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Pomegranate for Diabetes and its' Complications Amelioration

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ABSTRACT

Diabetes mellitus is a metabolic disease affecting various body organs and system sca using several complications such as cardiovascular diseases, stroke, nephropathy, retinopathy, neuropathy, erectile dysfunction and diabetic foot ulcer development. Diabetic patients are under oxidative stress and inflammation that play crucial role in complications development. Pomegranate, containing various phytochemicals with antioxidant, anti-inflammatory, antihyper glycemic, antihyper lipidemic, antihypertensive, anti at her ogenic, antimicrobial, cardio protective, neuro protective, regenerative, wound healing and immune modulatory bioactivities, at the same time is sufficiently treating diabetes and its' complications without any side effects as safety tests have shown. Pomegranate treatment improves antioxidant status of the patients, reduces inflammation, ameliorates diabetes improving insulin sensitivity, increasing insulin production and secretion, reducing blood glucose levels, inhibiting hemoglobin glycosylation, protecting pancreas and is contributing to pancreatic islets regeneration and stimulation. It also ameliorates cardiovascular complications decreasing total cholesterol, triglycerides, LDL, lipid peroxidation, atherosclerosis, increasing the beneficial HDL. Pomegranate also enhances wound healing by reducing bacterial count, inhibiting quorum sensing and biofilm formation, increasing collagen production, up regulating the EGF, VEGF and TGF-β1 levels and leading to excellent epithelialization and neovascularization. Besides, pomegranate treatment ameliorates nephropathy reducing serum creatinine, urine albumin, blood urea nitrogen, urine albumin to creatinine ratio, renal fibrosis, glomerular sclerosis, hypertrophyand interstitial hyperplasia. Pomegranate treatment is preventing or ameliorating neuropathy and also improving erectile function by increasing intracavernosal blood flow and MICP/MAP percentage in patients with atherosclerosis. Moreover, pomegranate ameliorates retinopathy reducing the oxidative stress biomarker 8-OHdG, decreasing the levels of sialic acid, malondialdehyde, improving retina cell layers and delaying cataract onset. Is important to be mentioned that while in diabetic individuals the values of several biochemical parameters were significantly improved, at the same time there were not changes on the healthy individuals' biochemical parameters which were normal, indicating except its' excellent pharmaceutical properties also a regulatory role of pomegranate as it is acting only whenever it is needed for health achievement and maintenance. These results indicate that pomegranate can offer an alternative, safe, holistic treatment for diabetes and its' complications, improving the general health and quality of life of the patient.

KEYWORDS: pomegranate, antioxidant, anti diabetic, diabetes-complications, phytochemicals, health-maintenance, homeostasis.

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INTRODUCTION

Among all body organs, there are various multidirectional interactions to maintain the whole-body energy homeostasis and set the organism's adaptability to external cues. Through this inter-organ communication, in order to respond to various metabolic demands, one tissue can affect metabolic pathways of an another, distant tissue. Dysregulation of these communication lines leads to human pathologies such as diabetes mellitus, liver disease, obesity and atherosclerosis which in their turn, aggravate further this dysregulation.[1,2]

Diabetes mellitus is a metabolic, complex disease, which affects various body organs and systems causing many implications that contribute to other severe diseases development including cardiovascular diseases, nephropathy, neuropathy, retinopathy and diabetic foot ulcer development.[3] Commercial anti diabetic drugs may cause several undesirable side effects such as hematological, heart, liver, kidney disturbances and coma. Ameliorating diabetes and complications with less or without any adverse effects is still a challenge to the medical system.[4] In many studies, traditional herbal medicines are mentioned to be very effective in diabetes prevention and treatment with less side effects.[5]Pomegranate, containing in all of its' plant parts in high concentrations various categories of bioactive compounds, has been used since ancient year sin Ayurvedic, Unani, Islamic, Persian, Chinese, Greek, Roman traditional medicine in order to prevent or treats ever al diseases, some of which are complications of diabetes. More specifically, pomegranate plant part extracts were used for treatment of hyperglycemia, atherosclerosis, hypertension, hyperlipidemia, inflammation, allergies, several types of cancer, microbial infections, cerebral disease, weakness, gastrointestinal problems, peptic ulcers and oral diseases. Besides, pomegranate plant part extracts were used for their anthelmintic, anti urogenic and wound healing properties.[5, 6,7, 8]

DIABETES

Diabetes mellitus or diabetes is a metabolic diseases group characterized by high blood sugar levels that is caused due to body' s deficiency to produce enough insulin or due to cells disability to respond to the produced insulin [9]. Insulin is a two-peptide hormone which is secreted by β cells of the pancreatic islets of Langerhans. It facilitates glucose uptake, regulates lipid, protein and carbohydrate metabolism and promotes cell growth and division through its' mi to genic effects. [10]. There are two main types of diabetes, type 1 diabetes, or insulin dependent diabetes and type 2 diabetes or non-insulin-dependent diabetes (90% of diabetes cases), and also gestational diabetes and a range of other diabetes types such as cystic fibrosis diabetes, monogenic diabetes and diabetes caused by rare syndromes.[11] Type 1 diabetes is usually diagnosed in children and teens, yet it also may be diagnosed in adults. Type 2 diabetes which is one of most common metabolic disorders is diagnosed in adults, yet it may be diagnosed in children after the puberty onset. [12, 3,13]

Insulin dependent diabetes mellitus (type I diabetes) is an autoimmune disease that is characterized by altered beta cell function and also by beta cell death after exposure to inflammatory products. In this type of diabetes, the cells that release insulin are destroyed by the body's immune system and thus insulin production is eliminated [14]. Macrophages infiltrate in the islets of Langerhans, islands of endocrine cells that are scattered through pancreas and are involved in the regulation of glucose homeostasis. Through this procedure, reactive oxygen species such as H_2O_2 are generated and harm beta cells and mitochondrial oxidative metabolism. Mitochondria play a key role in the nutrient-induced insulin endocytosis control. Nitric oxide (NO) is also a free radical that is produced by macrophages, and it harms mitochondrial DNA which is very sensitive to oxidative stress (more than nuclear DNA). NO also suppresses mitochondrial activity and results in defective insulin release in response to nutrient secret a gogues.[15]

Type 2 diabetes is associated with high oxidative stress, impaired insulin release, impaired glucose and lipid metabolism.[16] The main etiology of the disease development is the combination of two factors: the defective insulin secretion by β-cells and also the fact that the insulin-sensitive tissues are unable to respond appropriately to insulin, condition that is called insulin resistance. Aggravation of the disease results in less insulin production. This is called insulin deficiency.[17]

Type 1 and 2 diabetes distinction is not always very clear. In recent scientific reports is mentioned that type 1 diabetic patients can also develop insulin resistance that is considered to be the main characteristic of type 2 diabetes. Aggravation of type 2 diabetes leads into less insulin production by pancreas, that mostly characterizes type 1 diabetes.[18, 19]

Gestational diabetes is a diabetes type that develops during pregnancy and usually disappears after giving birth.[20]

Cystic fibrosis related diabetes is a diabetes form characterized by markedly morbidity and mortality. The main etiology is relative insulin insufficiency, pancreatic islets destruction and other factors that affect the normal function of the remaining β cells. More than 50% of patients with cystic fibrosis develop this disease.[21] Monogenic diabetes is caused by a mutation in a single gene that cause changes in beta cells structure and accounts 1% of diabetes cases.[22, 23]

Elevated blood glucose, the common result of uncontrolled diabetes, may affect over time various body systems as it damages heart, blood vessels, nerves, kidneys and eyes causing heart disease, peripheral vascular diseases, neuropathy, nephropathy and retinopathy. [3] Is referred that diabetes complications mostly develop through damage and deterioration of blood vessels that are supplying the organs. Diabetic patients most often die from renal-related and cardiovascular complications than from causes directly related to diabetes as hypoglycemia or ketoacidosis.[24]

POMEGRANATE PHYTOCHEMICALS

Pomegranate plant parts contain various categories of phytochemicals such as flavanols, flavones, flavovols, flavanones, isoflavones, proanthocyanidins, anthocyanidins, phenolic acids, hydroxy-cinnamic acids, complex polysaccharides, hydrolysable and non-hydrolysable tannins and alkaloids. Each plant part contains different bioactive compounds, or same in different concentrations. [25]

Pomegranate peel is very rich in flavonoids as cyanidin, catechin, kaempferol, naringin, luteolin, epicatechin, epigallocatechin 3-gallate, prodelphinidin, pelargonidin, quercetin,flavan-3-ol,rutinand tannins as ellagic acid, gallic acid, methyl gallate, punicalagin, casuarinin, granatin A, granatin B, methyl gallate, pedunculagin and punicalin. [25]

Pomegranate juice contains in high concentrations flavonoids such as quercetin, catechol, catechin,procyanidin, epicatechin, pelargonidin 3-O-glucoside and isoquercetin, tannins such as gallic acid, ellagic acid and alkaloids such as tryptamine, serotonin and melatonin.[25]

In seeds are contained linoleic acid, punicic acid, palmitic acid, linolenic acid, linoleic acid, stearic acid, palmitic acid, phytosterols, 3,3'-di-O-methylellagic acid, 3,3',4'-tri-O-methylellagic acid,eleostearic acid and catalpic acid. [25]

Leaves contain the flavonoidsapigenin, luteolin 3′-O-xylopyranoside,luteolin 7-O-glucoside,apigenin 4′-O-glucopyranoside,the tannins ellagic acid, gallic acid, brevifolin, punicalagin, punicalin, brevifolin carboxylic acid and corilagin. [25]

In pomegranate flowers are found various polyphenols and metabolites such as gallic acid, quercetin, punicalagin, punictannin A, punictannin B, luteolin, apigenin, kaempferol, tricetin, tricin,ursolic acid, oleanolic acid, galloyl-glucoside, digalloyl-glucoside, cyaniding-3,5-O-diglucoside, pelargonidin-3,5-O-diglucoside, ethyl gallate, brevifolin, ellagitannin, luteolin-7-O-glucoside, isoquercetin, luteolin-7-O-glucoside, apigenin-7-O-glucoside, pentagalloyl-glucoside, kaempferol-O-glucoside.[26, 27]

In pomegranate plant roots are foundthe alkaloids pelletierine, N-methylpelletierene, pseudopelletierene, norpseudopelletierene, sedrinide, N-acetyl-sedrinide, norhygrine, hygrine, 2-(2'-hydroxypropyl)Δ1-piperidine and 2-(2'-propenyl)Δ1-piperidine. The tannins punicalin and punicalagin are also contained in the roots.[28]

These phytochemicalsshow more than one bioactivityincluding antioxidant, anti-inflammatory, antimicrobial, anti diabetic, neuro protective, cardio protective and wound healing efficacy. Due to these properties plant extracts are benefiting various body systems and functions and improve or contribute into maintenance of general health.[25]

POMEGRANATE AS AN ANTI-DIABETIC AGENT THROUGH ITS ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

Inflammatory mechanisms play important role in type-I diabetes pathogenesis. Chronic low-grade inflammation is mentioned to be involved in type 2 diabetes pathogenesis. Mediators of inflammation such as interleukin-6 (IL-6) family of cytokines, IL-18, IL-1 and certain chemokines are involved in both forms of diabetes pathogenesis. IL-6 in high levels affects glucose homeostasis and metabolism directly or indirectly by action on hepatocytes, adipocytes, skeletal muscle cells, neuroendocrine cells and pancreatic β-cells.[29]

Nuclear factor kappa B (NF-κB) family consists of DNA-binding protein factors which are needed for the transcription of most prionflammatory molecules such as chemokines, cytokines, adhesion molecules and enzymes. Is referred that NF-κB activation is a key event in diabetes pathobiology and thus NF-κB is involved in diabetes pathogenesis and is associated with its complications. [30]

There are various pomegranate phytochemicals, that are mentioned to have strong antioxidant and anti-inflammatory properties and prevent from vascular inflammation development, including gallic acid, ellagic acid, luteolin, quercetin, caffeic acid, chlorogenic acid,β-sitosterol, apigenin,epigallocatechin gallate, myricetin, kaempferol, betulinic acid, methyl gallate, ethyl gallate. propyl gallate and octyl gallate.[24]

Several studies have shown that pomegranate plant part extracts and isolated compounds are significantly down regulating the expression of the pro-inflammatory cytokines TNFα, IFN-γ, IL-8, IL-6, IL-5, IL-18, IL-1βand IL-10, NF-κB, MPO.COX, MMPs and NO levels are also markedly down regulated and thus the risk of chronic inflammation, tissue damage, auto inflammatory and autoimmune diseases, infections, eosinophil mediated inflammation and pathogenic processes as cancer is reduced.[31]

Oxidative stress induced by hyperglycemia induces endothelial dysfunction that plays important role in micro vascular pathogenesis causing neuropathy, nephropathy and retinopathy and macro vascular pathogenesis leading to cardiovascular comorbidities. It increases the expression of pro-inflammatory and co coagulant factors and induce nitric oxide release and apoptosis. Moreover, oxidative stress is involved to atherosclerosis and cause phenotypic alterations to smooth muscle cell too.[32] Reducing oxidative stress through antioxidant therapy improves the clinical picture of diabetic patients and reduces diabetes complications which are mostly induced due to oxidative stress.[33]

Catalase is an antioxidant enzyme that is the main regulator of hydrogen peroxide (H_2O_2) as it catalyzes the H_2O_2 breakdown into water and oxygen. In diabetic patients there are low levels of the enzyme. Catalase deficiencies may cause elevated hydrogen peroxide concentrations, and this leads to severe complications.[34,35]Hydrogen peroxide in low concentrations acts as a cellular messenger in insulin signaling but in high concentrations is toxic especially in pancreatic β cells where it changes mitochondrial activation and insulin secretion. The insulin secretion control in pancreatic β cells depends on the precise tuning of the glucose metabolism leading to signal transduction. Impaired metabolism secretion coupling can cause defective blood glucose homeostasis. There are various reasons that may cause dysfunction of pancreatic beta cell signal transduction and among them oxidative stress plays a critical role.[14] Excessive amounts of hydrogen peroxide may also cause severe damages to DNA, RNA, protein and lipids. [34,35]. In study pomegranate flower polyphenols extract at doses of 50

and 100 mg/kg administrated in male Sprague-Dawley ratsweighing200 ± 20 g for 4 weeks increased and almost brought to healthy control group levels catalase activity.[5]Low levels of glutathione peroxide and superoxide dismutase lead to complications due to oxidative stress. The extracts at both doses increased the enzymes levels reducing oxidative stress.[35, 5]

Malondialdehyde (MDA) is a highly toxic product formed by lipid oxidation derived free radicals that is used in as an oxidative stress biomarker and is found increased in diabetic patients. It reacts both reversibly and irreversibly with phospholipids and proteins stiffening the collagen of the cardiovascular system which becomes very resistant to remodeling. This is important to be controlled as the initial collagen modification by sugar adducts leads to the formation of glycation end-products which stimulate lipids breakdown to more malondialdehyde leading to further collagenous tissues stiffening.[33] Moreover, is referred to be mutagenic in human cells.[36] In low levels malondialdehyde is referred to regulate glucose-stimulated insulin secretion in islets via Wnt signaling pathway and also to regulate gene expression.[37, 38] Hydroxynonenal, that also is considered as an oxidative stress marker is a toxic compound produced by fatty acid oxidation and it is involved in various pathologies such as metabolic diseases, cancers and neuro generative diseases. These pathologies are explained by the fact that hydroxylnonenal modulates various cell processes such as cell proliferation, transformation, oxidative stress and cell death. While hydroxynonenal at high concentrations lead to pathogenesis, at low concentrations is mentioned to disturb cellular calcium homeostasis.[39, 40] In a study, type 2 diabetic patients and healthy individuals were administrated twice a day for 4 weeks with pomegranate polyphenols extract rich in ellagic acid capsules (753 mg polyphenols per capsule) and significant decrease of malondialdehyde and hydroxynonenal has been observed in the diabetic patients while in healthy individuals the values were not affected. The blood glucose, blood lipids and C-reactive protein were not affected in both groups.[41]

High-sensitivity C-reactive protein is an inflammation marker which predicts peripheral arterial disease, stroke, incident myocardial infraction, sudden cardiac death among individuals without history of cardiovascular diseases and recurrent events of death in patients with stable or acute coronary syndromes. High-sensitivity C-reactive protein confers additional prognostic value at cholesterol levels, Framingham coronary risk score, severity of the metabolic syndrome and blood pressure.[42] In a research, 48 overweight and obese participants aged 30-60 years with body mass index 25-40 received either pomegranate extract at dose of 1000 mg or a placebo for 30 days. Anthropometric parameters, plasma concentrations of IL-6, high-sensitivity C-reactive protein, malondialdehyde, serum glucose and lipids levels were determined in order to evaluate the effect of pomegranate on inflammatory biomarkers, glycemic indices and lipid profile of overweight and obese patients. The results showed that pomegranate through ameliorating the systemic inflammation and lipid profile, decreased complications that are caused due to overweight and obesity. More specifically, there was remarkable decrease in IL-6,high-sensitivity C-reactive protein and malondialdehyde. Mean serum levels of glucose, Low-Density Lipoprotein (LDL) and total cholesterol were also markedly reduced while there was a significant increase of the beneficial High-Density Lipoprotein (HDL) levels. [43]

Pomegranate juice intake by hemodialysis patients for a year lead to remarkable decrease of all inflammatory and oxidative stress biomarkers compared to placebo group with a significant decrease in TNF-αand IL-6. [44]

In another study, two groups of patients aged 40-65 years with type 2 diabetes (placebo and test group) were treated daily for 12 weeks with 250 mg/kg pomegranate juice or a control beverage and biochemical markers such as inflammatory markers, insulin and fasting plasma glucose were assayed. On 12th week, a remarkable decrease in interleukin-6 (IL-6) and in plasma C-reactive protein (hs-CRP) by 30 and 32% respectively has been observed. Pomegranate juice did not affect insulin resistance index, plasma TNF-α concentration and fasting plasma glucose.[44]

POMEGRANATE'S ANTIDIABETIC ACTIVITY

It is mentioned in various studies that pomegranate flower compounds are very effective in decreasing blood glucose and lipid levels and ameliorate insulin resistance in diabetic animal models. The antihyper glycemic activity of pomegranate flower extracts are due to activating peroxisome proliferator-activate dreceptoramma. [5]Peroxisome proliferator activated receptors (PPAR) are steroid-type nuclear receptors that act as transcription factors which stimulate protein synthesis in various processes such as cellular differentiation, energetic metabolism and proliferation and thus are considered as key regulators of lipid and glucose metabolism and homeostasis. [45] The mean PPAR values in healthy subjects are significantly higher than those in diabetic or obese subjects.[46]Low PPAR values lead to several diabetes complications such as diabetic nephropathy, micro vascular and macro vascular complications. Activation of PPAR-γ improves insulin receptors' sensitivity. In order to treat metabolic syndromes and prevent diabetes complications are used PPAR agonists, compounds that act upon PPAR and improve insulin resistance and dyslipidemia, while they also are found to suppress oxidative stress, inflammation, lipotoxicity and activate the renin-angiotensin system.[47, 48, 49]

Pomegranate flower, used in Unani medicine for diabetes treatment, is referred to act as a dual activator of PPAR-α and PPAR-γ and to improve hyperlipidemia, hyperglycemia and fatty heart of Zucker diabetic fatty rats (type 2 diabetes and obesity genetic animal model).[50] In a study, type 2 diabetic mice were fed with punicic acid in order to evaluate its'

activity as a PPAR agonist. The results showed improvement in fasting glucose levels and glucose normalizing abilities that are contributed to upregulation of PPAR α and γ levels and their responsible genes. Moreover, inflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α) have been suppressed. Punicic acid by activating PPAR α and γ, suppressing TNF-α and NF-κB DNA-binding activity in white adipose tissue and liver, reduced obesity-related inflammation and insulin resistance. Due to these results, punicic acid can be considered as a dual PPAR α/γ agonistic compound with PPAR α/γ activating properties in both skeletal, muscle and white adipose tissue. [51] Another study showed a dose dependent ability of punicic acid in activating PPAR α and γ in 3T3-L1 cells while PPAR δ was not activated. [51]

Zucker lean rats and Zucker diabetic fatty rats (type 2 diabetes genetic model) were orally administrated with methanolic extract of pomegranate flowers at dose of 500mg/kg per day for 6 weeks. This treatment inhibited glucose loading-induced increase of plasma glucose in Zucker diabetic fatty rats whereas such inhibition has not been observed in Zucker lean rats. Real time polymerase chain reaction (RT-PCR) results showed that flower extract treatment in Zucker diabetic fatty rats increased cardiac PPAR-γ mRNA expression and also restored completely the down regulated cardiac glucose transporter GLUT-4 mRNA. In vitro studies showed that pomegranate flower extract increased PPAR-γ mRNA and protein expression and also enhanced PPAR-γ-dependent mRNA activity and expression of lipoprotein lipase in the human THP-1-differentiated macrophage cells. Phytochemical investigation showed that the compound that is most responsible for these results is gallic acid that exists in high concentration in pomegranate flowers. [49]

Pomegranate flower polyphenol extracts were administrated at doses of 50 and 100 mg/kg on rats with diabetes type II for 4 weeks. At the same time the normal control group consisted by healthy rats was treated with saline, the diabetic model control group was treated with the same volume of saline and the metformin group consisted by diabetic rats was treated with metformin at dose of 150 mg/kg for 4 weeks. In the 4th week it was found that in both diabetic model groups treated with metformin (150mg/kg) and pomegranate flower extract (50 and 100 mg/kg) there was similar marked decrease compared to diabetic controls in fasting blood plasma glucose with the pomegranate flower 100mg/kg extract and metformin to show the same efficacy. Is important to be mentioned that both flower extracts 50 and 100mg/kg gave very similar results on 4th week and during 2nd and 3rd week the lower concentration extract was doing even better than the higher (100mg/kg) indicating that the efficacy is not proportional to the (used) dosages and that increasing the dosage from one point onwards does not further reduce the fasting blood plasma glucose. In order to evaluate the ability of animals to dispose a glucose challenge, Oral Glucose Tolerance Test (OGTT) has been performed and was found that pomegranate flower extracts at both doses improved remarkably the impaired glucose tolerance, with the lower dose to give slightly better results, phenomenon that is called hormesis. The efficacy of both extracts was comparable to metformin's. Insulin Tolerance Test (ITT) showed that pomegranate flower extracts in both doses improved insulin sensitivity in diabetic rats, giving results comparable to metformin's. The efficacy of pomegranate flower extract in the improvement of insulin sensitivity was further tested with Homeostatic Model Assessment for Insulin-Resistance (HOMA-IR) index, which is used to quantify insulin resistance and is calculated on the basis of fasting blood glucose and fasting insulin. The index was 4 times higher in diabetic controls over the healthy controls for the insulin resistance. Pomegranate extracts (50 and 100 mg/kg) showed remarkable efficacy comparable to metformin's. In this case too, both high and low concentration extracts gave similar results.[5] Diabetic patients are under oxidative stress due to hyperglycemia and the influence of free radical production may lead in cardiovascular complications.[52]In diabetic rats, catalase was reduced. Both pomegranate flower extract treatments increased catalase giving similar results with metformin. The blood lipid profile was found to be significantly improved in pomegranate flower treated groups with the results to be similar to those of metformin treatment.[5]

In a study Sprague - Dawley rats with type 2 diabetes were daily administrated with pomegranate seed oil at doses of 200mg/kg and 600mg/kg and after 28 days the blood glucose, serum insulin levels, glutathione peroxidase (GDH-P x) and malondialdehyde were determined. Glutathione peroxidase prevents cellular damage that is caused due to reactive oxygen species. It was found that the serum insulin in diabetic rats treated with 200 mg/kg was significantly higher than this of diabetic rats receiving vehicle and was approaching the level of insulin of the healthy controls. Treatment with 600 mg/kg also improved markedly the serum insulin levels compared to diabetic controls yet the results of 200 mg/kg treatment were better, as well as the results of soya bean oil 200 mg/kg were found to be better than those of 600 mg/kg treatment that have been carried out in the same research.[16, 53] This phenomenon, hormesis, indicates a probably complicated mechanism of medicinal activity (more complicated than the dose dependent manner)where other factors are also involved and contribute to the configuration of these results and further research would provide with useful information on the mechanism that the pomegranate seed oil and soya been oil act. Pomegranate seed oil at both dosages decreased markedly oxidative stress while blood fasting glucose, glutathione peroxidase and malondialdehyde were not much affected. [16]

In a study, two groups of male C57BI/J6 mice were administrated for 12 weeks as following: The first group (control) was put on high fat diet with the 45,3% of the energy to be derived from fat. The second group was also on the same

fat diet where 1g of fat per each 100g of food, has been replaced by 1g pomegranate seed oil. During the 12 weeks of same energy intake, the body weight of the control mice was increased more than the seed oil treated group (8.5 ± 3.1 g and 5.7 ± 2.9 g of body weight for control and seed oil group respectively), while DEXA analysis showed a decreased fat mass in the seed oil fed animals. Moreover, pomegranate seed oil treatment increased peripheral insulin sensitivity but did not increase liver insulin sensitivity. [54]

In research, 4 groups of albino rats were orally treated for a week as following: Group-A (normal control) received normal saline 10mg/kg/day, group-B (non-insulin-dependent diabetic control) received normal saline 10mg/kg/day, group-C (diabetic test) was treated with pomegranate leaf ethanolic extract 500mg/kg/day and group-D (diabetic standard) received glibenclamide 0.5 mg/kg/day. In pomegranate leaf extract and glibenclamide treated groups, there was remarkable increase in the glycogen content of cardiac muscle, liver and skeletal muscle as compared to diabetic control, with the results of group D to be a slightly better than in the pomegranate group. In another study, pomegranate leaf ethanolic extract has been orally administrated in rats with type 2 diabetes at the dosage of 500mg/kg/day, while the control group received normal saline, 10 ml/kg/day, and the standard group was treated with metformin 90mg/kg p.o. In both pomegranate and metformin groups there was remarkable decrease in blood sugar levels. Pomegranate group gave better results than metformin group, with the percentage of decrease in blood glucose on the eighth day to be 70.52% and 68.45% respectively. Both pomegranate and metformin groups showed remarkable decrease in intestinal glucose absorption compared to normal control group. Serum total cholesterol, low density lipoprotein, triglycerides and at herogenic index were markedly decreased at both pomegranate leaves and metformin group, with the leaf extract to give mostly better results. Serum high density lipoprotein has been significantly increased in both pomegranate and metformin groups with the values to be 35 ± 2.19 and 34 ± 2.44 respectively. These results indicate that pomegranate leaves can be considered as a promising anti diabetic agent and also as a protective agent against the development and progression of atherosclerosis and possible related cardiovascular complications. [55]

In research, fresh pomegranate juice at dose of 1,5ml/kg have been administrated in 28 healthy individuals and 28 patients with impaired fasting glucose, and blood specimens were collected 5 minutes before and 1 and 3 hours after administration in order to evaluate its effects on glucose and insulin levels. The results showed that pomegranate juice consumption exerted an antihyperglycemic response after 3 hours only in the patients with impaired fasting glucose and not in the healthy individuals. Moreover, there was a decrease in insulin resistance in impaired fasting glucose group as it has been assessed by Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). In both groups were found higher insulin concentrations. Is referred that the main mechanism through pomegranate juice affects the diabetic conditions and especially insulin resistance ones, is by neutralizing the accumulated reactive oxygen species such as ·OH, H_2O_2, and $O2\cdot^{-}$, decreasing thus the cellular oxidative stress. [56]

Glucose transporter proteins 2 (GLUT2) and 4 (GLUT4) play key roles in energy metabolism and glucose transport.[57]GLUT2 is expressed in pancreatic β cells, liver, kidney, intestine, neurons, tanycytes, astrocytes and plays key role in various regulatory mechanisms. Its' expression is required for physiological control of glucose sensitive genes and its' inactivation results in impaired glucose stimulated insulin secretion. [58] GLUT4 is found in adipose tissues, cardiac and skeletal muscle. Its' function is to permit the facilitated diffusion of glucose down its' concentration gradient into fat and muscle cells. Once it enters the cells, glucose is phosphorylated by glucokinase in liver and by hexokinase in other tissues and forms glucose-6-phosphate which enters glycolysis, or it is polymerized giving glycogen. [59] Defective expression of GLUT4 or translocation to the peripheral cell plasma membrane blocks the entrance in cells of the necessary for energy production glucose. [60] Insulin increases GLUT4 and GLUT2 mRNAs expression and insulin defection stimulates pathways which contribute to constant hyperglycemia.[61]

In research, healthy and diabetic (due to alloxan administration) Wistar rats were orally administrated with pomegranate fruit extract (peeled off pomegranate without separating seeds) at various doses for 21 days in order to evaluate its' anti diabetic activity. Healthy rats were treated with 100mg/kg body weight, 200mg/kg body weight and 300mg/kg body weight pomegranate extract and diabetic rats with 100mg/kg body weight, 200mg/kg body weight and 350mg/kg body weight. Healthy and diabetic control groups were administrated with vehicle. Diabetic control group showed remarkable decrease in insulin mRNA expression in comparation with normal control and thus in diabetic controls, serum insulin levels were significantly lower than those of healthy controls. Pomegranate treatment amplified insulin mRNA levels 3 to 3.5-fold in the group treated with 350mg/kg body weight. There was significant increase in insulin production and secretion in the diabetic groups that were treated with 200 and 350 mg/kg body weight, about 46.5 % and 74.41 % respectively with gallic acid and ellagic acid that are in high concentrations in pomegranate extract, to play important role to that. Glycemia normalization that have been observed in the pomegranate treated groups could also be attributed to anthocyanin and quercetin polyphenols which suppress the glucose intestinal absorption. Quercetin is also mentioned to be involved in pancreatic islets regeneration and secretion of insulin. Insulin regulates mediators which are involved in glycogen synthesis, gluconeogenesis and glycolysis. Pomegranate treatment raised the levels of glycogen storages which in the diabetic control were very

low, bringing value close to this of the normal control group. In order to study glucose homeostasis, the mRNA expression of GLUT-4 and GLUT-2 have been evaluated in all groups. In diabetic control group there was significant reductions in transcription levels of both GLUT-4 and GLUT-2. Pomegranate treatment increased in both healthy and diabetic groups the expression of GLUT-4 and GLUT-2 dose dependently, with the changes in the diabetic groups to be bigger than those in healthy groups. Results also showed that pomegranate treatment decreased plasma free fatty acids and triglyceride levels compared to diabetic controls with the best results to be given at the 350mg/kg body weight treated group. [61]

Oral administration of pomegranate seed methanolic extract on streptozotocin diabetic adult albino Wistar rats at doses of 300 and 600 mg/kg and studies on the variation of blood glucose levels after streptozotocin / seed extract administration have been carried out in order to evaluate the pomegranate seeds' hypoglycemic activity. In the same study a third group of rats was treated with the commercial hypoglycemic agent chlorpropamide which is used for type 2 diabetes treatment, at 200mg/kg. It was found that pomegranate seed extract at dose of 300mg/kg lowered significantly blood glucose concentration within 2 h by 7% with a pick activity at 12h by 47%. Seed extract at dose of 600mg/kg decreased remarkably blood glucose concentration by 15% within 2 h with a pick activity at 12 h by 52%. Pomegranate seed extract treatment at both doses gave better results than chlorpropamide at 200mg/kg. [62]

α-amylase is an enzyme thatplays important role in digestion of carbohydrates. It hydrolyses starch molecules and produces dextrins and smaller glucose unit-composed polymers that cause hyperglycemia and type 2 diabetes mellitus development.[63] By inhibiting α-amylase's activity, glucose production is decreased and thus is possible to regulate blood glucose levels. In an in vitro study, 2mg of starch azure was suspended in 0.2 ml of Tris-HCl buffer, 0.5M (pH=6.9) containing $CaCl_2$ 0.01M (substrate solution). The tubes with substrate solution were boiled for 10 min and then they were preincubated for 5 min in 37ºC. Pomegranate peel dry extracts (extracted by methanol) were dissolved in concentrations of 20, 40, 60, 80 and 100μg/ml in DMSO and were added into the tubes containing substrate solution. Then 0.2 ml of porcine pancreaticα-amylase in Tris-Hcl buffer were added in each tube and the reaction was carried out for 10 minutes in 37ºC. The reaction was stopped by adding in the tubes 0.5ml of acetic acid 50%. The mixture was centrifuged for 5 min at 3000 rpm at 4ºC and the absorbance of the resulting supernatant was measured at 595nm. As standardα-amylase inhibitor was used acarbose and the percentage of inhibition was calculated with the formula: %inhibition=[(Abs.control-Abs.sample)/Abs.control]∗100%. The results showed that extract in concentration of 100μg/ml inhibited markedly the activity ofα-amylase at the percentage of 56%.Hemoglobin of red blood corpuscles tends to bound on glucose and form glycosylated hemoglobin. High amount of glycosylated hemoglobin is associated with diabetes complications as retinopathy and it should not be higher that 12%. High blood glucose concentration means high amount of glycosylated hemoglobin and thus the glycosylated hemoglobin amount is used as a guide for the blood glucose concentration. In in-vitro study, hemoglobin 0.06%, gentamycin 0.02% and glucose 2% solutions were prepared in phosphate buffer 0.01M with pH=7.4. 1ml of each solution was mixed in a test tube. Pomegranate peel dry extract was dissolved in DMSO and solutions with concentrations 20, 40, 60, 80 and 100μg/ml were prepared. 1 ml of each concentration was added in the mixture hemoglobin-gentamycin-glucose. After 72 h incubation in dark place, the degree of hemoglobin glycosylation was measured calorimetrically(520nm). As standard inhibitor was used acarbose and the %inhibition was calculated using the formula:%inhibition=[(Abs.control-Abs.sample)/ Abs.control]∗100%. It was found that the extract with concentration 100μg/ml showed a good inhibitory activity on hemoglobin glycosylation with IC50 value to be 91.4μg/ml and inhibition percentage 55%.[64]

In a study, type 2 diabetic patients aged 30-60 years with diabetes duration 1-10 years were treated twice a day with 5 g pomegranate seed powder or placebo for 8 weeks and fasting blood glucose, glycated hemoglobin, triglycerides and total cholesterol were measured. After the 8-week period of treatment, the mean value of fasting blood glucose of the intervention group was found to be 135.83 ± 35.92 mg/dl, markedly lower than the corresponding value in placebo group, which was 158.84 ± 39.04 mg/dl. Moreover, the between-group comparison of post-intervention glycated hemoglobin values of the test and placebo groups that were 6.94 ± 0.77 mg/dl and 7.53 ± 0.98 mg/dl respectively, revealed a remarkable difference. However, at doses used no significant changes in triglycerides and total cholesterol have been observed.[65]

In order to evaluate the efficacy of pomegranate rind extract, its' spray dried bio polymeric dispersions with chitosan or casein and also of gallic acid, the main compound of the extract against diabetes, various doses of the extract, its' dispersions and of gallic acid were administrated on alloxan-induced diabetes mouse model and then acute (6 h) and subacute (8 days) were determined. Furthermore, the in-vivo antioxidant activity was assessed using serum catalase level. The results showed that pomegranate rind extract at all doses used (25, 50 and 100 mg/kg) exhibited remarkable hypoglycemic activity decreasing blood glucose levels after 6 hours by 36.4, 34.2 and 48.4% respectively. Pomegranate extract dispersion with chitosan, at doses used equivalent to 25, 50 and 100 mg extract/kg reduced blood glucose levels after 6 hours by 40.6, 40.0 and 52.4% respectively. Extract dispersion with casein at doses equivalent to 25. 50 and 100 mg extract/kg, after 6h reduced blood glucose levels by 30.2, 42.6 and 40.6% respectively. Gallic acid, at doses of 6 and 12

mg/kg showed strong hypoglycemic effect decreasing blood glucose levels by 36.1 and 36.4%. At dose of 3 mg/kg no significant activity has been observed. On the 8th day of 100mg pomegranate extract/kg administration, blood glucose level was decreased by 54.2% while at 25 and 50mg/kg the reduction was by 1.77 and 6.4% respectively. Pomegranate extract dispersion with chitosan at doses equivalent to 25, 50 and 100mg extract/kg, blood glucose levels were decreased by 40.2, 40.9 and 50.5% respectively. Similarly, pomegranate extract dispersion with casein, decreased blood glucose levels by 43.7, 44.1 and 49.0% respectively. Gallic acid also showed a remarkable hypoglycemic effect at all doses (3, 6 and 12 mg/kg) decreasing blood glucose levels by 51.6, 53.3 and 43.7% respectively. These results indicate hormesis as gallic acid in the highest dose shows lower activity than in lower doses. The antioxidant activities of the pomegranate extract and its' polymeric dispersions were in vivo evaluated by monitoring catalase levels of each mouse on 1st, 3rd, 5th and 8th day after administration. Pomegranate extract treatment at doses of 25, 50 and 100 mg/kg caused a gradual rise in serum catalase activity to reach a remarkable difference on 5th day (1.0, 9.2 and 15.1% respectively) and on 8th day (4.1, 6.1 and 15.5% respectively) compared to diabetic control group. Polymeric dispersion with chitosan at doses equivalent to 25, 50 and 100 mg extract/kg, reached remarkable differences on 5th day (2.7, 8.7 and 14.5% respectively) and on 8th day (5.2, 6.1 and 19.8% respectively) compared to diabetic control. Similarly, polymeric dispersion with casein at doses equivalent to 25, 50 and 100 mg extract/kg, reached remarkable difference on the 8th day, increasing serum catalase activity by 3.3, 4.0 and 10.9% respectively. Gallic acid at doses of 6 and 12 mg/kg also showed a gradual rise in serum catalase activity to reach a remarkable difference on the 8th day (3.4 and 3.1% respectively) compared to diabetic controls. [66]

POMEGRANATE SUFFICIENTLY PREVENTS OR TREATS CARDIOVASCULAR COMPLICATIONS DUE TO DIABETES

Hyperglycemia plays important role in cardiovascular damage working through various mechanisms such as activation of protein kinase C, hexosamine and polyol pathways and production of advanced glycationend-products. These pathways and also mitochondrial dysfunction induced by hyperglycemia lead to reactive oxygen species (ROS) overproduction that cause cellular damage and contribute to complications. ROS damage DNA, RNA, proteins, lipids and also modulate intracellular signaling pathways such as mitogen activated protein kinases and redox sensitive transcription factors leading to protein expression changes and to irreversible oxidative modifications.[52, 32, 3]

Various studies have shown that pomegranate plant part extracts and isolated phytochemicals due to their antioxidant activity offer many benefits to cardiovascular health. The Low-Density Lipoprotein oxidation is the major cause of cardiovascular diseases. Pomegranate phytochemicals reduce oxidative stress and improve the antioxidant status of the patients. It decreases total cholesterol, LDL levels, triglycerides, increases the beneficial HDL levels, acts as antihypertensive and protects against atherosclerosis. Is mentioned to reduce cardiac fibrosis in diabetic patients, reduce weight and improve the cardiovascular health of aged people. [67]

The above-mentioned transcription factor NF-κB is associated with cardiovascular health as it is influencing myocardial ischemia, reperfusion injury, ischemic preconditioning, heart hypertrophy, vein graft disease, atherosclerosis and heart failure. Depending on physiological and cellular content, it may prevent cardiovascular tissue injuries or lead to pathogenesis. Research showed that polyphenols of pomegranate peel suppress the activation of TLR4/NF-κ B pathway and thus inhibit the lipopolysaccharide induced NF-κB activation and inflammation in macrophages.[67]Other in vitro studies showed that pomegranate flower extract and its' components gallic acid, ursolic acid and oleanolic acid that are potent antioxidants, inhibited the lipopolysaccharide induced activation of NF-κ B in macrophages.[50]

Endothelin 1 (ET-1) is an endogenous vasoconstrictor, secreted by endothelial cells, which act through 2 types of receptors, ETA and ETB. In high concentrations ET-1 causes fibrosis in the vascular cells, stimulates reactive oxygen species production and induces pro-inflammatory mechanisms through increasing cytokine secretion and superoxide anion production. Is referred that ET-1 is involved in the expression of pro-inflammatory cytokines as IL-1, IL-6, TNF-α and in the activation of transcription factors as NF-κB. [68]Diabetic heart is characterized by increased fibrosis that impairs cardiac function. NF-κB and endothelin interactively regulate the fibroblast growth. [50] C-jun N-terminal kinase is an inflammatory kinase that responds to several cellular stress signals activated by cytokines, hyperglycemia and free fatty acids. It mediates both β-cell dysfunction and insulin resistance and is a key mediator between obesity and type 2 diabetes.[69]Inhibitory kappa B kinases are key regulators for NF-κB cascade and represent a point of convergence for various extracellular agents which activate this pathway. Their main function is to transduce pro-inflammatory and growth stimulating signals which contribute to important cellular processes and in specific conditions they also play key role in various human diseases pathogenesis.[70]

In research, pomegranate flower extract was administrated at dose of 500mg/kg to Zucker diabetic fatty rats for 6 weeks. It was found that the flower extract diminished cardiac fibrosis in Zucker diabetic fatty rats at least in part by modulating NF-κ B and ET-1 signaling. The up regulated cardiac mRNA expressions of endothelin-1, ETA, c-jun and NF-κB were decreased and the down regulated mRNA expression of

inhibitor-kappaBalpha which inhibits NF-κB, was normalized.[50]

Increased fatty acid oxidation and excess triglyceride accumulation in the diabetic heart leads to cardiac dysfunction. In research, long term (6 weeks)oral administration of pomegranate flower methanolic extract at dose of 500 mg/kg in Zucker lean and Zucker diabetic fatty rats has been carried out in order to investigate the effects and mechanisms of action of the flower extract on abnormal cardiac lipid metabolism. The control groups (Zucker lean and Zucker diabetic fatty rats) received vehicle. Plasma levels of triglycerides, total cholesterol and nonesterified freefatty acids were measured in the beginning of the study and on 4th week without fasting and on 5th week under fasting conditions. The pomegranate flower extract treatment significantly reduced fasting plasma none sterified free fatty acid levels in both Zucker lean and Zucker diabetic fatty rats. On week 6, heart total cholesterol content was markedly decreased in Zucker diabetic fatty rats while in Zucker lean rats there was no change. Plasma total cholesterol levels were also markedly decreased in Zucker diabetic fatty rats while in Zucker lean rats there was not significant change. The treatment reduced liver weight in Zucker diabetic fatty rats while no such affect has been observed in Zucker lean rats. In order to investigate the molecular mechanism through which the flower extract improved the abnormal cardiac lipid metabolism, mRNA expression of cardiac lipogenic genes has been examined. The results showed up regulation of the expression of cardiac mRNA sencoding FATP, CPT-1, PPAR-α, ACO and AMPKa2 while ACC mRNA expression was down regulated in the left ventricle of Zucker diabetic fatty controls compared to Zucker lean controls where lower effect has been observed. To further understand the mechanism of action of the pomegranate flower extract in lipid metabolism regulation, it's efficacy on PPAR-αluciferase activity was investigated in in-vitro studies that have been carried out. The extract dose dependently enhanced the activity of PPAR-αluciferase in HEK293 cell line transferred with PPAR-αreporter gene. These results were comparable the results of the commercial PPAR-αactivator fenofibrate. In another in-vitro studies the effects of the isolated constituents of pomegranate oleanolic acid, ursolic acid and gallic acid were tested on PPAR-αluciferase activity on the same cell lines. It was found that oleanolic acid dose dependently increased PPAR-αluciferase activity while no such effect has been observed in case of ursolic and gallic acid. [71]

Obesity is a risk factor for cardiovascular diseases and diabetes mellitus development. Frequently, it is associated with abnormalities in insulin secretion and insulin resistance.[72]Hyper lipid emic high-fat diet obese ICR mice were treated with pomegranate leaf extracts at doses of 400 and 800 mg/kg/day for 5 weeks. The results showed remarkable decrease in total cholesterol (TC), triglycerides and TC/HDL-C ratio. Moreover, pomegranate leaf extract administration decreased the appetite in obese high-fat diet mice group but there was not such effect on the normal diet mice.[67]

Insulin dependent diabetes was caused to male Swiss albino rats after streptozotocin injection (50mg/kg) and they were treated orally with pomegranate peel powder at dose of 200mg/kg/day for 20 days. Another diabetic group was treated once every 48h with the standard drug insulin (6IU/kg. S.C) for 20 days. On the 21stday biochemical parameters and his to pathological analysis were carried out in order to evaluate the medicinal activity of pomegranate peel powder. In both pomegranate and insulin treated groups significant improvement in all biochemical parameters with values to be close to healthy control group and pomegranate group in most cases to give better results than insulin treated group. Remarkable reduction in cholesterol has been observed in both pomegranate and insulin groups with values to be 55.74±3.523 and 65.82±2.524 mg/dl respectively while values of healthy and diabetic control group were 50.41±2.866 and 74.38±7.86 mg/dl respectively. Triglycerides in pomegranate group, insulin, healthy control and diabetic control group were 55.77±2.298, 56.97±6.758, 46.68±9.503 and 81.59±6.823 mg/dl respectively. LDL was markedly decreased in pomegranate and insulin group with values to be 22.5±5.590 and 29.1±3.645 mg/dl while in diabetic group was 47.16±8.930mg/dl. HDL was increased compared to the diabetic control with values to be 21.99±1.287, 25.77±2.279, 25.96±3.045 and 10.50±2.166 mg/dl in pomegranate, insulin, healthy and diabetic control group respectively.[73]Alanine transaminase and aspartate transaminase are liver enzymes released when there is liver or muscle damage, and they are used as liver function indicators. Low levels of the enzymes indicate a healthy liver. [74] In diabetic control, alanine transaminase was significantly increased compared to healthy control (104.67 IU/l and 66.27 IU/l in diabetic and healthy control respectively). In both pomegranate and insulin treated groups alanine transaminase was markedly lower (73.93 IU/l and 77.8 IU/l respectively). Aspartate transaminase in diabetic and healthy control groups was found to be 144.71 and 89.81 respectively while in insulin and pomegranate treated groups was 102.01 IU/l and 90.75 IU/l respectively. The antioxidant enzyme superoxide dismutase was remarkably decreased in the diabetic control group compared to healthy control group (50.06 U/ml and 283.5 U/ml respectively) and it was increased in both insulin and pomegranate treated groups (170.3 U/ml and 182.8 U/ml respectively).Total antioxidant capacity was decreased in diabetic control group in comparison with healthy control (0.604mM /l and 1.63mM respectively). In both pomegranate and insulin treated groups total antioxidant capacity was markedly increased (1.404 mM /l and 1.58mM /l respectively). His to pathological analysis in pancreas showed that in diabetic control group there was a shrinkage in the islets of Langerhans and also degeneration and necrosis of component cells while in both pomegranate

and insulin groups islets of Langerhans had normal size and a minor b cells degeneration has been observed in pomegranate group. His to pathological analysis of liver in diabetic control group showed increased vacuolation in hepatocytes' cytoplasm. In insulin treated group his to pathological analysis in liver showed normal hepatocytes with normal architecture. A mild vacuolation of hepatocytes has been observed. The pomegranate peel powder treated group gave the best results of treated groups. His to pathological analysis showed normal hepatocytes that were arranged in normal sheets or cord around the central vein.[73] Methanolic extract of pomegranate leaves at doses of 200, 400 and 600mg/kg/day and the commercial anti-diabetic drug glibenclamide 1mg/kg/daywere orally administrated for 45 days in type-2 diabetic rats and the anti-diabetic and antioxidant effect of the extract was evaluated and compared to glibenclamides' activity. Anti-diabetic activity was examined by measuring plasma insulin, glycated hemoglobin and blood glucose levels. The antioxidant effect of the extract was determined by analyzing renal and hepatic antioxidant markers as catalase, superoxide dismutase, reduced glutathione, glutathione peroxidase and lipid peroxidation. Total cholesterol, HDL, triglycerides, aspartate transaminase, alanine transaminase, urea, creatinine and alkaline phosphatase were also studied. The results showed that type 2 diabetes remarkably altered these parameters which pomegranate flower extract oral administration remarkably ameliorated them. The fasting blood glucose levels in diabetic control group were markedly increased while insulin was decreased. Administration with pomegranate leaves extracts and glibenclamide resulted in significant decrease of blood glucose levels and also to significant increase of insulin levels, with the extract at 600mg/kg dosage to give comparable results with glibenclamide. In rats treated with extract 600 mg/kg and glibenclamide, hemoglobin was markedly higher and glycated hemoglobine lower compared to diabetic control group. The hepatic function of the rats was studied by estimating aspartate transaminase, alanine transaminase and alcaline phosphatase. Oral intake of the flower extract resulted in a remarkable reduction of these biomarkers with the 600mg/kg dosage of pomegranate and glibenclamide to show similar activity. The renal function was studied in terms of creatinine and urea levels. Both creatinine and urea were markedly decreased in pomegranate and glibenclamide groups with pomegranate flower extract at 600mg/kg to show similar efficacy to glibenclamide. Catalase, superoxide dismutase, glutathione peroxidase and reduced glutathione were significantly increased, with pomegranate extract 600mg/kg to show similar potency with glibenclamide. Lipid peroxidation expressed as malondialdehyde was markedly increased in parenchymal cells of both kidney and liver of diabetic control rats. Oral extract administration ameliorated these changes dose-dependently and even restored the normal values at 600mg/kg. Total cholesteral and triglycerides were significantly decreased in pomegranate flower extract treated groups. Extract at dose of 600mg/kg gave better results than glibenclamide. HDL was markedly increased in extract treated groups with better results to be achieved by 600mg/kg dosage which was better than glibenclamides' results. Histological examination of healthy rats' pancreas showed normal architecture of islets of Langerhans while in diabetic controls there were pathological changes in pancreatic tissue parenchymal cells. Pomegranate at 600mg/kg and glibenclamide administration restored the general architecture of pancreas with better results to be given by pomegranate as the group treated with it had nearly normal architecture of pancreas. Safety tests showed that the leaf extract shows no oral toxicity or mortality at doses up to 2000mg/kg for 21 days that is much higher than what is needed in order to exhibit medicinal activity and this indicates the safety of pomegranate leaf extract for prolonged use.[75]

In a research, 19 patients with type 2 diabetes were administrated twice a day with capsules containing pomegranate peel extract (250mg dry extract per capsule) for 8 weeks in order to evaluate its' effects on blood pressure, lipid profile and fatty acid levels. HPLC analysis of the dry extract showed that it contained gallic acid 10.46 ± 0.04 mg/g, ellagic acid 23.83 ± 0.07 mg/g, punicalagin 69.67 ± 0.72 mg/g and punicalin 30.41 ± 0.11 mg/g. Total tannins were spectrophotometrically detected according to European Pharmacopoeia 8.0 and were found to be 11.8%. Placebo group (18 participants) was treated with placebo capsule. The pomegranate peel extract intake lowered markedly both systolic and diastolic blood pressure by 6.06 mmHg and 2.10 mmHg (4.45% and 2.53%) respectively, while no differences have been observed in the placebo group. Total cholesterol was not affected, yet triglycerides, LDL/HDL ratio and lipid peroxidation index, thiobarbituric acid reactive substances (TBARS) that are produced due to lipid peroxidation were significantly decreased. At the same time HDL levels were markedly increased. Moreover, pomegranate peel extract treatment reduced total saturated fatty acids such as palmitic and stearic acid which in high concentrations indicate increased risk of cardiovascular diseases, atherosclerosis and stroke, and at the same time increased arachidonic acid levels, which is a polyunsaturated fatty acid, biological cell membrane constituent that gives flexibility and fluidity and thus is necessary for all cells functions, especially in skeletal muscle, nervous system and immune system. In placebo group no such differences have been observed. Anthropometric parameters such as body weight, fat mass, fat free mass and BMI were not significantly affected, yet there was a remarkable effect on waist circumference (-2.46cm).[76, 77, 78]

In research, pomegranate juice at doses of 1, 2, 4 ml/200g body weight and glibenclamide 0.1 mg/kg body weight were administrated in streptozotocin- induced diabetic rats for 4 weeks and the antioxidant and hypoglycemic effects were evaluated by measuring lipid peroxidation, malondialdehyde,

blood glucose level and advanced glycation end-product before and after treatment. Lipid profile was also studied before and after treatment. In glibenclamide and in all pomegranate juice treated groups there was remarkable decrease in blood glucose levels. All 3 doses of pomegranate juice showed the same efficacy in decreasing blood glucose levels which was similar toglibenclamide's efficacy. Serum malondialdehyde level was markedly reduced after 4 weeks treatment with juice at dose of 2ml/200g body weight, while other 2 doses also showed a tendency towards reduced malondialdehyde level. At doses used, there was not significant reduction in serum concentration of advanced glycation end-product, total cholesterol, and triglycerides levels with neither glibenclamideor pomegranate juice. [79]

Pomegranate peel aqueous extract was administrated in healthy and alloxan induced diabetic rats weighing 110-130g at dosage of 0.43g daily, equivalent to human therapeutic dose for 4 weeks. There was a remarkable weight loss in diabetic controls while in diabetic rats treated with pomegranate extract, an improvement in body weight has been observed. Is important to be mentioned that the body weight of healthy rats was not affected, and this probably may indicate that the prevention of body weight loss is achieved by its' anti-diabetic activity that is observed only in diabetic rats. In diabetic controls there was a significant decrease of insulin levels and increase of plasma glucose. Moreover,β cell necrosis, significant decrease of theβcells number and intracellular vacuolation has been observed. In diabetic rats treated with pomegranate extract there was significant decrease of blood glucose and significant increase of insulin levels. The number ofβ cells was increased yet vacuolation still was observed in some cells. The mechanism of anti-diabetic action of the pomegranate peel extract is through protecting pancreas, regeneratingβ cells increasing their number and stimulating them that means subsequent insulin release. Pancreasβ cell protection is achieved through the antioxidant activity of pomegranate as pancreas is especially susceptible to free radical damage and pomegranate acts as a free radical scavenger and protects thusβ cells from damage. [4]

High fat and glucose – induced obese rats were administrated by gavage for 12 weeks with 250 mg/kg body weight of one of 3 pomegranate extracts, peel, leaves and juice in order to determine their preventive effects on oxidative stress and insulin resistance. At the end of 12th week treatment, plasma fasting glucose level in the high fat diet control group was increased by 30% compared to the standard diet control group. Treatment with all pomegranate extracts led to significant decrease of fasting glucose levels. More specifically, fasting glucose levels were found to be 104.83 ± 9.17,114 ± 9.40 and 113.66 ± 14.26 mg/dl in juice, leaf and peel treated groups respectively, while in normal and high fat diet control groups the values were 90.5 ± 9.02 and 129 ± 6.32mg/dl respectively.The homeostatic index of insulin resistance which was markedly elevated in high fat diet control group compared to the normal control, was decreased by half with juice and leaves extract treatment and by 39.1% with peel extract treatment, indicating a remarkable improvement in insulin resistance by all pomegranate extracts. In the high fat/fructose diet control group, α-amylase activity was found to be increased by 70% compared to the normal diet control group. Pomegranate peel, leaf and juice treatment reduced amylase activity by 50%, 49% and 30% respectively.[80]Lipoprotein lipase plays important role in metabolism of both high-density lipoproteins and triglyceride-rich particles and is a determinant of both HDL and triglyceride concentrations. In humans, the enzyme activity is insulin dependent in both skeletal muscle and adipose tissue and thus it varies in diabetic patients according to insulin sensitivity and ambient insulin level. In type 1 diabetes, the enzyme activity is low and increases upon insulin therapy. In type 2 diabetes the average enzyme activity in postheparin plasma and adipose tissue is normal or subnormal. In both type 1 and 2 diabetes, changes of lipoprotein lipase activity are related to alterations in lipoprotein pattern. In type 1 diabetic patients with low lipase activity, HDL is reduced while total cholesterol and triglycerides are increased. In type 2 diabetes with subnormal lipase activity, HDL is reduced, and serum triglycerides are increased. [81]Compared to normal diet control, in the high fat diet control group, a potent increase of plasma lipase activity has been observed. However, the long-term treatment with pomegranate juice, leaves and peel extract, reverted back the lipase activity in plasma by 37%, 28% and 51% respectively. Besides, oxidative stress was significantly reduced. Pomegranate extract treatment improved markedly the levels of hepatic antioxidant enzyme superoxide dismutase with peel and leaf extracts to give better results and glutathione peroxidase with the leaf extract to give the best results.[80]

In research, Wistar rats were divided into 7 groups that were treated as following: Group 1, healthy control, received only distilled water. Group 2 rats received streptozotocin that induces diabetes (70 mg/kg, only once), group 3 was treated with streptozotocin (70 mg/kg, once) and angiotensin II that induces hypertension (150 μg/kg at the end of 4 weeks), group 4 was treated with streptozotocin (70 mg/kg, once) and pomegranate juice (100 mg/kg/day for 4 weeks), group 5 received streptozotocin (70 mg/kg, once) and pomegranate juice (300 mg/kg/day for 4 weeks), group 6 was treated with streptozotocin (70 mg/kg, once), pomegranate juice (100 mg/kg/day for 4 weeks) and angiotensin II (150 μg/kg at the end of 4 weeks), group 7 was administrated with streptozotocin (70 mg/kg, once), pomegranate juice (300 mg/kg/day for 4 weeks) and angiotensin II (150 μg/kg at the end of 4 weeks). The results showed that pomegranate juice treatment at both doses suppressed and in some cases even reversed various biochemical changes induced by diabetes and angiotensin II.[82]Angiotensin converting enzyme (ACE), is a component of the renin-angiotensin-aldosterone

system, a hormone system that regulates body fluids volume, electrolyte balance and blood pressure. It converts angiotensin I to the active vasoconstrictor angiotensin II, and overactivity leads to high blood pressure. ACE activity is very high in patients with diabetes and cardiovascular diseases, and it is a key drug target for cardiovascular diseases treatment.[83, 84] In all pomegranate treated groups, serum angiotensin converting enzyme activity was remarkably inhibited, with best results to be given by 300mg/kg dosage which were very similar to the healthy control results. In all pomegranate groups, mean arterial blood pressure was found to be markedly decreased, with values of 300mg/kg treated groups to be very close to healthy control group values. The prevention of high blood pressure development in diabetic rats is probably due to the antioxidant activity of pomegranate compounds that decrease oxidative stress induced by diabetes and angiotensin II and inhibit angiotensin conversing enzyme activity. In all diabetic and hypertensive diabetic rats, the levels of the antioxidant enzymes catsalase, superoxide dismutase and glutathione reductase were markedly decreased. In all pomegranate treated groups at both doses, the enzymes' values were significantly elevated, reaching natural levels. His to pathological studies showed normal morphology in control animals while in diabetic rats, sclerosis of capillaries, microangiopathy, hypertensive changes in blood vessels have been observed in kidneys. Moreover, tubular degenerative changes and increased eosinophilic material were found in diabetic rats. Pomegranate treatment at both dosages, prevented congestion, tubular degenerative changes and also hypertensive changes in kidneys, while mesangial protection has not been observed at the present doses used and treatment duration.[82]Thiobarbituric acid reactive substances (TBARS), lipid peroxidation byproduct, used as a lipid peroxidation generic metric in biological fluids and as an oxidative stress indicator. Patients with diabetes and cardiovascular diseases have significantly elevated levels of TBARS.[85] Both pomegranate doses treatments reduced significantly the TBARS in pancreas and kidney tissue of the diabetic and hypertensive diabetic rats. Hormesis has been observed as the 100mg/kg juice treatment gave better results than 300mg/kg treatment with TBARS levels to be almost as in the healthy controls.[82]

Type 2 diabetic patients were treated daily with 2 capsules containing each 753 mg pomegranate polyphenols. The results showed significant decrease in lipid peroxidation, while there was not such effect in healthy controls.[67]

Aqueous extract of pomegranate flowers at doses of 250mg/kg and 500mg/kg were daily administrated to diabetic albino Wistar rats in order to evaluate its' anti diabetic and cardio protective activity. After 21 days, blood glucose levels were significantly decreased compared to diabetic controls. More specifically, blood glucose values of pomegranate groups 250mg/kg and 500 mg/kg were 112.84 ± 7.39mg/100ml and 84.67 ± 10.17mg/100ml respectively, while the values in healthy and diabetic control groups were 82.33 ± 9.18mg/100ml and 352 ± 7.12mg/100ml respectively. In both pomegranate flower extract treated groups, LDL, VLDL, triglyceride levels and atherogenic index were markedly decreased dose dependently, with the 500mg/kg dose group to give results very similar to those of the healthy control group. HDL was significantly increased, reaching at 500mg/kg dose group almost the HDL levels in the healthy control group (48.67 ± 5.16mg/100ml and 50.83 ± 3.49mg/100ml respectively). Flower extract treatment resulted in significant elevation of the antioxidant enzymes catalase, glutathione reductase, glutathione peroxidase, glutathione-S-transferase and superoxide dismutase, compared to diabetic control group. Treatment with 500mg/kg of extract resulted in almost same levels of the antioxidant enzymes, as they are referred for the normal control group. [86]

Zucker diabetic fatty rats were orally administrated with pomegranate flower extract at dose of 500 mg/kg. The results showed an improvement of the abnormal cardiac lipid metabolism in the rats, that is due to activation of PPAR-α and the decrease of the circulatory lipid. The circulatory lipid cardiac uptake was found to be inhibited.[67]

Paraoxonase-1 (PON-1) is a serum enzyme related to HDL. It's breaking down harmful lipids that exist in atherosclerotic plaques, lipoproteins and macrophages and it prevents LDL oxidation.Paraoxonase-2 (PON-2) is found in tissues and shows antioxidant activity at the cellular, not humoral level. PON-1 and PON-2 are both protecting against atherosclerosis. Tannins and anthocyanins that are contained in pomegranate possess strong antiatherogenic properties as they are found to increase the activity of PON-1.Diabetic, hypertensive and hypercholesterolemic patientshave weak antioxidant status and increased oxidative stress that means high risk for atherosclerosis. Pomegranate polyphenols, increasing PON-1 and PON-2 activity are reducing oxidative stress and atherosclerosis.[67]

Research in mice and humans showed that pomegranate juice consumption reduced markedly atherogenic modifications of LDL. Pomegranate administration to humans resulted in remarkable LDL levels decrease while serum paraoxonase was increased by 20%. Administration in mice decreased LDL oxidation up to 90%. These results were due to decreased cellular lipid peroxidation and less superoxide release. In both mice and humanatherosclerotic lesions size was decreased. In mice, pomegranate group, lower number of foam cells were found compared to control group.[67]

In a study on how pomegranate juice affects healthy individuals was found that the levels of hemoglobin, red blood cell count and hematocrit were raised and there was no effect on triglycerides, LDL, HDL, total cholesterol and complete blood count. [67]

POMEGRANATE TREATMENT PREVENTS OR AMELIORATES DIABETIC NEPHROPATHY

Diabetic nephropathy is a quite frequent microvascular complication that is observed in both type 1 and type 2 diabetic patients which at a percentage of 10-20% succumb to kidney failure. The generation of advanced glycation end products (AGE) that is associated to hyperglycemia plays important role in the disease pathophysiology. AGE receptor (RAGE) engagement with its' ligands leads to oxidative stress and chronic inflammationin nephritic tissue ending up with kidney function loss. Furthermore, activation of RAGE leads to activation of different intracellular signaling pathways such as NF-κB,MAPK/ERKandPI3K/Akt. PI3K/AKT is a signaling pathway that is required for normal metabolism and its imbalance leads into obesity and type 2 diabetes development-which causes insulin resistance, that in turn exacerbates the PI3K/AKT pathwayforming thus a vicious cycle. MAPK/ERK signaling regulates insulin sensitivity and increased activation is related with impaired insulin signaling in subcutaneous microvascular endothelial cells.[87, 88, 89,24]Prolonged hyperglycemia activates oxidase 4 (NOX4), an enzyme which is generating ROS at intracellular membranes and leads to oxidative stress that may cause renal damage. Mitochondria become dysfunctional and superoxide radical production is increased. MnSOD, is a mitochondrial enzyme that is involved in the regulation of antioxidant response and in diabetic patients is inhibited. Besides, catalase, superoxide dismutase and reduced glutathione in tissues and biological fluids are in low levels due to oxidative stress. Increased oxidative stress promotes the formation of vasoactive mediators which affect renal functions and reduce glomerular filtration rate.[90, 91]

In research, pomegranate peel extract in various concentrations stabilized on gold nanoparticles was administrated on adult BALB/c diabetic micewith nephropathy and biochemical parameters as blood glucose, plasma insulin, glycated hemoglobin HbA1C, total cholesterol, triglycerides, renal intracellular reactive oxygen species (iROS), renal lipid peroxidation, renal reduced glutathione level, renal superoxide dismutase activity, tissue nitrite (NO), proinflammatory cytokine level, protein expression and biodistribution of PPE-AuNP were determined. In the pomegranate peel extract were identified by LC-ESI/MS analysis 17 phenolic compounds namely gallic acid, ellagic acid,ellagic acid glucoside,ellagic acid arabinoside,epigallocatechin gallate, 3′,4′,5,7-tetrahydroxyisoflavanone,gallagic acid, cyanidin 3-O-xyloside, 6″-O-acetyldaidzin,dihydroquercetin 3-O-hexoside, epigallocatechin, isopeonidin 3-O-sambubioside, methylgalangin, kaempferol 3-O-rhamnoside, kaempferol 3-(2G-rhamnosylrutinoside), leucopelargonidin and punicalin with ellagic acid and its' derivatives to be the most predominant phytochemicals. Group I was the healthy control. Group II mice were treated with a single intraperitoneal streptozotocin injection (200 mg/kg body weight) and diabetes was induced by 7 days after streptozotocin injection. Mice in groups III-V were treated with PPE-AuNP 5, 15 and 25 mg/kg body weight respectively, on 8^{th}, 10^{th}, 12^{th}, 14^{th}, 16^{th} day after diabetes injection. Moreover, in a separate experimentation set mice were administrated with pomegranate peel extract in concentrations of 5, 15, 25 and 100mg/kg for 5 alternate days, 8^{th}, 10^{th}, 12^{th}, 14^{th}, 16^{th} after streptozotocin injection in order to compare the efficacy of pomegranate peel extract and PPE-AuNP. Histopathological and immunohistochemical examinations were carried out. The plasma insulin decrease in diabetic rats, was gradually and dose dependently normalized, as for extract concentrations of 5, 15, 25 mg/kg body weight insulin was increased by 0.25-, 0.43- and 0.53-fold respectively. PPE-AuNP showed better efficacy than the native extract. The best results of native pomegranate peel extract were given by concentration of 100mg/kg body weight, which were similar to those of PPE-AuNP 25mg/kg body weight. Fasting blood glucose was also reduced dosed dependently with PPE-AuNP 25mg/kg body weight to give best results than native extract 100 mg/ kg body weight. The function of β-pancreatic cells was also evaluated using Homeostatic Model Assessment (HOMA-βassessment). Diabetes induction decreased markedly HOMA-β (0.02-fold) compared to healthy controls. PPE-AuNP application gradually, in a dose-dependent manner (5, 15, 25 mg/kg) increased β-cell function by 0.07-, 0.14-, 0.25-fold respectively. Furthermore, PPE-AuNP treatment suppressed streptozotocin-induced renal toxicity. Diabetic control mice had, compared to healthy controls, increased levels of creatinine (2.94-fold) and serum urea (3.24-fold). PPE-AuNP treatment decreased creatinine levels at 2.15-, 2.34- and 2.68-fold for 25, 15 and 15 mg/kg body weight respectively, while serum urea levels were reduced at 1.98-, 2.47- and 3.01-fold for25, 15 and 5 mg/kg body weight respectively. PPE-AuNP treatment also suppressed streptozotocin-induced formation of glycated hemoglobin. Streptozotocin administration augmented markedly hemoglobin glycation and in diabetic group, the level of glycated hemoglobin was increased by 2.57-fold compared to healthy control group. PPE-AuNP treatment reduced in a dose dependent manner HbA1C level (2.12-, 1.72- and 1.45-fold at 5, 15, 25 mg/kg respectively). Compared to the healthy control group, streptozotocin injection increased serum total cholesterol and triglycerides by 2.33-fold and 3.0-fold respectively. PPE-AuNP treatment decreased cholesterol levels at 2.22-, 1.82- and 1.68-fold and triglycerides at 2.75-, 2.53- and 2.25-fold for doses of 5, 15 and 25 mg/kg. With respect to healthy control group, streptozotocin injection enhanced by 3.35-fold iROS generation. PPE-AuNP administration (5, 15 and 25 mg/kg) progressively decreased iROS generationin nephritic tissue by 2.51-, 2.18- and 1.61-fold respectively. Diabetes increased lipid peroxidation on renal tissue by 3.41-fold compared to healthy control group. Thiobarbituric acid reactive-substances, by-products of lipid peroxidation, were

found to be increased by 3.41-fold compared to healthy controls and were decreased dose dependently with PPE-AuNP that enhanced endogenous antioxidants (1.86- and 1.55-fold for 15 and 25 mg/kg respectively). The activities of glutathione and superoxide dismutase were restored towards normalcy. Moreover, PPE-AuNP administration inhibited the production (due to streptozotocin injection) of cellular nitrite that is associated with several abnormalities. Histological examination of the pathological and functional changes due to streptozotocin injection, showed that in nephritic tubules there was a marked degree of vacuolar degeneration along with widened podocyte membrane and thickened basement membrane. PPE-AuNP administration decreased significantly the nephritic tubules vacuolar degeneration reducing the thickened basement membrane. In streptozotocin treated animals, glomerular sclerosis and tubulointerstitial fibrosis have been observed, which with PPE-AuNP administration at all doses were decreased. Diabetic nephropathy is also associated with renal fibrosis that is a state of excessive accumulation of extracellular matrix where fibrogenic factors as collagen IV and TGF-βare excessively accumulated. In order to determine the efficacy of PPE-AuNP to prevent this state, immunofluorescence has been carried out to assess the expression of TGF-βand collagen IV. The results showed that in diabetic controls TGF-βand collagen IV expression was markedly enhanced. PPE-AuNP treatment caused in a dose dependent manner a gradual decline of collagen IV and TGF-β. Moreover, PPE-AuNP treatment suppressed markedly the streptozotocin-induced AGE-RAGE, NF-κB and MAPK pathwaysactivation in nephritic tissue. PPE-AuNP treatment also reversed the inhibition of Mn-SOD expression due to diabetes and regulated the modulation of PI3K/AKT pathway in renal tissue. These results indicate that PPE-AuNP can sufficiently prevent oxidative stress and its' adverse effects that are induced at hyperglycemic conditions. [92]

In order to evaluate the pomegranate peel protective properties against diabetes-induced renal histopathological changes, 40 male albino rats with type 2 diabetes were orally administrated with pomegranate peel extract for 40 weeks and kidneys were sampled for histopathological, immuno-histo-chemical and ultra-structural examinations. The results showed an improving effect on renal parenchyma in kidneys of diabetic rats indicated by healed renal tubules, healed glomerular tuft, healed interstitial epithelial cells. The normal glomerular capillary wall thickness, the normally tubular basement membrane thickness and the normal glomerular tuft structures were restored. Moreover, there was significant improvement in total cholesterol, triglycerides and blood glucose levels. [93]

Sialic acid is an acetylated product of neuraminic acid which act as a cofactor of several surface receptors such as insulin receptors and it is positively related to most of the serum acute phase reactants. It is an important component of the serum and in diseases as diabetes or certain malignancies is elevated. Besides, high concentrations of sialic acid are markedly associated with various risk factors for cardiovascular diseases and are also seen in patients with diabetic retinopathy and nephropathy.[94]Protein carbonyl content results from protein oxidation and its' level in plasma and tissues is a relatively stable oxidative marker damage.[95]27 rats with streptozotocin-induced diabetes mellitus were administrated with either pomegranate juice or saline for 10 weeks and then their lungs were harvested for immune histochemical and histologic evaluation. Protein carbonyl content, sialic acid, reduced glutathione and superoxide dismutase activities were measured in pulmonary tissue as well as the presence of endothelial nitric oxide synthase through immunohistochemistry. Sialic acid was markedly elevated in diabetic group compared to controls and with pomegranate juice treatment it was significantly decreased. Moreover, protein carbonyl content, which was in high concentration in diabetic group, was remarkably decreased with pomegranate juice treatment. The activity of the antioxidant enzyme superoxide dismutase was found to be markedly reduced in diabetic group and was increased in pomegranate treated diabetic group while reduced glutathione activity was not significantly changed. Endothelial nitric oxide synthase was reduced with the pomegranate treatment compared to diabetic group. Histological study in diabetic lung tissues showed thickened basal membranes and also intense mononuclear cell infiltration, while in lung tissues of pomegranate juice treated diabetic rats, the inflammatory reaction was much more lesser.[96]

The intervention effect of punicalagin on diabetic nephropathy has been investigated using a diabetic nephropathy mice model treated with punicalagin at dose of 20 mg/kg/body weight/day for 8 weeks. The results showed that after punicalagin treatment, blood urea nitrogen, serum creatinine and urine albumin to creatinine ratio were markedly reduced compared to untreated diabetic nephropathy group mice. Moreover, the symptoms of glomerular hypertrophy and glomerular interstitial hyperplasia were alleviated. Pyroptosis is a manner of programmed cell death in inflammatory response.The study results showed marked decrease in the expression of proteins which are associated with pyroptosis, such as cysteinyl aspartate-specific protease-1 (caspase-1), gasdermin D (GSDMD), interleukin-1 (IL-1β) and nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing protein 3 (NLRP3), indicating that punicalagin administration at dosage used for 8 weeks inhibits markedly pyroptosis. Moreover, the expression of NOX4 NADP Hoxidasethat causes oxidative stress and is also related to retinal eye diseases has been decreased and mitochondria damage was alleviated. Low NOX4 expression inhibits the dissociation of thioredoxin and thioredoxin-interactingprotein (TXNIP)while NLRP3 inflammasome

activation that contributes into pyroptosis and other types of cell death were suppressed.[97]

Oleanolic acid, found in pomegranate flowers and ursolic acid in flowers and seed, are referred to exert very good pharmacological activity in diabetic patients.[27]. In research, diabetic Spague Dawley rats (150-200 gr) (4 weeks after perineal injection with streptozotocin that induces diabetes) were orally treated for 4 weeks with oleanolic acid at doses of 20, 40 and 60 mg/kg/day. The healthy and diabetic control groups were treated with vehicle. After 8 weeks, blood and 24h urine samples were collected in order to measure urine and serum levels of urea, albumin and creatinine for renal function assessment. Kidney samples were examined in order to determine weight changes, oxidative stress related parameters such as superoxide dismutase, catalase and reduced glutathione levels. Besides, one kidney of each rat has been examined with transmission electron microscopy. The results showed that the oxidative stress in kidneys has been significantly decreased in a dose dependent manner. The lipid peroxidation level was markedly increased in diabetic control group and was reduced with oleanolic acid treatment. This reduction of lipid peroxidation protected kidney cell membranes from oxidative damage. Oleanolic acid treatment improved dose dependently the reduced glutathione levels, yet this rise was remarkable only in the 60mg/kg dose treated group. The activities of superoxide dismutase and catalase were significantly lower in the diabetic control group than the healthy control and were increased by oleanolic acid treatment dose dependently. Urine albumin, a renal damage indicator was decreased with oleanolic acid treatment and creatinine that is associated with renal function was also improved in treated rats. The glomerular filtration rate of diabetic controls was significantly lower than the healthy animals and was dose dependently improved in oleanolic acid treated groups with the value of the 60mg/kg treated group to be same with this of the healthy controls. The average kidney weight in diabetic control group was lower than the healthy controls. In all doses oleanolic acid treated groups the kidney weight had almost the same value, which was the mean value of healthy and diabetic controls. The electronic microscopy results showed that oleanolic acid treatment at dose of 60mg/kg inhibited sufficiently the nephropathy induced alterations in podocytes integrity, basement membrane thickness and spacing between podocytes. Diabetes induced polydipsia and polyphagia were also inhibited with the treatment. Oleanilic acid is also referred to improve the insulin secretion and inhibit the advanced glycation end products generation which are contributing to vascular cell derangement. As oleanolic acid prevents or treats through multiple mechanisms diabetic nephropathy and also it is safe as safety tests have shown, it can be considered as an alternative drug in order to treat or prevent this diabetes complication. [90]

In research, pomegranate seed oil was daily administrated at doses of 0.4 and 0.8 ml/kg in streptozotocin induced diabetic rats and biochemical parameters such as urea, creatinine, malondialdehyde, LDL, triglycerides andglucose levels were determined on the 21st and 28th day of the study. In streptozotocin (diabetic)group there was remarkable elevation of urea, creatinine, LDL, triglycerides, glucose levels as well as malondialdehyde levels and urine markers compared to controls. Pomegranate seed oil administration resulted in a remarkable decrease in tissue malondialdehyde content, serum creatinine, urea as well as urine markers compared to the diabetic untreated group. Moreover, the lipid profile was improved after the seed oil treatment with the results to be better on 28th than on 21st day. [98]

POMEGRANATE AS A NEUROPROTECTIVE AGENT PREVENTS FROM DEVELOPING AND DETERIORATING OF DIABETIC NEUROPATHY

An amount of 50% of diabetic patients is referred to develop diabetic neuropathy that constitutes damage to the nerves and also to the micro-vessels that are supplying the nerves, tissue. Common symptoms are pain, numbness, tingling or weakness in hands and feet. Combined with decreased blood flow, neuropathy in foot increases the risk of foot ulcer onset and sometimes the eventual limb amputation. [24]

Peripheral nerve conduction deterioration is an important indicator for diabetic patients with peripheral neuropathy. In study, the effects on sensory function of pomegranate rind extract at doses of 25, 50 and 100mg/kg, rindextract biopolymeric dispersions in chitosan or casein at doses equivalent to 25, 50 and 100 extract/kg and gallic acid at doses of 3, 6 and 12mg/kgon diabetic mice, were examinedby thermal latency measurement with hot plate and tail flick tests, on 8th week after alloxan injection. Pomegranate rind extract treatment improved significantly the thermal latency test results compared to vehicle group. For doses of 25, 50 and 100mg/kg, there was improvement by 33.3, 73.5 and 85.1% respectively. Moreover, significant improvement in tail flick latency has also been observed, that was by one-, two- and threefold for 25, 50 and 100mg pomegranate extract/kg respectively, compared to vehicle group. After 8 weeks treatment, extract dispersion with chitosan at doses equivalent to 50 and 100 mg extract/kg, improved markedly hot plate latency, by 15.7 and 84.3% respectively, while the lower dose did not give significant results compared to vehicle group. Extract dispersion with chitosan improved markedly tail flick latency compared to vehicle control. Doses that were equivalent to 25, 50 and 100 mg/kg were found to improve tail flick latency by 1.5-, 1.7- and 3-fold respectively compared to controls. Treatment with extract dispersion with casein at doses equivalent to 50 and 100 mg extract/kg improved remarkably hot plate latency by 45.5 and 60.8% respectively while tail flick latency was improved by 0.3- and 0.9-fold compared to controls. At the lowest dose, the extract dispersion with casein did not improve markedly

tail flick or the hot plate results. In addition, gallic acid treatment at doses of 3, 6 and 12 mg/kg, showed a significant improvement in tail flick latency by 1.5-, 2- and 2.4- fold and in hot plate latency by 70.6, 78.6 and 83.6% compared to vehicle group.[66]

DIABETIC WOUNDS - POMEGRANATE ENHANCES WOUND HEALING PROCESS DUE TO ITS' STRONG ANTIMICROBIAL, REGENERATIVE AND IMMUNE STIMULATING PROPERTIES.

Diabetic wounds

Diabetic chronic wounds are multifactorial and complex. Diabetes is affecting many body systems causing various complications which further influence the wound healing process. [99]

Sensory neuropathy that is observed in diabetic patients results into loss of the protective function of discomfort and pain which are the alert for possible injuries and thus there is a high risk of Charcot's foot deformity development, destruction of joints and bones of the foot that may raise further the pressure on new points of the foot and lead in further ulceration. Motor neuropathy is possible to place undue pressure onto the insensate foot and in combination with the poor vascular supply may result in foot arching and toes clawing, altering this way the foot pressure points and causing the formation of callus and ulceration at these new pressure points of the foot. Autonomic neuropathy leads in reduced sweating that gives rise to dry skin cause fissures that are an entry for microbes.[100]

High blood glucose level is stiffening arteries and narrowing blood vessels causing poor circulation as narrowed blood vessels lead to reduced blood flow and oxygen to the wound. Moreover, high blood glucose level reduces the function of the red blood cells which carry nutrients to the wound tissue. As there are not sufficient nutrients and oxygen, the wound healing process occurs slowly.[99]

Exposed subcutaneous tissues consist favorable substrates for various microorganisms to contaminate and colonize and in case the involved tissue is devitalized (e.g., hypoxic, necrotic or ischemic) and the immune system of the host is compromised, there is microbic growth and infection. Wound contaminants may originate due to microorganisms from the environment, the surrounding skin (including normal skin microflora members such as propionibacteria, *Staphylococcus Epidermidis*, skin diphteroids, micrococci) and endogenous sources such as mucous membranes. In diabetic wounds and ulcers mostly are found *Bacteroides spp., Proteus spp., Peptostreptococcus spp., Klebsiella pneumoniae, Pseudomonas spp., Acinetobacter spp., Escherichia coli, Enterobacter spp., Staphylococcus aureus, Staphylococcus epidermidis*and *Bacteroides fragilis group*. [101,102] In chronic wounds such as diabetic wounds, bacteria mostly exist in biofilms, membranous tissues constructed by extracellular polymer substances that are formed due to quorum sensing signaling of the bacteria and intracellular cyclic dimeric guanosine monophosphate (c-di-GMP) signaling. Biofilm microorganisms are protected from immune response and adverse environmental factors and is mentioned to be more virulent (80% of infections are caused due to biofilms) and about1000 times more resistant to antibiotics than their planktonic counterparts.[25]

Furthermore, diabetes decreases the immune system efficacy to defend against infection. Due to high blood glucose levels the immune cell's function become ineffective and this raises the risk of infection. Infection slows the overall healing process and also raises several health concerns.[99]Poorly treated diabetic wounds and ulcers may lead to gangrene, osteomyelitis and limb amputation, while there is an increased mortality risk for the amputees and increased bacterial resistance in survived patients.[102,103]

Pomegranate wound healing properties

Pomegranate phenolic acids, tannins, flavonoids-especially anthocyanins possess various biological properties such as antioxidant, antimicrobial, anti-inflammatory, neuro protective, immune modulatory and thus are benefiting and enhancing wound healing process by managing at the same time the bacterial count, the inflammation and infection that occur, while the clinical picture of vascular and nervous system is improved, and immune system is stimulated. Besides, pomegranate plant part extracts are mentioned to enhance significantly re-epithelization, collagen production and neovascularization processes.[25]

Pomegranate's strong antimicrobial properties against various pathogens are mentioned in several reports as well as its' anti-quorum sensing and anti-biofilm activity. More specifically, pomegranate plant part extracts and also isolated phytochemicals show strong antimicrobial activity against *Acinetobacter, Aggregatibacter, Bacillus, Helicobacter, Clostridium,Porphyromonas, Escherichia coli, Citrobacter, Cryptococcus, Bacteroids, Alcaligenes, Cronobacter, Prevotella, Achromobacter, Proteus, Pseudomonas, Enterococcus, Treponema, Yersinia, Serratia, Klebsiella, Lysteria, Shigella, Mycobacterium, Streptococcus, Salmonella, Vibrio* and*Staphylococcus*.Pomegranate is decreasing the bacterial count showing similar efficacywithvariouscommercial antibiotics - sometimes even higher, and also through its' anti-quorum sensing activity prevents the communication among bacteria that leads in biofilm formation and increased bacterial virulence and resistance against antibacterial agents. Furthermore, studies have shown that pomegranate treatment reduces significantly the biomass of already existing biofilms.Is important to be mentioned that although pomegranate extracts and isolated phytochemicals act as strong antibacterial agents against pathogens, at the same time they are protecting and enhancing the beneficial bacteria in contrast to antibiotics which are harming the beneficial gut micro biota causing dysbiosis and various other adverse effects that may be particularly severe. [104, 25] Moreover, is referred to be an association between

disrupted gut micro biota and high risk of development or aggravation of obesity and type 2 diabetes. [105]

In a study, diethyl ether extracts of Punica Granatum Linn flowers and Malva sylvestris Linn flowers were administrated on wounded diabetic Wistar rats in order to evaluate their wound healing efficacy. Group I - non-diabetic wounded rats and group II(control) -wounded diabetic rats received simple ointment base. In groups III and IV diabetic rats were treated each one by one of the extracts. In group V diabetic rats were administrated with a 1:1 mixture of the extracts and in group VI diabetic rats received the standard drug nitrofurazone. Histopathological studies results showed that treatment with all natural extracts decreased significantly the wound aria compared to nitrofurazone and control groups. The best results among the natural extracts were given by the pomegranate flower extract, as the rats treated with this extract were on 18th day completely healed. On 18th day, the extract mixture had given remarkably better results compared to nitrofurazone group, as the wound aria in extract group was 0.112 ± 0.033 cm^2 and in nitrofurazone group 0.711 ± 1.004 cm^2.[25] Is important to be mentioned that nitrofurazone may cause adverse effects such as allergic contact dermatitis with clinical presentations to be dyshidrosiform, erythroderma, excoriated plaques and papules, while pomegranate which has higher wound healing efficacy not only is safe but also is antiallergic and through its' antioxidant anti-inflammatory and regenerative properties is benefiting the skin. [106, 107]

In research, in order to evaluate the efficacy of pomegranate peel polyphenols to heal diabetic wounds, gel containing peel polyphenols (30% polyphenol mass fraction) was administrated for 21 days on alloxan-induced diabetic rats with cutaneous wounds. The wound healing process was significantly shortened in the pomegranate treated rats compared to controls. Histological examination showed significant collagen regeneration enhancement and increased epithelialization, vascularization and fibroblast infiltration in the wound aria, indicating that pomegranate peel polyphenols could possibly be used as an alternative medicine for diabetic wounds treatment.[25]

The vascular endothelial growth factor (VEGF) is a signal protein which stimulates the blood vessel formation. Overexpression or low levels of VEGF is possible to lead to several disorders such as pulmonary emphysema, vascular diseases or cancer. Decreased production of VEGF and reduced angiogenesis contribute into delayed and impaired wound healing. In studies is referred that VEGF treatment of diabetic wounds accelerates healing process. Transforming growth factor beta 1 (TGF-β1) is a cytokine that belongs to transforming growth factor superfamily and plays important role in wound healing process as it performs various cellular functions such as the control of cell proliferation, growth, differentiation and apoptosis. TDF-β1, through its' effect on mesenchymal cells, is acting as a key regulator of the production and remodeling of extracellular matrix. Treatment of diabetic wounds with TGF-β1 is referred to accelerate significantly wound healing process. Epidermal growth factor (EGF) is a protein that binds on its' receptor (EGFR), stimulates cell growth and differentiation and accelerates markedly the wound healing process. It is used for diabetic foot ulcer treatment. [25]

In study, in order to evaluate the wound healing activity of Saudi pomegranate peel on diabetic wounds, gel containing methanolic extract of peels (5% w/w)was administrated for 21 days in diabetic rats with excision wounds. Rats were divided into pomegranate peel extract-gel treated group, gel alone group, non-treated group and TGF-β1, VEGF, EGF, nitric oxide (NO) and NO synthase (NOS) were estimated in wound lysates. Moreover, skin histopathology study has been carried out to evaluate the re-epithelization neovascularization and anti-inflammatory effects of the extract. The results showed that pomegranate gel accelerated remarkably the excisional wound healing process as it increased significantly epithelization, fibroblast proliferation, neovascularization, granulation tissue and collagen deposition. Hydroxyproline content, VEGF, TGF-β1 and EGP expressions were upregulated while NO levels were found significantly lower in pomegranate group compared to the vehicle group. Moreover, NOS activity was markedly lower in pomegranate group than in vehicle group and this indicates that pomegranate peel extract contributes into the wound healing process also through NOS activity and NO production down regulation.[25]

Pomegranate as an immune system stimulant and immunomodulatory agent

Immune system contributes to homeostasis by preparing the body to fight against antigens, infections, harmful agents that may disturb health maintenanceand also it is enhancing the healing process in case of any harm. A strong immune system is a perquisite for maintenance of health. As much important for wound healing is the efficacy of the medical agents that are used for treatment, that much and even more important is the ability of the body itself to fight against microbes, infections, and other destructive and harmful agents and conditions that are threatening health.[108]

Lymphocytes, white blood cells, are immune systems' part. There are two main types of lymphocytes: B cells that are producing antibodies that attack invading bacteria, viruses and toxins and T cells which destroy body cells that became cancerous or are taken over by viruses. [108]Diabetic patients are immunocompromised due to short-term and chronic hyperglycemia.[109]In in-vitro study, human lymphocytes isolated from peripheral blood (10^6 cells/ml, 80µl suspension) were incubated at 32^{o}C for 72 hours with 20µl pomegranate extract with final concentrations of the extract to be 0.1, 0.25 and 0.5 µl/ ml. It was found that the stimulation index of the pomegranate was 1.2 times higher than the index of a commercial immune-stimulant at the same concentration as the % stimulation index values for pomegranate and

commercial immune-stimulant were 1245 and 1024 respectively. [108]

In research, BALB mice were administrated with high calorie biscuit with either pomegranate extract that was equal to 400g/day consumed by 70 kg man or commercial immune-stimulant or no immune stimulant (control group). Immunoglobulin is a sensitive immune marker that represents a specific humoral immune response due to infections. Total serum immunoglobulin was measured in week 0, 2, 4and 8 in order to study the humoral immunity stimulation. It was found that at the end of the 8th week in both pomegranate extract and commercial immune-stimulant groups there was a remarkable and comparable increase of total serum immunoglobulin G.[108]

In another study, administration of immunocompromised mice with pomegranate peel polysaccharides at doses of 100, 200 and 400 mg/kg per day increased the immune organ index.Hepatic antioxidant capacity and the antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase were also remarkably increased, indicating that pomegranate peel polysaccharides could possibly be used as an effective immunostimulant agent in immunocompromised patients.[110]

Macrophages are a phenotype of phagocytes that playsimportant role in defense and maintenance of host tissues. The first step of macrophages' response to invading bacteria is phagocytosis. Immunological tests that have been carried out showed that pomegranate beverage with echinacea and spirulina havingtotal flavonoids 2.084 ± 0.55 mg catechin/ ml and total phenolics 2.294 ± 0.64 mg gallic acid /mlincreased remarkably phagocytosis. [111]M1 and M2 are macrophage phenotypes which play different roles in the immune system. M1 phenotype produces nitric oxide (NO), reactive oxygen species (ROS) and reactive oxygen intermediators (ROI) in order to attack bacteria and viruses. M2 phenotype plays anti-inflammatory role and resolves inflammation which favors oxidative metabolism. NO and ROS overproduction by M1 phenotype results in oxidative stress which causes inflammation and various disease and thus macrophage polarization plays crucial role for tissue fate. In healthy subjects M1 and M2 phenotypes remain in a balance state. In research, administration of pomegranate juice rich in ellagic acid and gallic acid in mice inhibited M2 to M1 shifting, favoring the anti-inflammatory M2 phenotype. In in-vitro study on macrophage-like cell line J774A1 was found that pomegranate polyphenols and juice decreased in a dose dependent manner the macrophage response to M1 pro-inflammatory activation, as a remarkable decrease of TNF-α and IL-6 secretion has been observed in response to stimulation by INF-γ and Lipopolysaccharide. Besides, pomegranate juice and punicalagin promoted dose dependently the anti-inflammatory M2 phenotype indicating the immunomodulatory properties of pomegranate.[112]

POMEGRANATE IMPROVES ERECTILE FUNCTION OF DIABETIC PATIENTS

Erectile dysfunction is a common diabetes complication. The prevalence of impotence in the diabetic men is over 50%. Diabetes-induced dysfunction pathophysiology is multifactorial. The proposed mechanisms that cause this complication include atherosclerosis, increased levels of oxygen free radicals, high levels of advanced glycation end-products, impaired nitric oxide synthesis and neuropathic damage.[113]

Several studies have shown that pomegranate prevents or treat sufficiently atherosclerosis, reduces oxidative stress and improves the antioxidant status of the patient, it is a neuroprotective agent andreduces the production of glycation end-products. [67, 66, 90]

In research, New Zealand white rabbits with atherosclerosis-induced erectile dysfunction and age-matched control animals, weighing 3.5kg each, were daily administrated with pomegranate extract liquid containing 30, 60 or 120 mg polyphenols or placebo for 8 weeks. As placebo, drinking water has been used. Atherosclerosis decreased significantly blood flow compared to age-matched controls. Arterial pressure of rabbits receiving the dosage of 120 mg polyphenols was markedly lower than placebo administrated animals. Pomegranate extract consumption increased markedly intracavernosal blood flow in atherosclerotic and age matched controls in comparison with placebo groups. However, intracavernosal blood flow in animals with atherosclerosis that received pomegranate extract was lower than control animals that were receiving pomegranate extract or placebo. These findings indicate that pomegranate extract intake improves significantly intracavernosal blood flow in animals with atherosclerosis compared to those (atherosclerotic animals) that received placebo, yet blood flow was not normalized to the levels of control animals. Pomegranate extracts at all concentrations showed the same efficacy in increasing blood flow. Electrical stimulation of cavernosal nerve in control animals increased significantly intracavernosal pressure and led to full erection. MICP/MAP percentage in erectile dysfunction groups which received pomegranate extract at all concentrations was markedly increased compared to placebo erectile dysfunction animals but still it was lower than control animals that received pomegranate extract or placebo. Pomegranate extract intake did not affect MICP/MAP percentage in control animals. These observations suggest that pomegranate extract improved significantly erectile function of erectile dysfunctional animals, yet there was not normalization up to the levels recorded in control animals. Extracts at all concentrations showed similar efficacy in improving erectile response of the treated animals. In erectile dysfunction group receiving placebo, endothelium-dependent relaxation of the erectile tissues was markedly less than the control group receiving placebo. Pomegranate extract treatment increased markedly endothelium-dependent relaxation in both erectile

dysfunction and control groups in comparison to placebo receiving groups, yet there was not complete normalization. Pomegranate extracts at all concentrations gave similar results. Diffused fibrosis and significant loss of smooth muscle were observed in erectile dysfunction group. Masson's trichrome-stained penile sections histomorphometric analysis in erectile dysfunction group showed marked decrease in smooth muscle percentage compared to the control group that received placebo. Pomegranate extract treatment did not affect significantly the smooth muscle content in control group. In erectile dysfunction group treated with pomegranate extract the smooth muscle percentage was higher than this of erectile dysfunction group treated with placebo but also markedly less than the control group that received placebo. The oxidatively modified product isoprostane-8-epi-PGF2αlevels were markedly higher in the erectile tissue of the erectile dysfunction group treated with placebo than those of the control group treated with placebo, indicating oxidative stress. Consumption of pomegranate extract containing 30 mg polyphenols was sufficient to decrease isoprostane-8-epi-PGF2α levels to those of age-matched control group that received placebo.Moreover, pomegranate extract containing 30mg polyphenols reversed the molecular changes that have been observed in erectile dysfunction group to the age-matched control levels. Electron microscopy showed that pomegranate extract (30mg polyphenols) protected mitochondrial and endothelial structural integrity and diminished caveolae levels in the erectile tissues of erectile dysfunction group. [114]

In another study, diabetic adult male Sprague-Dawley rats were treated daily with pomegranate juice 100mg/kg for 10 weeks and the in vivo erectile, that is a ratio of intracavernosal pressure/mean arterial pressure (ICP/MAP) and ex vivo corpus cavernosum (CC) responses were determined. The results showed that the ICP/MAP value was lower in diabetic rats than in controls and after pomegranate treatment it partially was improved. In the diabetic group, electrical field stimulation EFS-induced relaxant responses in CC were markedly reduced after pomegranate juice treatment. Moreover, pomegranate juice normalized malondialdehyde levels in diabetic CC samples. Oxidative stress and fibrosis were markedly reduced.[115]

POMEGRANATE TREATMENT PREVENTS DIABETICOCCULAR STRUCTURAL CHANGES

Diabetic retinopathy, an important cause of blindness, occurs as a result of long-term damage of the small blood vessels in retina. Is referred that an amount of 10% of diabetic patients is possible to develop severe visual impairment after 15 years of diabetes.[24]

In a study, diabetic adult Sprague-Dawley rats weighing 230-250g were treated with pomegranate juice 100 μL/ day for 10 weeks. The healthy control and diabetic group received 25 ml of saline. Retina tissues were evaluated for antioxidant enzymes, lipid peroxidation and oxidative DNA damage. [53]Oxidized nucleoside 8-hydroxyguanosine (8-OHdG) is a biomarker of cellular oxidative stress and also a risk factor for atherosclerosis, diabetes and cancer. Elevated 8-OHdG is found in diabetic patients and patients with various types of cancer.[116]Malondialdehyde and 8OHdG levels were markedly increased in the retina of diabetic group animals compared to healthy controls. Pomegranate juice treatment decreased both malondialdehyde and 8OHdG levels compared to diabetic group, however the values yere still higher than those of healthy controls. The antioxidant enzymes' glutathione peroxidaseand superoxide dismutase activities were markedly reduced in diabetic group animals' retinacompared to healthy controls. The activity of reduced glutathione was also reduced yet the difference was not significant. Pomegranate juice intake increased the activities of all three antioxidant enzymes. In healthy control group, pomegranate juice intake did not change the activities of the antioxidant enzymes. In the diabetic group, glutathione peroxidase activity was increased remarkably, yet the activities of the other enzymes did not have significant change.[53]

In research, 40 diabetic male Wistar rats were orally administrated with pomegranate peel extract at dose of 500mg/kg body weight/dayand clinical examinations of fundus and lens have been carried out. On 8^{th}, 12^{th}, 16^{th}, 20^{th}, 24^{th}, 28^{th}, 32^{nd}, $36^{th}$and 40^{th}week after diabetes induction biochemical analysis has been carried out to determine total cholesterol, triglycerides, blood glucose, and glycated hemoglobin. In healthy control group retina and lens were normal. In the diabetic untreated group, 12 of a total of 40 rats showed a cataract onset at 16^{th} week after diabetes induction, which was increasing gradually. Inter-retinal microvascular abnormalities, tortuous and dilated vessels, microaneurysms and small "dot and blot" hemorrhage have been observed. In pomegranate treated group only 7 of a total of 40 rats showed cataract onset on 24^{th} week after diabetes induction. Furthermore, hemorrhage spots and abnormal vascularization that have been found in pomegranate group were less numerous compared to diabetic untreated group. Biochemical analysis showed marked elevation of blood glucose (261.36 mg/dl) compared to healthy control group (77.83 mg/dl). In treated with pomegranate diabetic group there was a significant decrease (173.07 mg/dl) compared to the untreated group. Remarkable improvement of total cholesterol has been observed in pomegranate treated group with a mean value of 163.12 mg/dl compared to untreated diabetic group with 202.32 mg/dl. At the end of the treatment, there was a significant decrease in triglycerides in treated group (125.50 mg/dl) compared to untreated diabetic group (163.42 mg/dl). Moreover, at the end of the treatment, a significant decrease of the percentage of glycated hemoglobin has been observed. Histopathological analysis in healthy control group showed normal capillaries and normal highly organized layers, while in untreated diabetic group retinal

cells were remarkably reduced and retina was disorganized with impaired layers. Moreover, in pomegranate treated diabetic group, a significant amelioration of retinal cell layers has been observed. Transmission electron microscope observation showed a normal capillary wall at the healthy control group rats while in the diabetic untreated group there was a significantly thickened capillary basement membrane with focal fiber accumulation in some parts. In pomegranate treated diabetic group there was a nearly normal capillary wall yet with some focal thickness in capillary wall with few focal accumulations of fibers in some parts.[117]

SIDE EFFECTS OF MEDICINES USED FOR DIABETES AND ITS' COMPLICATIONS TREATMENT

As diabetes is affecting various organs and systems causing several implications that may also be severe diseasessuch as cardiovascular diseases, nephropathy, diabetic foot- delay in wound healing-infected woundsect, diabetic patients are following medication for these diseases too.

Side effects of antidiabetic drugs

In the currently available pharmacotherapy for diabetes are included insulin and oral hypoglycemic agents. These medicines act by either increasing insulin secretion from pancreas or decreasing plasma glucose concentrations by reducing gluconeogenesis. These drugs cannot restore normal glucose homeostasis for long periods and also are not free of adverse effects which may be severe, such as hypoglycemia, kidney diseases, hepatotoxicity, insulinoma, and gastrointestinal problems.[9]

Insulin is used for type 1 diabetes treatment. It is regulating glucosehomeostasis through glycogen synthesis enhancement and stimulating liver glucose uptake. As insulin side effects are referred to be hypoglycemia in case of inappropriate dosage, weight gain, bumps, rashes or swelling at the injection site. Hypoglycemia symptoms are dizziness, fatigue, pale skin, confusion, trouble in speaking, sweating, seizure, twitching muscles and loss of consciousness. [9, 118]

Thiazolidenediones are synthetic antidiabetic medicines that act as agonists of PPAR γ increasing the sensibility of liver, muscle and adiposity tissues to the insulin action and are used for the type 2 diabetes mellitus treatment. They cause various side effects such as edema, anemia, increased weight, congestive cardiac failure and pulmonary edema while their use is also related to an increased myocardium infract risk.[45]

Fenofibrate is a PPARA αagonist. Its' side effects include liver problems, respiratory disorder, asthma, myalgia, pancreatitis, flatulence, abdominal pain, dyspnea, headache, neuralgia, dizziness, eczema, dyspepsia and gastroenteritis.[119]

Chlorpropamide is an antihyperglycemic agent used for type 2 diabetes treatment that may cause severe side effects including difficulty in breathing, swelling of throat, face, tongue, lips, skin pain, burning in eyes, yellowing of skin or eyes, severe skin rush, unusual bleeding, red or purple skin rash which spreads and causes peeling and blistering, confusion, severe weakness, loss of coordination, unsteady feeling, slurred speech, nausea, full feeling and heartburn. [120]

Metformin is an antidiabetic drug that reduces blood glucose levels. Its' side effects include diarrhea, nausea, nausea and vomiting, painful or difficult urination, muscle pain or cramping, decreased appetite, heartburn, fast or shallow breathing, lower back or side pain, general feeling or discomfort, flatulence and lactic acidosis. Other side effects that are referred are reduced vitamin B12 serum concentration and asthenia. Metformin-associated lactic acidosis may cause hypotension, hypothermia, resistantbradyarrhythmias or even death.[121]

Glibenclamide is used in order to reduce blood glucose levels in patients with type 2 diabetes. Its' side effects include hypoglycemia, sweating, heartburn, nausea, abnormal fulness, fast heartbeat, unusual weakness and tiredness, unusual bleeding, fever or chills, yellow colored skin or eyes. [122]

Glipizide is an antidiabetic medicine that reduces blood sugar levels in type 2 diabetic patients. It may cause mild or severe side effects. Mild side effects include nausea, dizziness, flatulence, headache, diarrhea, drowsiness, constipation andabdominal pain. Severeside effects may include changes in blood cells levels as anemia, leukopenia thrombocytemia with symptoms to be fatigue with anemia, fever or infections with leukopenia and bruising or bleeding easily with thrombocytopenia. Glipizide may also affect liver, increasing liver enzymes as alanine aminotransferasewith symptoms to include itchy skin, light colored stools, yellowing skin and eyes. Syndrome of inappropriate-antidiuretic-hormone (SIADH) is a possible serious side effect. Symptoms of SIADH mayinclude nausea and vomiting, confusion, fluid buildup, seizures or coma in severe cases. Furthermore, cardiovascular problems, hypoglycemia and allergic reaction are referred as possible severe adverse effects of glipizide.[123]

Side effects of antihyperlipidemic, antihypertensive antiatherogenic drugs

Antihyperlipidemic drugs' side effects may include dizziness, tightness in the chest, unusual weakness or tiredness, cough, hives, itching, skin rush, puffiness or swelling of the eyelids, headache, fatigue, abnominal pain, back pain, muscle aches, kidney damage or kidney failure, tachycardia and gastric disturbances.[124]

Antihypertensive drugs may cause side effects that include dry mouth, dizziness, dizziness upon standing up, headache, insomnia, nasal congestion, depression, mental or mood changes, nausea, stomach upset, vomiting, constipation or diarrhea, slow heart rate, muscle weakness and feeling faint. [125]

Side effects of antiatherogenic drugs may includemuscle pain, myopathy, fever, nausea, abdominal pain, weakness and kidney damages that may lead to death. [126]

Side effects of diabetic-nephropathy medications

Canagliflozin is used in order to slow diabetic kidney disease. It is a sodium-glucose co-transporter 2 inhibitor which when added to standard care (angiotensin converting enzyme inhibitor and angiotensin II receptor blockers), decreases the risks for cardiovascular events and kidney failure. Side effects of canagliflozin may include acute kidney injury, diabetic ketoacidosis, amputations, fractures and SGLT2 inhibition.[127]

Losartan is used in order to treat diabetic nephropathy. Mild side effects of the drug include dizziness, stuffy nose, fatigue, high or low blood pressure, hypoglycemia and back pain. Serious side effects include high potassium blood levels. This condition symptoms may be slow heart rate, heart rhythm problems and muscle weakness. Kidney disease with symptoms to include ankles, hands and feel swelling and allergic reactions with symptoms to be face or lips or throat swellingare also referred.[128]

Telmisartan is used for diabetic nephropathy and high blood pressure treatment. Its' side effects include fast heartbeat, heart burn and back pain. [129]

Side effects of antibiotics

Antibiotics may strongly affect the gut microbiota and cause various severe long-term consequences. The gut microbiota has an important metabolic function and acts as a real bogy organ as it is stimulating the immune system, regulating inflammatory response and contributing into the gut homeostasis maintaining through the symbiotic interactions among the microorganisms and gastrointestinal tract. Moreover, is inhibiting intestinal and urogenic pathogensgrowth. Gut microbiota disturbance is called dysbiosis. Microbial diversity is reduced, and opportunistic pathogens growth is enhanced. As every other organ disturbance leads to diseases, dysbiosis is associated to various intestinal and extraintestinal pathogenesis and disorders asinflammation, metabolic syndrome, aggravation of diabetes, obesity, Inflammatory Bowel Disease, Irritable Bowel Syndrome, asthma, psoriasis, cardiovascular diseases, coeliac disease and also is associated with colorectal cancer. [104]

Antibiotics usage may also cause stomach pain, gastrointestinal discomfort, diarrhea, rashes, fever, nausea, vomiting, anemia, allergic reactions and arthropathies. Antibiotics are also affecting kidneys, liver, heart rate and ear function leading to severe diseases. Bacterial antibiotics are mentioned to promote ROS formation and induce alterations to cellular redox state 77 that increases the risk of the development of diseases related to oxidative stress. [104]

POMEGRANATE SAFETY

Pomegranate plant part extracts at doses needed to showpharmaceuticalactivityand were used in traditional medicine, were found to be safe in the safety tests that have been carried out. Extract of pomegranate fruit, standardized to 30% punicalagins was found to have oral lethal dose 50 (LD50) higher than 5g/kg body weight. Intraperitoneal LD50 in rats and mice were 217 and 187 mg/kg body weight respectively. In a study, Wistar rats were treated with pomegranate extract standardized to 30% punicalagins at doses of 0 (control group), 60, 240 and 600 mg/kg per day. The extract administration lasted for 90 days followed by a recovery phase of 28 days. In comparison to the control group, no important toxicological changes have been observed in the clinical picture, clinical observations, body weight, organ weights, feed consumptions, ophthalmic examinations, body weight gains and clinical pathology evaluations .Hematology and serum parameters were found within the normal laboratory limits of statistically significant changes in comparison to controls, considered as biological variations, not as toxic effects. In the terminal necropsy, no histopathology findings or treatment related gross have been observed. According to these results, the extract's no observable adverse effect level (NOAEL), is 600 mg/kg body weight, which is the highest dosage tested. Moreover, is referred that punicalagin administration in subjects weighing 60 kg at dosage of 180mg/kg per day for 90 days, does not cause any adverse effects. [25]

Safety tests showed that pomegranate seed oil at dose of 2 g seed oil/kg body mass did not cause any side effects. LD50 could be considered as higher than 5g/kg body weight. According to OECD 423 assessment, no categorizing or marking of oral toxicity is needed for the seed oil. NOAEL of punicic acid is found to be 50,000 ppm that is equivalent to 4.3 g seed oil/kg body mass per day. [25]

Pomegranate peel extracts at doses of 0.5, 1.9 and 7.5 mg/kg per day were administrated for 22 days in Balb/c miceand also a single intradermal injection at dose of 224mg/kg was done. There were not any toxic effects in the epithelial cells' layer of tongue, trachea and larynx. Moreover,no mortality, side effects or behavioral changes have been observed. Repeated administrations with the extract did not cause any local alterations or irritations of oral mucosa, indicating that pomegranate extract has no toxicity and can be used for its' pharmaceutical properties.[25]

In research, no oral toxicity or mortality has been observed with pomegranate leaf extract administration at doses up to 2000mg/kg for 21 days, while the extract has shown very good pharmaceutical activity at 600 mg/kg.[75]

DISCUSION

The health issues that diabetic patients have to deal with are in addition to the lack of insulin and hyperglycemia, also the complications of diabetes that arise due to depletion of the

cellular antioxidant defense system which leads to body organ in juries which may result into other severe diseases.

Due to hyperglycemia, diabetic patients are under oxidative stress. Free radical production induces inflammation and inflammation produces more oxidative stress forming thus a vicious circle that leads to complications or aggravation of already existing ones. Pomegranate through its' strong antioxidant and anti-inflammatory activity reduces oxidative stress and inflammation, improves the antioxidant status of the patients and prevents the development or aggravation of diabetes complications. The antioxidant enzymes catalase, glutathione reductase, superoxide dismutase and glutathione peroxidase are increased, decreasing oxidative stress in diabetic patients, while they are not affected in healthy subjects. Moreover, pomegranate significantly down regulates the expression of the pro-inflammatory cytokines IL-1β, TNFα, IFN-γ, IL-6, IL-5,IL-8, IL-18, and IL-10, NF-κB, MPO while MMPs, COX and NO levels are also markedly down regulated.

Pomegranate treatment reduces markedly blood glucose levels, improves insulin sensitivity and increases insulin production and secretion in diabetic patients while there are no such changes in healthy subjects. Through its' potent antioxidant activity, pomegranate affects diabetic conditions by neutralizing the accumulated oxygen species asH_2O_2·OH and O_2·$^-$, reducing thus cellular oxidative stress. Through its' antioxidant activity, pomegranate is protecting also pancreas that is especially susceptible to the radical damage, is involved in regeneration of pancreatic islets, increases the number of β-cells and stimulates them resulting in subsequent insulin release. Pomegranate treatment increased the activities of antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase, improvedhepatic function decreasing the biomarkers alanine transaminase, aspartate transaminase and alkaline phosphatase, suppressed glucose intestinal absorption, reduced amylase enzyme activity decreasing thus glucose production, reduced malondialdehyde, inhibited markedly hemoglobin glycosylation that is associated with diabetes complications and suppressed inflammatory cytokines as TNF-αand NF-κB. Is referred that the glucose normalizing activity of pomegranate is due to PPAR-αand PPAR-γ upregulation as low PPAR values indicate diabetic conditions and obesity. Activation of PPAR-γis also mentioned to improve insulin receptor's sensitivity. Besides, pomegranate was found to increase the mRNA expressions of GLUT-4 and GLUT-2 contributing to glucose homeostasis. In both diabetic and healthy individuals there were changes in these mRNA expressions, yet thechanges were bigger in diabetic patients. Moreover, the diabetes-induced polydipsia and polyphagia were decreased.

Prolonged hyperglycemia may lead to cardiovascular diseases through various mechanisms, such as activation of protein kinase C, hexosamide and polyol pathways, production of glycation end-products. Furthermore, mitochondrial dysfunction due to hyperglycemia leads to ROS overproduction that causes cellular damage and complications. ROS damage RNA, DNA, proteins, lipids, modulate intracellular signaling pathways, lead to protein expression changes and to irreversible oxidative modifications. Pomegranate is a potent antioxidant. Treatment with pomegranate plant parts or isolated compounds upregulated the antioxidant enzymes glutathione reductase, glutathione peroxidase, superoxide dismutase and glutathione-S-transferase up to levels of healthy subjects, inhibited NO production and increased the activity of enzymes PON-1 and PON-2, which are both reducing oxidative stress and protect against atherosclerosis. Histological studies have shown a significant decrease on lipid peroxidation generic metric TBARS in pancreas and kidney tissue of diabetic and hypertensive patients. There was significant decrease intriglycerides, LDL, VLDL, total cholesterol, lipid peroxidation, observed only in diabetic patients and not in healthy subjects. Beneficial HDL was markedly increased only in diabetic patients with cardiovascular complications and not in healthy subjects. Pomegranate reduced both systolic and diastolic pressure through its' antioxidant activity and by reducing the activity of angiotensin converting enzyme that converts angiotensin I to angiotensin II which leads to high blood pressure. Moreover, itreversed various biochemical changes induced by diabetes and angiotensin II, decreased atherogenic modifications on LDL, decreased fat mass, obesity-related inflammation, inhibited circulatory lipid cardiac uptake, down regulated mRNA expression of ET1 that is associated with fibrosis in vascular cells and mRNA expression of NF-κB associated with atherosclerosis, myocardial ischemia, reperfusion injury and heart failure and also decreased generation of glycation end-products that lead to vascular derangement. Abnormal cardiac metabolism was improved due to PPAR-αactivation, while PPAR-γ activation contributes to the antilipidemic activity of pomegranate. Furthermore, sialic acid, a risk factor for cardiovascular diseases, diabetic nephropathy and retinopathy was decreased.

Prolonged hyperglycemia activates NOX4 enzyme that produces ROS, leading in oxidative stress and mitochondria dysfunctionality. Increased oxidative stress promotes the formation of vasoactive mediators that affect renal functions and decrease glomerular filtration rate. Pomegranate plant part extracts treatment prevented or inhibited by multiple mechanisms diabetic nephropathy. Pomegranate reversed the diabetes-induced inhibition of the mitochondrial enzyme MnSOD, which is involved in the regulation of antioxidant response, and regulated PI3K/AKT pathway in renal tissue, that is required for normal metabolism and its' imbalance leads to obesity and aggravation or type-2 diabetes inducement. The expression of NOX4 NADPH oxidase, related to oxidative stress and retinopathy was reduced. The levels and the activity of antioxidant enzymes were increased

only in patients while there was not any change on healthy subjects. Endothelial synthesis of NO, oxidative stress in kidneys, lipid peroxidation that damage kidneys and inflammation were significantly decreased. Furthermore, sialic acid, tissue malondialdehyde levels, glycated hemoglobin, serum creatinine, blood urea nitrogen, urine albumin, urine albumin to creatinine ratio were markedly decreased. Histopathological studies after treatment with pomegranate plant part extracts have shown improving effect on renal parenchyma in kidneys, healed glomerular tuft, healed interstitial epithelial cells and renal tubules, with the normally tubular basement membrane thickness and glomerular tuft structures to be restored.Nephritic tubules vacuolar degeneration, glomerular sclerosis, fibrogenic factors collagen IV and TGF-β and renal fibrosis as well, were markedly reduced.

Neuropathy constitutes damage of the nerves and alsoof the micro-vessels that are supplying the nerves, tissue. Pomegranate has neuroprotective properties and prevents or ameliorates neuropathy symptoms. Neuropathy leads to loss of the protective function of pain and discomfort that is alert for possible injuries. Prolonged hyperglycemia and oxidative stress are narrowing blood vessels and stiffening arteries, causing thus poor circulation that means poor oxygen and nutrients supply to the wound, making wound healing process prolonged. Open wounds combined to compromised immune system of diabetic patients are favorable substrates for various microorganisms and infections. Pomegranate constituents, through their antioxidant, antimicrobial, anti-inflammatory, neuroprotective, regenerative and immunomodulatory properties are enhancing wound healing process as at the same time reduce oxidative stress, inflammation, infection, bacterial count, inhibit biofilm formation, improve the clinical picture of vascular and nervous system, stimulate immune system, shows immunomodulatory activity and enhance re-epithelialization, collagen production and neovascularization process. Pomegranate treatment increased VEGF levels that when in low levels is associated to delayed wound healing, upregulated the expression of TGF-β1 cytokine, which plays important role in wound healing as it performs many cellular functions as control of cell differentiation, proliferation, growth and apoptosis and also increased EGF that stimulates cell growth and differentiation and accelerates wound healing. Moreover, NOS activity and NO production were downregulated decreasing thus oxidative stress and inflammation.As important is the wound healingagent that is used for wound treatment, that and even more important is the immune system's efficacy to perform healing processes. Pomegranate treatment increased immunoglobulin, immune marker that represents humoral immune response due to infections, increased the stimulation index values better than commercial immune stimulants in immunocompromised patients and inhibited M2 macrophage phenotype to M1, favoring the anti-inflammatory M2 phenotype.

Diabetes erectile dysfunction is a complication with multifactorial pathophysiology with the proposed mechanisms that lead to this complication to be oxidative stress, atherosclerosis, high levels of advanced glycation end-products, impaired nitric oxide synthesis and neuropathic damage. Pomegranate constituents possessing strong antioxidant, anti-inflammatory and anti-atherogenic propertiesreduce oxidative stress, improve antioxidant status of the patient, reduce inflammation, prevent or treat sufficiently atherosclerosis and reduce the production of advanced glycation end-products. Moreover, the studies showed that pomegranate improved significantly intracavernosal blood flow in individuals with atherosclerosis, yet the blood flow was not normalized up to the levels of healthy individuals. Pomegranate treatment protected from mitochondrial and endothelial structural integrity, reversed the molecular changes that are observed due to diabetes induced erectile dysfunction and increased MICP/MAP percentage in diabetic patients with erectile dysfunction, yet it did not affect MICP/MAP percentage of healthy subjects while ICP/MAP partially was also improved. Moreover, pomegranate increased the smooth muscle percentage but did not affect this value in healthy subjects.

Retinopathy occurs as a result of long-term damage of small blood vessels in retina due to prolonged hyperglycemia, oxidative stressand high levels of glycosylated hemoglobin. Pomegranate treatment increased the activity of antioxidant enzymes, reduced oxidative stress with the biomarker of cellular oxidative stress 8-OHdG and malondialdehyde to be significantly lower than in untreated subjects, decreased blood glucose levels, sialic acid, improved significantly retina cell layers, decreased the percentage of glycated hemoglobin and delayed cataract onset. Pomegranate treatment subjects had an almost normal capillary wall while in untreated subjects, abnormalities were observed. Hemorrhage spots and abnormal vascularization were also numerous less after pomegranate treatment.

Is important to be mentioned that pomegranate plant part extracts and isolated compounds gave comparable, and in many cases even better results in diabetes and its' complications treatment than commercial drugs tested, while pomegranate is not showing any side effects as it was found in safety tests that have been carried out. Moreover, pomegranate at the same time is benefiting all body systems that are affected due to diabetes, constituting thus a holistic treatment that is indeed improving the general health and quality of life of the patients. In contrast with pomegranate, commercial drugs show adverse effects which may be severe and end up in serious diseases inducement. These drugs are improving the values of specific biochemical parameters of a body system but at the same time are aggravating values of biochemical parameters of another body systems, causing injuries to body organs. For example, antidiabetic drugs that are reducing hyperglycemia which is one of the main reasons of cardiovascular disease development and thus there is a

need to be reduced, may cause as side effects cardiovascular problems or even heart failure. Antihyperlipidemic, antihypertensive and antiatherogenic drugs that are used in order to ameliorate cardiovascular complications, are harming the kidneys. Kidney damage is already a diabetes complication which is also through the cardiovascular medical treatment induced or aggravated. Canagliflozin that is used in order to slow diabetic kidney disease may through a side effect harm the kidneys which the therapy aims to protect. Losartan is used for high blood pressure and diabetic nephropathy treatment. As side effects are referred heart rhythm problems.

A very important information that has come up is that pomegranate in several cases was found to affect and change-improve biochemical parameters of diabetic patients, for example, LDL, triglycerides, atherosclerosis, blood glucose levels were reduced, HDL was increased, insulin sensitivity was improved, antioxidant enzymes levels and activities were increased ect, but at the same time these values were not affected in healthy individuals.

The antioxidant enzymes catalase, glutathione reductase, glutathione peroxidase, glutathione- S-transferase and superoxide dismutase levels and activities were increased in diabetic patients but not affected in healthy individuals. Reactive oxygen species play an important role in the immune system functions. Lack of ROS may reduce the body's ability to fight against microbes and lead to various disorders, while ROS overproduction and accumulation also leads to inflammation and several diseases. Acute inflammation is the first stage of a healing process, and its' function is to neutralize and destroy toxic agents and restore tissue homeostasis. Yet persistent, chronic inflammation leads to many diseases and disorders. Hyperglycemia is the main cause of other severe diseases onset, yet very low blood glucose levels lead to hypoglycemia that may cause coma and even death. Hydroxynonenalat high concentrations leads to pathogenesis and is also used as an oxidative stress marker, yet in very low concentrations too is mentioned to disturb cellular calcium homeostasis. Malondialdehyde is a toxic product of lipid peroxidation that is used as an oxidative stress biomarker, yet in low levels is referred to regulate glucose-stimulated insulin secretion in islets via Wnt signaling pathway and also to regulate gene expression. Low HDL levels are associated with increased incidence of myocardial infraction. High LDL levels protects from atherosclerosis and endothelial dysfunction, yet very high HDL levels may increase the risk of heart disease. High LDL increases the risk of coronary artery disease, peripheral artery disease and heart diseaseand at very low levels increases the danger of stroke and cancer. Within specific value levels, LDL has a beneficial role as it is binding to bacteria and endotoxins and is neutralizing them before they affect negatively the host. [67, 130]

A living being, as every complex functional system is an equilibrium of various individual equilibria of many factors and parameters that interact directly or indirectly each other leading to the functional unity. There is not good or bad factor or function. Everything can be beneficial or harmful and whether it will act one or other way depends on the dose or the extent to which it exists and consequently affects the system equilibrium and functionality. Disruption of any of these equilibria leads to disruption of body homeostasis that means disease.

The mechanisms of action of pomegranate phytochemicals in extracts or isolated is very important to be further researched, as they seem not only to participate in body's chemical reactions and change biochemical parameters even towards the desired direction (as reducing blood glucose levels, increasing insulin, reducing LDL ect), but seem also to act only when it is necessary for the homeostasis maintenance, changing these parameters' values only in diabetic patients, and whenever it is not necessary (in healthy individuals) because of already health conditions existing, no change-that would mean balance disturbance, is observed. This indicates a regulatory activity of pomegranate which is higher and more important than the simple medicinal activity that changes parameter values yet without that "sense" of balance towards health achievement and maintenance.

The absence of side effects of pomegranate extracts may probably be related to this regulatory capacity, this "sense" of equilibrium of biochemical parameters, organs and functions of the body, as well as to its immunomodulatory capacity too, as the presence of side effects (non-healthy condition), presupposes a disturbance of this equilibrium that does not seem to occur accordingto the biochemical parameters values when already are "correct" for health maintenance. Further experiments and investigation could possibly show if this hypothesis is true and may also provide useful information about the mechanisms of action of the extracts for diabetes and its complications treatment.

CONCLUSION

The multiple pathogenic disturbances that are present in diabetic patients indicate that a therapeutic agent with multiple therapeutic actions is required in order to maintain normoglycemia and provide a good quality of life. Pomegranate through its' antioxidant, anti-inflammatory, antimicrobial, neuroprotective, regenerative, cardioprotective, vasoprotective, wound healing, immune-stimulating properties, is benefiting body systems that are affected by diabetes, ameliorating oxidative stress, inflammation, cardiovascular disease, nephropathy, neuropathy, retinopathy, improving erectile function in diabetic patients, stimulating immune system and enhancingwound healing process without side effects, indicating that it could be considered as a possible alternative and safe therapeutic agent for diabetes and it's complications.

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