinin on streptozotocin diabetic rats: First Novel Comparative Assessment of Structural Recoveries of Histological and Ultrastructural Changes on Islets of Langerhans, B-Cells, Mitochondria and Nucleus. *International Journal of Pharmacy and Pharmaceutical Sciences, 8*, 69-76.

Eltayeb A, Al-Ansari AS, Roddick JG (1997). Changes in the steroidal alkaloid solasodine during development of *Solanum nigrum* and *Solanum incanum*. *Phytochemistry, 46*, 489-494.

Erel O (2004). A novel automated method to measure total antioxidant response against potent free radical reactions. *Clinical biochemistry, 37*, 112-119.

Estruch M, Miñambres I, Sanchez-Quesada JL, Soler M, Pérez A, Ordoñez-Llanos J, Benitez S (2017). Increased inflammatory effect of electronegative LDL and decreased protection by HDL in type 2 diabetic patients. *Atherosclerosis, 265*:292-298.

Fang YZ, Yang S, Wu G (2002). Free radicals, antioxidants, and nutrition. *Nutrition, 18*, 872-879.

Fedewa MV, Gist NH, Evans EM, Dishman RK (2014) Exercise and insulin resistance in youth: a meta-analysis. *Pediatrics,* 133, 163-174.

Ferreira SM, Lerner SF, Brunzini R, Evelson PA & Llesuy SF (2004). Oxidative stress markers in aqueous humor of glaucoma patients. *American journal of ophthalmology,* 137, 62-69.

Foyer CH, Lelandais M, Kunert KJ (1994). Photooxidative stress in plants. *Physiologia Plantarum,* 92, 696-717.

Gupta AK, Ganguly P, Majumder UK, Ghosal S (2009). Improvement of lipid and antioxidant status in hyperlipidaemic rats treated with steroidal saponins of *Solanum nigrum* and Solanum xanthocarpum. *Pharmacologyonline,* 1, 1-14.

Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M (1998). Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *New England journal of medicine,* 339, 229-234.

Haldar S, Rowland I, Barnett Y, Bradbury I, Robson P, Powell J, Fletcher J (2007). Influence of habitual diet on antioxidant status: a study in a population of vegetarians and omnivores. *European journal of clinical nutrition,* 61, 1011.

Halliwell B (1994). Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *The lancet,* 344, 721-724.

Harasym J, Oledzki R (2014). Effect of fruit and vegetable antioxidants on total antioxidant capacity of blood plasma. *Nutrition,* 30, 511-517.

Heo KS, Lee SJ, Lim KT (2004) Cytotoxic effect of glycoprotein isolated from *Solanum nigrum* L. through the inhibition of hydroxyl radical-induced DNA-binding activities of NF-kappa B in HT-29 cells. *Environmental Toxicology and Pharmacology,* 17, 45-54.

Hou TH, Chung JP, Chen SS, Chang TL (2013). Antioxidation and antiglycation of 95% ethanolic extracts prepared from the leaves of black nightshade (*Solanum nigrum*). *Food Science and Biotechnology,* 22, 839-844.

Hsu JD, Kao SH, Tu CC, Li YJ, Wang CJ (2009). *Solanum nigrum* L. extract inhibits 2-acetylaminofluorene-induced hepatocarcinogenesis through overexpression of glutathione S-transferase and antioxidant enzymes. *Journal of Agricultural and Food Chemistry,* 57, 8628-8634.

Hu K, Kobayashi H, Dong A, Jing Y, Iwasaki S, Yao X (1999), Antineoplastic Agents III: Steroidal Glycosides from Solanum Nigrum. *Planta Medica,* 65, 35-38.

Huang Q, Feng J, Wu R, Yang Y, Dai C, Li J, Liao Y, Xiang M, Wang D, Du XB (2017). Total Oxidant/Antioxidant Status in Sera of Patients with Esophageal

Cancer. *Medical science monitor: international medical journal of experimental and clinical research,* 23, 3789.

Hung HY, Qian K, MorrisNatschke SL, Hsu CS, Lee KH (2012). Recent discovery of plant-derived anti-diabetic natural products. *Natural product reports,* 29, 580-606.

Iso H, Date C, Wakai K, Fukui M, Tamakoshi A (2006). The Relationship between Green Tea and Total Caffeine Intake and Risk for Self-Reported Type 2 Diabetes among Japanese AdultsGreen Tea Reduces Diabetes Risk. *Annals of Internal Medicine,* 144, 554-562.

Jagadeeshan S, David D, Jisha S, Manjula S, Nair SA (2017). *Solanum nigrum* Unripe fruit fraction attenuates Adriamycin resistance by down-regulating multi-drug resistance protein (Mdr)-1 through Jak-STAT pathway. *BMC complementary and alternative medicine,* 17, 370.

Jakus V (2000). The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. *Bratislavske lekarske listy,* 101, 541-551.

Jansen EH, Ruskovska T (2013). Comparative analysis of serum (anti) oxidative status parameters in healthy persons. *International journal of molecular sciences* 14, 6106-6115.

Jinu U, Jayalakshmi N, Anbu AS, Mahendran D, Sahi S, Venkatachalam P (2017). Biofabrication of cubic phase silver nanoparticles loaded with phytochemicals from *Solanum nigrum* leaf extracts for potential antibacterial, antibiofilm and antioxidant activities against MDR human pathogens. *Journal of Cluster Science,* 28, 489-505.

Johansen JS, Harris AK, Rychly DJ, Ergul A (2005). Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovascular diabetology*, 4, 5.

Karmakar UK, Tarafder UK, Sadhu SK, Biswas NN, Shill MC (2010). Biological Investigations of Dried Fruit of *Solanum nigrum* Linn. *Stamford Journal of Pharmaceutical Sciences,* 3, 38-45.

Khanna S (2000). Thiol Antioxidants. Ph. D. Dissertation. Department of Physiology University of Kuopio, Kuopio, Finland.

Knapp S (2002). Solanum section Geminata (Solanaceae). *Flora Neotropica*, 84.

Knight JA (2000). Free radicals, antioxidants, and the immune system. *Annals of Clinical & Laboratory Science,* 30, 145-158.

Knowler W (2002). Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, and Nathan DM. *Reduction in the incidence of type* 2, 393-403.

Kontush A, Chapman MJ (2010). Antiatherogenic function of HDL particle subpopulations: focus on antioxidative activities. *Current opinion in lipidology* 21, 312-328.

Kumar P, Kumar J, Kumar R, Dubey R (2016). Studies on phytochemical constituents and antimicrobial activities of leaves, fruits and stems of *Solanum nigrum* L. *Asian Journal of Plant Science and Research,* 6, 57-68.

Kumar VP, Shashidhara S, Kumar M, Sridhara B (2001). Cytoprotective role of *Solanum nigrum* against gentamicin-induced kidney cell (Vero cells) damage in vitro. *Fitoterapia,* 72, 481-486.

Lee SJ, Ko JH, Lim K, Lim KT (2005). 150kDa glycoprotein isolated from *Solanum nigrum* Linne enhances activities of detoxicant enzymes and lowers plasmic cholesterol in mouse. *Pharmacological research,* 51, 399-408.

Lipinski B (2001). Pathophysiology of Oxidative Stress in Diabetes Mellitus. *Journal of Diabetes and its Complications,* 15, 203-210.

Mabberley DJ (1997). *The plant-book: a portable dictionary of the vascular plants.* Cambridge university press.

Maharana L (2011). Investigation of the Hypoglycemic/Antidiabetic Potential and Toxicity Profile of Some Plants in Control of Blood Glucose Level in Experimental Animal Models. Pharma*cologyonline,* 1, 942–963.

Mackey RH, Mora S, Bertoni AG, Wassel CL, Carnethon M., Sibley CT, Goff DC (2015). Lipoprotein particles and incident type 2 diabetes in the multi-ethnic study of atherosclerosis. *Diabetes care,* 38, 628-636.

Mayne ST (2003). Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. *The Journal of nutrition,* 133, 933-940.

McGarry JD (2002). Banting lecture 2001. *Diabetes* 51, 7-18.

Meera R, Sivaranjani K (2017) Anti-Obesity and Hypolipidemic Activity of Siddha Drugs: A Review. *World Journal of Pharmaceutical Sciences*, 5, 1-12..

Mohan H (2005). *Textbook of pathology.* Jaypee brothers medical publishers New Delhi.

Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ (2006). Leads from Indian medicinal plants with hypoglycemic potentials. *Journal of Ethnopharmacology,* 106, 1-28.

Munari CC, de Oliveira PF, Campos JCL, Martins SdPL, Da Costa JC, Bastos JK, Tavares DC (2014). Antiproliferative Activity of *Solanum Lycocarpum* Alkaloidic

Extract and Their Constituents, Solamargine and Solasonine, in Tumor Cell Lines. *Journal of Natural Medicines,* 68, 236-241.

Napoli N, Chandran M, Pierroz DD, Abrahamsen B, Schwartz AV, Ferrari SL (2017). Mechanisms of diabetes mellitus-induced bone fragility. *Nature Reviews Endocrinology,* 13, 208-219.

Neuman JC, Fenske RJ, Kimple ME (2017). Dietary polyunsaturated fatty acids and their metabolites: Implications for diabetes pathophysiology, prevention, and treatment. *Nutrition and Healthy Aging,* 4, 127.

Nyeem MAB, AKMMU R, Nowrose M, Hossain MA (2017). *Solanum nigrum* (Maku): A review of pharmacological activities and clinical effects. *International Journal of Applied Research,* 3, 12-17.

Opande GT, Musyimi DM, Cherono KL, Jiveri BK, Buyela DK (2017). Antimicrobial Activities of Crude Leaf Extract of *Solanum nigrum* on Fusarium Oxysporum and Pseudomonas Syringae Cultures in Maseno (Kenya). *Journal of Asian Scientific Research,* 7, 271-278.

Ozougwu J, Obimba K, Belonwu C, Unakalamba C (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *Journal of Physiology and Pathophysiology,* 4, 46-57.

Pandey KB, Rizvi SI (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity,* 2, 270-278.

Parameswari K, Aluru S, Kishori B (2012). In vitro antibacterial activity in the extracts of *Solanum nigrum*. *Indian Streams Research Journal,* 2, 1-4.

Patel D, Kumar R, Laloo D, Hemalatha S (2012). Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pacific Journal of Tropical Biomedicine,* 2, 411-20.

Patel D, Kumar R, Prasad S, Hemalatha S (2011). Pedalium murex Linn (Pedaliaceae) fruits: a comparative antioxidant activity of its different fractions. *Asian Pacific Journal of Tropical Biomedicine,* 1, 395-400.

Patel D, Kumar R, Prasad S, Sairam K, Hemalatha S (2011). Antidiabetic and in vitro antioxidant potential of Hybanthus enneaspermus (Linn) F. Muell in streptozotocin–induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine,* 1, 316-322.

Pham-Huy LA, He H, Pham-Huy C (2008). Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS,* 4, 89.

Poongothai K, Ahmed KSZ, Ponmurugan P, Jayanthi M (2010). Assessment of antidiabetic and antihyperlipidemic potential of *Solanum nigrum* and Musa

paradisiaca in alloxan induced diabetic rats. *Journal of Pharmacy Research,* 3, 2203-2205.

Russo LM, Nobles C, Ertel KA, Chasan-Taber L, Whitcomb BW (2015). Physical activity interventions in pregnancy and risk of gestational diabetes mellitus: a systematic review and meta-analysis. *Obstetrics & Gynecology,* 125, 576-582.

Salisbury E.J. (1961) Weeds and Aliens. Collins, London,

Sanchez-Quesada J, Perez A, Caixas A, Ordonmez-Llanos J, Carreras G, Payes A, Gonzalez-Sastre F, De Leiva A (1996). Electronegative low density lipoprotein subform is increased in patients with short-duration IDDM and is closely related to glycaemic control. *Diabetologia,* 39, 1469-1476.

Schaan B, Dall'Ago P, Maeda CY, Ferlin E, Fernandes T, Schmid H, Irigoyen M (2004). Relationship between cardiovascular dysfunction and hyperglycemia in streptozotocin-induced diabetes in rats. *Brazilian journal of medical and biological research,* 37, 1895-902.

Scheffer P, Bos G, Volwater H, Dekker J, Heine R, Teerlink T (2003). Associations of LDL size with in vitro oxidizability and plasma levels of in vivo oxidized LDL in type 2 diabetic patients. *Diabetic medicine,* 20, 563-567.

Schweiger M, Romauch M, Schreiber R, Grabner GF, Hütter S, Kotzbeck P, Benedikt P, Eichmann TO, Yamada S, Knittelfelder O (2017). Pharmacological inhibition of adipose triglyceride lipase corrects high-fat diet-induced insulin resistance and hepatosteatosis in mice. *Nature communications,* 8, 14859.

Scobie IN (2006). *Atlas of diabetes mellitus*. CRC Press.

Sen S, Chakraborty R, De B (2016). *Diabetes Mellitus in 21st Century*. Springer.

Shalaurova I, Connelly MA, Garvey WT, Otvos JD (2014). Lipoprotein Insulin Resistance Index: a lipoprotein particle–derived measure of insulin resistance. *Metabolic syndrome and related disorders,* 12, 422-429.

Sies H (1985). Hydroperoxides and thiol oxidants in the study of oxidative stress in intact cells and organs. *Oxidative stress*, 73-90.

Singh RP, Sharad S, Kapur S (2004). Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants. *Journal of Indian Academic and Clinical Medicine,* 5, 218-225.

Sohrabipour S, Kharazmi F, Soltani N, Kamalinejad M (2013). Effect of the administration of *Solanum nigrum* fruit on blood glucose, lipid profiles, and sensitivity of the vascular mesenteric bed to phenylephrine in streptozotocin-induced diabetic rats. *Medical science monitor basic research,* 19, 133.

Sohrabipour S, Kharazmi F, Soltani N, Kamalinejad M (2014). Biphasic effect of *Solanum nigrum* fruit aqueous extract on vascular mesenteric beds in non-diabetic and streptozotocin-induced diabetic rats. *Pharmacognosy research,* 6, 148.

Somogyi A, Rosta K, Pusztai P, Tulassay Z, Nagy G. (2007). Antioxidant measurements. *Physiological Measurement,* 28, 41.

Sparks JD, Sparks CE, Adeli K (2012). Selective hepatic insulin resistance, VLDL overproduction, and hypertriglyceridemia. *Arteriosclerosis, thrombosis, and vascular biology,* 32, 2104-2112.

Sperling MA (2003). *Type 1 diabetes: etiology and treatment*. Springer Science & Business Media.

Stahl W, Sies H (2005). Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* 1740, 101-107.

Sun R, Zhou Q, Wang X (2006). Relationships between cadmium accumulation and organic acids in leaves of *Solanum nigrum* L. as a cadmium-hyperaccumulator. *Huan jing ke xue= Huanjing kexue,* 27, 765-769.

Tiwari AK, Rao JM (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current science*, 30-38.

Tiwari VK, Jain S (2017). Hypoglycemic Activity of Ethanolic Extract of *Solanum nigrum* Linn. Leaves on Alloxan Induced Diabetes Mellitus in Rats. *International Journal of Pharmaceutical and Phytopharmacological Research,* 2, 26-28.

Toprak I, Kucukatay V, Yildirim C, Kilic-Toprak E, Kilic-Erkek O (2014). Increased systemic oxidative stress in patients with keratoconus. *Eye,* 28, 285.

Tripathi K (2013). *Essentials of medical pharmacology*. JP Medical Ltd.

Turkez H, Aydin E, Aslan A (2012). Xanthoria elegans (Link)(lichen) extract counteracts DNA damage and oxidative stress of mitomycin C in human lymphocytes. *Cytotechnology,* 64, 679-686.

Ullah A, Khan A & Khan MI (2015). Diabetes mellitus and oxidative stress―a concise review. *Saudi Pharmaceutical Journal,* 24, 5, 547-553.

Umamageswari M, Karthikeyan T, Maniyar YA (2017). Antidiabetic Activity of Aqueous Extract of *Solanum nigrum* Linn Berries in Alloxan Induced Diabetic Wistar Albino Rats. *Journal of clinical and diagnostic research:* 11, 16-19.

Unnikrishnan R, Anjana RM, Mohan V (2016). Diabetes mellitus and its complications in India. *Nature reviews endocrinology* 12, 357-370.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology,* 39, 44-84.

Venkatesan D, Karrunakaran C (2010). Antimicrobial activity of selected Indian medicinal plants. *Journal of phytology,* 2.

Victor RP (2014). Diabetes: Oxidative Stress and Dietary Antioxidants. Elsevier Press: 32 Jamestown Road, London NW1 7BY, UK.

World Health Organization (1999). Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation. Part 1, Diagnosis and Classification of Diabetes Mellitus.

World Health Organization (2006). Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation.

Wallace JI (1999). Management of diabetes in the elderly. *Clinical diabetes,* 17, 19.

Wang Y, Xiang L, Yi X, He X (2017). Potential Anti-inflammatory Steroidal Saponins from the Berries of *Solanum nigrum* L.(European Black Nightshade). *Journal of Agricultural and Food Chemistry*, 31;65, 21,4262-4272.

Weseler AR, Bast A (2010). Oxidative stress and vascular function: implications for pharmacologic treatments. *Current hypertension reports,* 12, 154-161.

Witztum JL (1997). Role of modified lipoproteins in diabetic macroangiopathy. *Diabetes,* 46, 112.

Wu R, Feng J, Yang Y, Dai C, Lu A, Li J, Liao Y, Xiang M, Huang Q, Wang D (2017). Significance of Serum Total Oxidant/Antioxidant Status in Patients with Colorectal Cancer. *PloS one* 12, 1, e0170003.

Xiang S, Zhang Q, Tang Q, Zheng F, Wu J, Yang L, Hann SS (2016). Activation of AMPKα mediates additive effects of solamargine and metformin on suppressing MUC1 expression in castration-resistant prostate cancer cells. *Scientific reports,* 6, 36721.

Zubair M, Rizwan K, Rasool N, Afshan N, Shahid M, Ahmed VU (2011). Antimicrobial potential of various extract and fractions of leaves of *Solanum nigrum*. *International Journal of Phytomedicine* 3, 63.

CURRICULUM VITAE

Muhammad Nuradin Fathullah. I live in Kalar a distric from Al-Sulaimaniyah governorate in Northern region of Iraq. I was born on November 25, 1985, in Baghdad, capital city of Iraq. I have got marrid, I have a son (Anas). I have finished my Primary, Secondary and High Schools in Kalar. I also graduated from the university of Sulaymaniya/ College of Education, Department of Chemistry during 2007-2008. I started work in 2008 as chemistry teacher in Zewar High school in Kalar city, I'mstill working as a chemisrty teacher in Hamren high school. Finally, I have accepted to study master degree in Republic of Turkey at the Yuzuncu Yil University, Institute of Health Science Department of Vertarinary/ Biochemistry Science during the years (2014-2017).

ATTACHMENT

Attachment 1. Animal Information.

Date:	Case No:

Owner İnformation

Owner name	
Phone	
Address :	

Case İnformation

Ages:		Breed:	
Resp. Rate:		Heart Rate:	
Body Temp:		Sex:	
Notes:			

Clinical Signs:

Cough	
Dysnea:	
Emaciation	
Mastitis	
Arthritis	
Paralysis	

Attachment 2. Ethical Statement.

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

YUZUNCUYILUNIVERSITY (TURKEY)
ANIMAL RESEARCHES LOCAL ETHIC COMMITTEE
RESEARCH FINAL REPORT APPROVAL CERTIFICATE

AraştırmanınAdı	Deneysel diyabet modelinde *Solanumnigrum* meyve ekstresinin antioksidan ve antihiperlipidemik etkisi
Title of the Research	Antioxidantandantihyperlipidemiceffect of Solanumnigrumfruitextract in experimentaldiabetes model
Araştırıcı(lar) *Investigator(s)*	Yürütücü / *Chief investigator* :Yrd. Doç. Dr. Ahmet Cihat ÖNER
	Yardımcı Araştırıcı(lar) / *Co-investigator(s):* Prof. Dr. Fatmagül YUR Mohammed Noor Addin Fethullah

AraştırmanınBaşlamaTarihi / *Research Starting Date:* 11.07.2016

AraştırmanınBitişTarihi / *Research Completion Date:* 01.07.2017

ProjeSüresi / *Total Time of Project:*12 ay

Proje No / *Project Number:* TYL- 2016- 5135

AraştırmayıDestekleyenKuruluş (varsa)/ *Funding institution(s) (if available):*YYU BAP

Destek Şekli ve Miktarı / *Type and amount of funding:* 3748,86 TL

Karar:
Yukarıda bilgileri verilen planlanan araştırma projesi için Hayvan Deneyleri Etik Kurul Onayı gerekmemektedir. Tarih:30/11 /2017 ; Karar no: 2017/11

Decision:
The proposed research project detailed above does not need Animal Researches Ethic Committee Approval. Date: 30 /11 / 2017   Decision number 2017/11.

	BAŞKAN/CHAIR Prof. Dr. Semiha DEDE	
ÜYE Prof. Dr. N. Tuğba BİNGÖL	ÜYE Prof. Dr. Sıddık KESKİN	ÜYE Prof. Dr. Suphi DENİZ
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60

YÜZÜNCÜ YIL ÜNİVERSİTESİ
SAĞLIK BİLİMLERİ ENSTİTÜSÜ
LİSANSÜSTÜ TEZ ORİJİNALLİK RAPORU

Tarih: 23/11/2017

Tez Başlığı / Konusu:

"Antıoxıdant and antıhyperlıpıdemıc effect of *Solanum nıgrum* fruıt extract ın experımental dıabetes model"
Yukarıda başlığı/konusu belirlenen tez çalışmamın Kapak sayfası, Giriş, Ana bölümler ve Sonuç bölümlerinden oluşan toplam 50 sayfalık kısmına ilişkin, 22/11/2017 tarihinde şahsım/tez danışmanım tarafından Plagiarism Detection (www.turnitin.com) "turnitin" intihal tespit programından aşağıda belirtilen filtreleme uygulanarak alınmış olan orijinallik raporuna göre, tezimin benzerlik oranı %15 (**ONBEŞ**) tir.

Uygulanan filtreler aşağıda verilmiştir:
- Kabul ve onay sayfası hariç,
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- Gereç ve yöntemler hariç,
- Kaynakça hariç,
- Alıntılar hariç,
-Tezden çıkan yayınlar hariç,
- 7 kelimeden daha az örtüşme içeren metin kısımları hariç (Limit match size to 7 words)

Yüzüncü Yıl Üniversitesi Lisansüstü Tez Orijinallik Raporu Alınması ve Kullanılmasına İlişkin Yönergeyi inceledim ve bu yönergede belirtilen azami benzerlik oranlarına göre tez çalışmamın herhangi bir intihal içermediğini; aksinin tespit edileceği muhtemel durumda doğabilecek her türlü hukuki sorumluluğu kabul ettiğimi ve yukarıda vermiş olduğum bilgilerin doğru olduğunu beyan ederim.

Gereğini bilgilerinize arz ederim.

Tarih ve İmza

Adı Soyadı: Mohammed NoorAddin Fethullah
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Statüsü:Y.Lisans☐ Doktora☐

DANIŞMAN ONAYI
UYGUNDUR

Yrd. Doç. Dr. Ahmet Cihat ÖNER
(2.danışman)

(Unvan, Ad Soyad, İmza)

ENSTİTÜ ONAYI
UYGUNDUR

(Unvan, Ad Soyad, İmza)