The Role of Olive Leaves and Pomegranate Peel Extracts on Diabetes Mellitus Induced in Male Rats

Fahmy G. Elsaid[1,2,*], Khalid M. Alsyaad[1], Fatimah A. Alqahtani[1]
[1]Biology department, Science College, King Khalid University, KSA
[2]Zoology department, Faculty of science, Mansoura University, Egypt
*Corresponding author:Fahmy G. Elsaid,email; elsaidfg@gmail.com

ABSTRACT

Background: Metabolic diseases such as diabetes mellitus (DM) were progressively widespread all over the world and the alteration of oxidative stress and antioxidants associated with DM was established.

Aim of the work: The natural products were used in many ancient cultures to overcome the DM, so pomegranate peel and olive leaves will be investigated in this work.

Materials and Methods: Adult male albino rats (*Sprague Dawley*), weighing 220-250 g, were used in this study. Animals were classified into five groups, five rats in each group. Control group: rats were served as normal healthy control and were received no treatment but only normal chow diet and water *ad libitum* for 4 weeks. Diabetic group: rats were intraperitoneal injected with STZ at dose of 40 mg / kg b. wt. for a single dose. Diabetic and olive leaves extract group: diabetic rats were orally administered with watery extract of olive leaves at dose of 250 mg / kg b. wt. for 4 weeks. Diabetic and pomegranate peel extract group: diabetic rats were orally administered with pomegranate peel at 250 mg / kg b. wt. for 4 weeks. Diabetic and mix of olive leaves & pomegranate peel group: diabetic rats were orally administered with pomegranate peel and olive leaves at 250 mg / kg b. wt. for 4 weeks.

Results: The oxidative stress markers such as lipid peroxidation and hydrogen peroxide were increased but there was a decrease in the antioxidants makers such as glutathione peroxidase, superoxide dismutase, glucose-6-phosphate dehydrogenase, reduced glutathione and total antioxidants capacity in the liver of diabetic rats. The expression of insulin receptor and glucose transporter 2 genes was inhibited in liver of diabetic rats. Watery extracts of olive leaves and pomegranate improved the biochemical and molecular changes associated with DM.

Conclusion: The results showed a hypoglycaemic, hypocholesterolemic and hypolipidemic action of olive leaves and pomegranate peel.

Keywords: Diabetes mellitus, antioxidants, oxidative stress, glucose transporters, insulin receptor, pomegranate, olive leaves.

INTRODUCTION

There are many metabolic disorders and autoimmune diseases involved in the destruction of the β-cells of the pancreas which leads to subsequent insulin deficiency and aberrations that bring about resistance to insulin action. Streptozotocin (STZ), glucosamine-nitrosourea compound, is an antibiotic mediator that has been broadly used for inducing type-1 diabetes in many animals by affecting disintegration and necrosis of pancreatic β-cells[1]. Insulin shortage can be produced in different experimental animals by injecting STZ. Hyperglycemia leads to dyslipidaemia as an increase of triglyceride, total cholesterol and LDL-C levels in sera and affect lipid metabolism. Hence, high plasma triglyceride levels and lowered HDL-cholesterol are corporate with diabetes.

Hyperglycaemia possibly increases the oxidative stress by elevating glucose auto-oxidation, protein glycation, free fatty acids and initiation of the polyol pathway. The role of antioxidants is essential in diabetes and the decrease of plasma antioxidants develops the impacts of diabetes[2]. Hence, the levels of free radical scavengers reduced throughout the progression of diabetes. Reduced glutathione (GSH) and other relative antioxidants such as glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities enhance the elimination of superoxide anion, hydrogen peroxide (H_2O_2) and hydroxyl radicals[3]. The decreased glutathione-S-transferase (GST) activity in the liver of STZ-induced diabetic rats was detected[4]. So, diabetes produces deviation of lipid profiles and increases the oxidative stress markers like lipid peroxidation (LPO), H_2O_2 and decreases the antioxidants profile in the liver. Damasceno[5] reported that STZ produced oxidative stress and depletion of antioxidant systems in both blood and tissues particularly, liver. Free radicals are quenched by efficient systems of antioxidant enzymes such as SOD, CAT, GST and GPx as well as the nutrient-derived antioxidants flavonoids, carotenoids, terpenoids and GSH.

The movement of glucose through the plasma membrane is occurred by a carrier-mediated diffusion transport system. Glucose transporter 2 (GLUT2), the primary hepatic liver transporter, is a low affinity, high capacity transporter expressed in high levels on the sinusoidal membranes of hepatocytes, the basolateral membrane of intestinal epithelial cells, renal proximal tubule cells, and pancreatic beta-cells[6]. Hepatocytes and pancreatic β-

Received:20 /12 /2017
Accepted: 30/12 /2017
DOI: **10.12816/0046605**

cells express one of these isoforms which is a GLUT2. GLUT2 is a transmembrane carrier protein that allows the passive glucose transport throughout the cell membrane. Glucose transporters used to uptake and release glucose and other certain sugars. Thus it plays a vital role in glucose homeostasis, and it is a part of the glucose sensor mechanism in beta pancreatic and hepatic cells. GLUT2 is concurrently synchronized by glucose and by a lipogenic factor. Glucose influences its own metabolism by controlling GLUT2 gene transcription, but it is involved in other aspects in managing of diet glucose. Thus, the increases in GLUT2 gene expression are important in glucose maintenance in diabetes. However, increased gene expression of GLUT2 has been widely stated in diabetic experimental animal models[7]. Insulin hormone plays a vital role in decreasing blood sugar concentration and it is secreted by pancreatic β-cells. The insulin receptor (INSR) exists as a heterotetrameric ($\alpha_2\beta_2$) structure with the α-subunits containing the insulin binding site and the β-subunits holding the transmembrane domain and tyrosine kinase activity[8]. Insulin binding to the extracellular portion of INSR leads to auto-phosphorylation of specific intracellular tyrosine residues. This may be impairs the hepatic glucose uptake that can lead to metabolic derangement in diabetic rats. Insulin binding to insulin receptors (INSR) activates tyrosine kinases that facilitate the phosphorylation of insulin receptor substrate-1[9]. The blockers of insulin receptor might be produced by altering the assembly of the INSR-binding domain of insulin. The variation of the domain is probable to change its feature that quiet able to bind into INSR but without triggering the function of the receptor. The binding between the modified INSR-binding domains will block insulin bind with its receptor [10].

Several classical medicinal herb extracts have been used for the manag-ement of diabetes mellitus. Many extracts displayed proper free radical scavenging activity. Olive (*Olea europaea L.*) leaves and fruits contain many clusters of ingredients, including polyphenols, flavonoids, flavones, oleuropein and carbohydrates[11]. These constituents have a substantial pharmacological action including antioxidant activity and little toxicity. Olive leaves have medical usages in the controlling of diabetes, and the anti-diabetic action of olive leaves or oleuropein has previously been proved in animal models[12]. Pomegranate (*Punica granatum Linn*) is belonging to the *Punicaceae* family. Nearly all parts of a pomegranate have vital activities and are used for the handling of several diseases. In Unani and Ayurvedic folk medicines practiced in India, pomegranate is accustomed to manage type 2 diabetes mellitus[13]. Different parts of pomegranate fruit have valuable components, the functional and medicinal actions such as antioxidant activities has been described. Also pomegranate peel extract have high antioxidant activity in *vitro*, in corresponding to the high concentration of polyphenols[14]. Upon of these, the study aimed to investigate the role of olive leaves and pomegranate peel extracts in the biochemical and molecular changes associated with induced diabetes mellitus in rats.

MATERIALS AND METHODS

Experimental Animals

Adult male albino rats (*Sprague Dawley*), weighing 220-250 g, were obtained from the animal house centre, King Khalid University, Saudi Arabia, were used in the present study. The local ethical committee approved the design of the experiment and the protocol conforms to the guidelines of the National Institutes of Health (NIH). All measures were taken to minimize the number of rats used and their suffering. Animals were kept in standard polyethylene cages under good ventilation in a room with a 12:12 h light/dark cycle, control temperature (22±1ºC), relative humidity (40-60%) and received a balanced laboratory chow diet and water was added *ad libitum* throughout the experimental period.

Induction of Diabetes:

Diabetes was induced by intraperitoneal injection of streptozotocin (STZ) (Sigma, St Louis, MO, USA) into the abdomen cavity at a dose of 40 mg / kg body weight. STZ was dissolved in 0.1 M cold sodium citrate buffer, pH 4.5[15]. The animals were considered as diabetic, if their blood glucose values were above 280-300 mg / dl serum on the 72 hours after STZ injection. The treatment was started on the 4th day after STZ injection and this was considered as 1st day of treatment. The treatment was continued for four weeks.

Natural Product Extracts and Mode of Treatment:

The leaves of 100 g air dried olive (*Olea europaea, Family: Oleaceae*) and 100 g air dried peel of pomegranate (*Punica granatum L. Family: Punicaceae*) were extracted by adding 1000 ml of distilled water and boiled at 80ºC for 30 min. The extracts were filtered by filter paper and the filtrates were evaporated using a rotary evaporator under reduced pressure to dryness [16]. Then 5 g of the dried extracts for each was dissolved in 100 ml of distilled water before the administration and each aqueous extracts was orally administered by stomach tube at a dose of (250 mg / kg b. wt.) to for 4 weeks.

Experimental Protocol:

Experimental animals were randomly divided into five groups, five rats in each group. Control group: rats were served as normal healthy

control and were received no treatment but only normal chow diet and water *ad libitum* for 4 weeks. Diabetic group: rats were intraperitoneal injected with STZ at dose of 40 mg / kg b. wt. for a single dose. Diabetic and olive leaves extract group: rats were injected with STZ at dose of 40 mg / kg b. wt. and orally administered with watery extract of olive leaves at dose of 250 mg / kg b. wt. for 4 weeks. Diabetic and pomegranate peel extract group: rats were injected with STZ at dose of 40 mg / kg b. wt. and orally administered with pomegranate peel at 250 mg/kg b. wt. for 4 weeks. Diabetic and mix of olive leaves & pomegranate peel group: rats were injected with STZ at dose of 40 mg / kg b. wt. and orally administered with pomegranate peel and olive leaves at 250 mg / kg b. wt. for 4 weeks.

Sampling and Tissue Extraction:

At the end of the treatment period, overnight fasted rats were sacrificed. Blood was collected in a clean centrifuge tubes. These tubes were centrifuged for 15 min at 3000 rpm. Non-haemolized sera were separated and were labelled and kept at -20ºC for later biochemical analysis. The animals were dissected; specimens from liver tissues were collected from each animal and weighed. Samples of liver tissues were homogenized in 10% (phosphate buffered saline (PBS) pH: 7.4) for biochemical analysis. Samples of investigated tissues were frozen in dry ice and stored at -80℃ for later molecular study.

Biochemical Study:

Determination of total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) level was detected according to Richmond [17] and Gordon[18] respectively. Determination of total lipids (TL) level was detected by according to Zollner[19]. The determination of lipid peroxidation product by thiobar-bituric acid reactive substances (TBARS) and hydrogen peroxide (H_2O_2) was estimated by Buege[20] and Fossati[21] respectively. Glutathione peroxidase (GSH-Px) activity was determined by the method of Habig[22]. Also, reduced glutathione (GSH) content was estimated according to the method of Beutler[23]. Determination of superoxide dismutase (SOD) activity was estimated according to Niskikimi[24]. Glucose -6- phosphate (G-6-PhD) activity and antioxidant capacity (TAC) was determined according to Beutler[25] and Koracevic[26] respectively.

Molecular Study:

Total RNA Extraction:

RNA isolation from tissues was carried out using RNeasy kit according to manufacturer's instructions (QIAGEN, Germany).

Complementary DNA (cDNA) Preparation:

First-strand cDNA was synthesized using Moloney Murine Leukemia virus reverse transcriptase (Fermentas, USA). Reverse transcription reactions were performed using oligo dT primer. Each 25 µl reaction mixture containing 2.5 µl of 5x buffer with $MgCl_2$, 2.5 µl of 2.5 mM dNTPs, 1 µg of primer, 2 µg RNA and 200 U reverse transcriptase enzyme. RT-PCR amplification was performed in a thermal cycler (Eppendorf) programmed at 42°C for 1 hr and 72°C for 10 min. cDNA was then stored at -20°C until used.

Polymerase Chain Reaction (PCR) Amplification of the INSR and GLUT2 Gene Using Specific Primers:

One µl randomly primed cDNA (from total RNA of healthy, infected tissues) were added to 2.5 µl Taq polymerase buffer 10x (Promega, Madison, USA) containing a final concentration of 1 mM $MgCl_2$, 0.2 Mm dNTPs, 0.2 mM each specific primer and 0.2 µL Taq polymerase (5 U / µL) in a final reaction volume of 25 µl. PCR conditions were; Initial denaturation at 95°C for 5 min, followed by 35 cycles at 52°C for 1 min, 55°C for 1 min and 72°C for 1 min. Final extension at 72°C was done for 10 min. Amplification products were electrophoresed in 1% agarose gels run in 0.5x TBE buffer.

GLUT 2	**F:5\'** GTCCAGAAAG CCCCAGATACC	**R:** 3\'GTGACATCCTCA GTTCCTCTTAG
INSR	**F:5\'** GCCTCTACAAC CTGATGAAC	**R:** 3\'ACAGATGTCTCC ACACTCC

Agarose Gel Electrophoresis of PCR Products

1% agarose was prepared by using 1x Tris borate EDTA (TBE) buffer containing 0.5 µg / mL ethidium bromide and poured onto a gel tray. The 0.25 µg / µL of DNA Ladder (100 bp) was loaded into the first well. 10 µL of each PCR product was mixed with the 4 µL 6x loading dye and 5 µL of it was loaded into each well. The gel was run for 60 min and then visualized and photographed using ultraviolet transilluminator gel documentation system[27].

Statistical Analysis

The biochemical data were expressed as mean±SD and statistical and correlation analyses were performed using the one way ANOVA followed by a post-hoc least significant difference (LSD) test. *P*-values <0.05 were considered as statistically significant. Statistical analyses were performed with the statistical package for the social sciences for Windows (SPSS, version 16.0, Chicago, IL, USA).

RESULTS

Biochemical results:

Table (1): Serum glucose and lipid profile in different animal groups

Parameters	Control	Di	Di +Olive	Di + Pom	Di+Mix
Glucose (*mg/dl*)	84.4±1.7	302±6.9***	201±2.5^{***+++}	213±5.1^{***+++}	192±3.2^{***+++}
TC (*mg/dl*)	169.4±1.4	217.5±1.70***	170.4±1.7^{+++}	180.7±1.4^{***+++}	197.37±2.0^{***+++}
HDL-C (*mg/dl*)	38.1±0.3	31.5±0.7***	41.3±0.4^{***+++}	38.2±0.4^{+++}	34.1±0.5^{***+++}
LDL-C (*mg/dl*)	131.3±1.3	186.9±1.6***	129.3±1.8^{+++}	142.4±1.5^{***+++}	163.6±1.7^{***+++}
Liver TL (*mg/dl*)	337.2±15.9	409.4±6.9***	301.6±5.9^{**+++}	362.6±2.3^{*+++}	360.1±2.7^{+++}

Values were expressed as means ±SE of five animals in each group.
$^{**=}$ P<0.01, ***=P<0.001(very high significant), when all groups compared with control group.

$^{+++}$ = P<0.001(significant), when Di+ Olive, Di + Pom groups and Di+ Mix compared with diabetic group.

The results in table (1) indicated that, serum glucose and lipid profile markers concentration, except HDL-C, was significantly increased in diabetes (Di), diabetes + olive (Di + Olive), diabetes + pomegranate (Di + Pom) and diabetes + mix (Di + Mix) groups as compared to the control group. In addition, glucose and lipid profile markers level, except HDL-C, was decreased significantly in Di + Olive, Di + Pom and Di + Mix groups as compared to the diabetic group.

Table (2): Liver oxidative stress markers in different animal groups

Parameters	Control	Di	Di +Olive	Di + Pom	Di+Mix
TBARS(*nM/g*)	163.2±3.1	373.9±7.8***	278.9±3.6^{***+++}	283.3±7.8^{***+++}	315.1±4.1^{***+++}
H_2O_2 (mM/g)	48.2±2.5	91.2±1.9***	66. 1±2.9^{***+++}	67.6±4.3^{***+++}	68.8±3.9^{***+++}

Values were expressed as means ±SE of five animals in each group.
***=P<0.001(very high significant), when all groups compared with control group.
$^{+++}$ = P<0.001(significant), when Di+ Olive, Di + Pom groups and Di+ Mix compared with diabetic group.

The results in table (2) showed that liver lipid peroxidation and H_2O_2 concentrations were significantly increased in diabetes (Di), diabetes + olive (Di + Olive), diabetes + pomegranate (Di + Pom) and diabetes + mix (Di + Mix) groups as compared to the control group. In addition, lipid peroxidation and H_2O_2 levels were decreased significantly in Di + Olive, Di + Pom and Di + Mix groups as compared to the diabetic group.

Table (3): Liver antioxidants markers in different animal groups

Parameters	Control	Di	Di +Olive	Di + Pom	Di+Mix
GSH-Px *(U/g)*	17.3±0.6	12.2±0. 4***	17.6±0.2^{+++}	14.6±0.2^{***++}	13. 4±0.8***
GSH (*mg/g*)	54.0±1. 39	42.07±0. 83***	63.7±1.77^{***+++}	53.7±1.14^{+++}	53.1±0.9^{+++}
SOD (µmol/*g*)	17.1±0.16	11.07±0.19^{***+++}	13.25±0.28^{***+++}	14.7±0.32^{***+++}	14.4±0.2^{***+++}
G-6-PhD (m*U/g)*	8.8±65.9	6.9±22.9***	6.9±0.9***	6.6±30.1^{***+++}	8.6±35.0^{*+++}
TAC (*mM/g*)	80.1±2.2	65.1±1.1***	72.6±1.9^{**++}	81.9±1.9^{+++}	72.0±1.9^{**++}

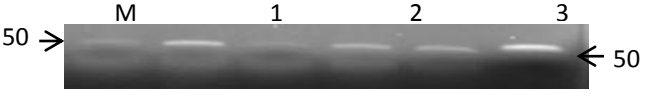
Values were expressed as means ±SE of five animals in each group.
$^{*=}$P <0.05, $^{**=}$ P<0.01, ***=P<0.001(very high significant), when all groups compared with control group.
$^{++=}$ P<0.01, $^{+++}$ = P<0.001(significant), when Di+ Olive, Di + Pom groups and Di+ Mix compared with diabetic group.

The results in table (3) showed that liver GPx, SOD and G-6-Ph-D activity and the concentration of GSH and TAC were significantly decreased in diabetes (Di), diabetes + olive (Di + Olive), diabetes + pomegranate (Di + Pom) and diabetes + mix (Di + Mix) groups as compared to the control group. In addition, liver GPx, SOD and G-6-Ph-D activity and the concentration of GSH and TAC were decreased significantly in Di + Olive, Di + Pom and Di + Mix groups as compared to the diabetic group.

Molecular results:

Insulin receptor (INSR) gene expression

When the recombinant genomic c-DNA of the liver was subjected to PCR amplification of the insulin receptor (INSR) gene, results presented in figure (1) showed that amplicone with approximately 500bp was observed with all the examined samples. The band intensity decreased in diabetic group (lane 2). The expression of INSR gene was increased in the all treated groups and it was more obviously in diabetes + mixture one (lane 5).



(Fig. 1): Liver insulin receptor gene (INSR) amplification using specific primer

Lane M: marker 100bp step ladder (Promega), lane 1: control group, lane 2: Diabetes group, lane 3:

diabetes + Olive group, lane 4: diabetes + Pomegranate group, lane 5: diabetes + Mix group.

Glucose transporter-2 (GLUT2) gene expression

In the figure (2), the amplicone with approximately 220 bp was observed with all the examined samples and the band intensity of GLUT2 gene decreased in diabetes and diabetes + olive groups and the intensity of bands increased in diabetes + pomegranate (lane 4) band diabetes + mix group (lane 5).

(Fig. 2): Liver glucose transporter 2 (GLUT2) gene amplification using specific primer

Lane M: marker 100bp step ladder (Promega), lane 1: control group, lane 2: Diabetes group, lane 3: diabetes + Olive group, lane 4: diabetess + Pomegranate group, lane 5: diabetes + Mix group.

DISCUSSION

Diabetes mellitus (DM) is a metabolic disorder, characterized by hyperglycemia due to low consumption of glucose in the liver cells. Also, hypercholesterolemia and hyperlipidemia are recognized complications of DM arising from increased mobilization of free fatty acids from the peripheral deposits in insulin deficiency and characterized by elevation of cholesterol, triacylglycerol and LDL-cholesterol levels but decreasing levels of HDL-cholesterol[28]. These results are in agreement with the present results that showed increase of lipid profile markers and decrease in HDL-C concentration when compared with control. Moreover, a study of Bagri[29] found that the administration of pomegranate aqueous extract reduce blood glucose, TC and LDL-C in compression with a diabetic group induced by STZ. As STZ induced the oxidative stress in pancreas so it plays a critical role in the induction of diabetes. Hyper-cholesterolemia and hyperlipidemia due to the hyperglycemia that induced by STZ may be aggregated the oxidative stress in liver tissues. The increase of lipid peroxidation (LPO) and H_2O_2 levels in diabetic group may be explain the oxidative stress status in liver of diabetic rats. Olive leaves extracts are rich in phenolic constituents and oleuropein is the most prominent phenolic compound[30]. The antioxidant potential of olive leaf extracts[31] has further verified the inhibitory potential of dietary olive leaves on LPO. Moreover, flavonoids content in olive leaves extract may account for the overall radical scavenging activity potential[32]. On the other hand, the antioxidants activity in liver of diabetic rats was highly inhibited as represented by the decreased in the activity of GPx, SOD and G-6-Ph-D, also the decrease in GSH concentration and total antioxidants capacity in liver of diabetic rats induced by STZ. In diabetic patients, low antioxidant enzymes levels or excess free radical production leads to defective ROS removal. These preceding changes enhance the physiological disorders associated with diabetes in rats after administration with STZ. These results suggest that olive leaves extract induces a protective effect by inducing the scavenging of ROS, thereby reducing diabetes-associated complications, which is in accordance with the results of previous studies. As, olive leaves and pomegranate peel extracts have antioxidants and antiradical scavenging properties31, so the improvement of the imbalance status of oxidants/antioxidants in liver of diabetic and treated group when compared with diabetic group was explained.

Insulin accelerates glucose clearance from the plasma by stimulating glucose uptake into peripheral tissues as well as through the inhibition of hepatic glucose assembly. Hepatic glucose transporter (GLUT2) facilitates glucose transport across the hepatocyte plasma membrane in both directions under insulin regulation[33]. It also enables the passage of dietary sugars, glucose, fructose, and galactose towards the blood stream. Interesting, olive leaves extract properties show a significantly increase of insulin receptor (INSRS) genes expression[34]. There are few studies concerned with the effect of olive leaves extract and the expression of GLUT2 gene. But the expression of GLUT2 gene in the liver are associated with olive oil enrichment in different experimental models was registered. A previous study demonstrated a defect in differentiation of adipocytes lacking INSRS, which could be restored by insulin receptor substrate-1 gene activation. There is a little publication concerned with the action of olive leaves on INSR. Also, there is a few publication focused directly with the role of pomegranate with the GLUT2 in diabetic rats. However, Kim[35] suggested that pomegranate could play a role in controlling the dietary glucose absorption at the intestinal tract by decreasing Na^+-dependent glucose transporter (SGLT1) expression, and may contribute to blood glucose homeostasis in the diabetic condition. However, the expression of brush border enzymes and GLUT such as SGLT1 in Caco-2 cells was down regulated in a dose dependent manner[35]. Li[36] showed that in white adipose tissue, oleanolic acid derived from pomegranate, enhanced mRNA expression of the genes encoding INSR and phosphatidylinositol 3-kinase. At the protein level, oleanolic acid upregulated total INSRS expression[37]. This may be explained the hypercholesterolemia and hyperlipidemia and the decrease insulin-stimulated

glucose uptake in the whole body, mainly in the liver which impairs insulin mediated suppression of endogenous glucose production[38].

Eisenberg[39] reported that because both INSR and GLUT2 are internalized from the hepatocyte plasma membrane upon insulin binding, the possibility exists that the two transmembrane proteins are physically linked and regulate hepatic glucose production as a complex. Moreover they indicated that the two transmembrane proteins are physically linked on the hepatocyte membrane. This suggests a mechanism which obligates a change in glucose transport after insulin binding to INSR, independent of INSR-mediated changes in intracellular glucose or glycogen metabolism. Both INSR and GLUT2 are removed from the plasma membrane and internalized to endosomal compartments upon insulin binding. The internalization serves to remove the glucose transporter from the hepatocyte plasma membrane and slow the release of hepatic glucose during hyperglycemic states. It is interesting to note that not all of the GLUT2 is internalized in the normal hepatic response to the post-prandial state. It has been shown that insulin exerts several mechanisms for controlling hepatic glucose production. Hepatic protein function is regulated at the transcriptional level for the hepatic enzymes of glucose metabolism, and GLUT2 expression is suppressed by elevated insulin and glucose levels[40]. The expression of INSR and GLUT2 genes was decreased in diabetic rats. The excessive ROS may lead to defective in insulin mRNA expression, decreased insulin content and reduced insulin secretion in islets of Langerhans, and consequent necrosis or apoptosis of islets. These may also be explained the decrement of intensity of bands in the INSR and GLUT2 genes in diabetic rats in liver tissues as a result of imbalance occurred in antioxidants/oxidants status. INSR and GLUT2 have hypoglycemic action by activating glucose uptake into peripheral tissues and by the suppression of hepatic glucose leakage. Move to the end, this study recommend both olive leaves and pomegranate peel extracts as supplementary stuff to alleviate hyperglycemia in the patients suffering from diabetes. This hypoglycemic action of olive leaves and pomegranate peel may be due to their antioxidant properties.

CONCLUSION

Oxidative stress was induced in the liver of diabetic rats represented by increase of lipid peroxidation and hydrogen peroxide. The decrease in the antioxidants activity in the liver of diabetic rats represented by a decrease in glutathione peroxidase, reduced glutathione, glutathione-S-transferase, superoxide dismutase, glucose-6-phosphate dehydrogenase, total antioxidant capacity. Also, gene expression of glucose transporter 2 and insulin receptor genes was decreased in diabetic rats. However, the therapeutic administration of aqueous olive and pomegranate peel extracts was capable of affording protection or modulating the onset and severity of diabetes. The effect of olive leaves and pomegranate peel extracts were beneficial in ameliorating the severity of some risk factors like the marked improvement in the antioxidants system and the inhibition of the high levels of oxidative stress resulted from induced diabetes. This action may be due to their phenolic constituents of of olive leaves and pomegranate peel. Moreover, the expression of glucose transporter 2 and insulin receptor genes has been increased on the cell surface of hepatocytes that significantly activated the uptake of glucose in the peripheral tissues and prevent the efflux of glucose from the liver. The results also revealed that olive leaves and pomegranate peel extracts have the ability to overcome the deflection in the antioxidant/oxidants system, lipid profile and molecular changes in liver of diabetic rats.

ACKNOWLEDGEMENT

My acknowledgement is sincerely extended to King Abdulaziz City for Science and Technology (KACST) with my deepest and faithful thanks from your student research number AT-34-239.

REFERENCES

1. **Merzouk H, Madani S, Chabane D *et al.* (2000):** Time course of changes in serum glucose, insulin, lipids and tissue lipase activities in macrosomic offspring of rats with streptozotocin-induced diabetes. Clin. Sci., 98: 21–30.
2. **Facchini F S, Humphreys, M H, Do Nascimento C, Abbasi A and Reaven GM (2000):** Relation between insulin resistance and plasma concentrations of lipid hydroperoxides, carotenoids, and tocopherols. Amer J Clinic Nutr., (72): 776-779.
3. **Soto C, Recoba R, Barron H, Alvarez C, Favari L (2003**): Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Comp Biochem Physiol., 136: 205–212.
4. **Dogan Abdulahad A, Ismail Celik B N and Mehmet S K (2015**): Antidiabetic properties of lyophilized extract of acorn (Quercus brantii Lindl.) on experimentally STZ-induced diabetic rats. J Ethnopharmacol., 176: 243–251.
5. **Damasceno D C, Volpato G T, Paranhos-Calderon M, Cunha- Rudge M V (2002):** Oxidative stress and diabetes in pregnant rats. Anim. Reprod. Sci., 72:235–244.
6. **Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Okazaki, H *et al*. (2004):** Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice. Diabetes, 53: 560–569.
8. **Briant L, Salehi A, Vergari E *et al.* (2016):** Glucagon secretion from pancreatic alpha-cells. Upsala J medical sci., 121: 113-119.
9. **Lee J, Miyazaki M, Romeo G R, Shoelson S E (2014):** Insulin receptor activation with transmembrane domain ligands, J. Biol. Chem., 289: 19769–19777.

10. **Galuh W, Permatasari Didik H, Utomo W (2016):** Designing peptide inhibitor of insulin receptor to induce diabetes mellitus type 2 in animal model Mus musculus, Comp Biol Chem., 64: 107–112.
11. **Ammar S, Zribi A, Ben Mansour A, Ayadi M, Abdelhedi R *et al.* (2014**): Effect of processing systems on the quality and stability of Chemlali olive oils. J Oleo Sci., 63(4): 311–323**.**
12. **Eidi A, Eidi M, Darzi R (2009):** Antidiabetic effect of *Olea europaea* L. in normal and diabetic rats. *Phytother Res*., 23: 347–350.
13. **Duman A D, Ozgen M, Dayisoylu K S, Erbil N, Durgac C (2009):** Antimicrobial activity of six pomegranate (Punica granatum L.) varieties and their relation to some of their pomological and phytonutrient characteristics. Molecules, 14: 1808-1817.
14. **Li Y, Guo C, Yang J, Wei J, Xu J *et al.* (2006):** Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem., 96:254-260.
15. **Elsaid, F G (2008):** Diabetes Mellitus and Some Egyptian's Volatile Oils. The Egyptian Journal of Hospital Medicine, 30: 39– 50.
16. **Verma P R, Deshpande SA, Kamtham YN, Vaidya LB (2012):** Hypolipidemic and antihyperlipidemic effects from an aqueous extract of Pachyptera hymenaea (DC.) leaves in rats. Food Chem., 132: 1251–1257.
17. **Richmond W (1973):** Preparation and properties of a bacterial cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem., 19: 1350.
18. **Gordon T, Castelli W P, Hjortland, MC, Kannel WB, Dawber TR (1977):** High density lipoprotein as a protective factor against coro-nary heart disease: The Framingham Study. Am J Med., 62:707-714.
19. **Zollner N and Kirsch K (1962):** Uber die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natiirlichen Lipoiden (allen bekannten Plasmali-poiden) gemeinsamen Sulphophosphovanillin Reaktion. Z ges exp Med., 135: 545-561.
20. **Buege J A and Aust SD (1978):** Microsomal lipid peroxidation. Methods Enzymol., 52, 302–310.
21. **Fossati P and Principe L (1982):** Serum triglycerides determined colorimeterrrically with an enzyme that produces hydrogen peroxide. Clin Chem., 28: 2077-2080.
22. **Habig W H, Pabst M J and Jakoby W B (1974):** Glutathione *S*-transferases: The first enzymatic step in mercapturic acid formation. J Biol Chem., 249**:**7130–7139.
23. **Beutler E *et al.* (1963):** Improved method for the determination of blood glutathione. J Lab Clin Med., 61: 882-890.
24. **Nishikimi M, Appaji N and Yagi K (1972):** The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun., 46: 849-854.
25. **Beutler *et al*. (1977):** International comittee for standardisation in Haematology : Recommended Methods for Red Cell Enzyme Analysis. J Haematol., 35:331-340.
26. **Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V (2001):** Method for the measurement of antioxidant activity in human fluids. J Clin Patho., 54: 356-361.
27. **Sambrook J, Fritsch E F and Maniatis T (1989):** Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
28. **Prabhakar P K and Doble M (2011):** Mechanism of action of natural products used in the treatment of diabetes mellitus. Chin. J. Integr. Med., 17:563.
29. **Bagri P, Ali M, Aeri V, Bhowmik M and Sultana S (2009):** Antidiabetic effect of *Punica granatum* flowers: effect of hyperlipidemia, pancreatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. Food Chem. Toxicol., 47, 1: 50-54.
30. **Bouaziz M, Fki I, Jemai H, Ayadi M and Sayadi S (2008):** Effect of storage on refined and husk olive oils composition: stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chem., 108: 253-262.
31. **Botsoglou E, Govaris A, Moulas A and Botsoglou N (2010):** Oxidative stability and microbial growth of turkey breast fillets during refrigerated storage as influenced by feed supplementation with olive leaves, oregano and/or α-tocopheryl acetate. British Poultry Sci., 51: 760–768.
32. **Taamalli A, Arráez-Román D, Ibañez E, Zarrouk M, Segura-Carretero A and Fernández-Gutiérrez A (2012):** Optimization of microwave-assisted extraction for the characterization of olive leaf phenolic compounds by using HPLC-ESI-TOF. doi: 10.1021/jf204233u
33. **Thorens B, Wu Y J, Leahy J L and Weir G C (2015):** The loss of GLUT2 expression by glucose-unresponsive beta cells of db/db mice is reversible and is induced by the diabetic environment. *J Clin Invest*., 90: 77–85.
34. **Okamoto Y, Tanaka S and Haga Y (2002):** Enhanced GLUT2 gene expression in an oleic acid-induced in vitro fatty liver model. Hepatology Research, 23: 138–144.
35. **Kim J J H, Xiao Y, Tan Z Z, Wang J, Paul Seale *et al.* (2009):** The effects and mechanism of saponins of Panax notoginseng on glucose metabolism in 3T3-L1 cells. Am. J. Chin. Med., 37: 1179–1189.
36. **Mahraoui L A, Rodolosse A, Barba E, Dussaulx A and Zweibaum M *et al.* (1994):** Presence and differential expression of SGLT1, GLUT1, GLUT2, GLUT3 and GLUT5 hexose- transporter mRNAs in Caco-2 cell clones in relation to cell growth and glucose consumption. Biochem. J., 298: 629–633.
37. **Li Y, Qi Y, Huang T H, Yamahara J and Roufogalis B (2008):** Pomegranate flower: a unique traditional antidiabeteic medicine with dual PPAR-alpha/-gamma activator properties. Diabetes Obes Metab., 10: 10-17.
38. **Xu K Z, Chenchen Z, Moon S K, Johji Y and Yuhao L (2009):** Pomegranate flower ameliorates fatty liver in an animal model of type 2. Antioxid Redox Signal, 21,(11): 1557–1570.
39. **Nakahara I, Matsuhisa M, Shiba Y, Akio A and Nakatani Y *et al.* (2004):** Acute elevation of free fatty acids impairs hepatic glucose uptake in conscious rats. Diabetes Research and Clinical. Practice, 66: 109-118.
40. **Park S Y, Kim M H, Ahn J H and Lee S J *et al.* (2014):** The stimulatory effect of essential fatty acids on glucose uptake involves both Akt and AMPK activation in C2C12 skeletal muscle cells. Korean J. Physiol. Pharmacol., 18 (3): 255–261.