Upregulation of GLUT4 and PI3K, and downregulation of GSK3 mediate the anti-hyperglycemic effects of proanthocyanidins

NAHLA E. EL-ASHMAWY, EMAN G. KHEDR, NEHAL H. ALFEKY and AMERA O. IBRAHIM

Department of Biochemistry, Faculty of Pharmacy, Tanta University, Tanta, Gharbia 31111, Egypt

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Abstract. Diabetes mellitus is the most common chronic metabolic disorder worldwide. The present study was designed to investigate the potential role of cinnamon bark extract oligomeric proanthocyanidins (OPCs) in controlling streptozotocin (STZ)-induced hyperglycemia and to clarify the underlying molecular mechanisms underlying its effects. For this purpose, 60 male rats were equally divided into six groups as follows: The normal control group; OPC control group (non-diabetic rats treated with OPC at 300 mg/kg orally for 21 days); the untreated diabetic control group; the wortmannin control group [diabetic rats treated with wortmannin at 1 mg/kg, intraperitoneal (i.p.) on the final day of the experiment]; the OPC diabetic group (diabetic rats treated with OPC at 300 mg/kg orally for 21 days); and the OPC diabetic + wortmannin co-treated group (diabetic rats treated with OPC at 300 mg/kg/day for 21 consecutive days and then 24 h after the final OPC dose treated with a single wortmannin injection at 1 mg/kg, i.p.). The results indicated that OPC ameliorated the diabetic state, as evidenced by a significant decrease in serum glucose levels, and a significant increase in the levels of insulin, amylin, insulin receptor phosphorylation, glycogen and glucose transporter-4 translocation; it also improved the lipid profile in STZ-diabetic rats. On the whole, the findings of the present study provide biochemical evidence that OPC treatment is effective as an anti-diabetic and anti-hyperlipidemic agent by enhancing glucose uptake through the activation of insulin receptor kinase activity and the PI3K/Akt pathway.

Correspondence to: Dr Amera O. Ibrahim, Department of Biochemistry, Faculty of Pharmacy, Tanta University, El-Geish Street, Tanta, Gharbia 31111, Egypt
E-mail: amera.ibrahim@pharm.tanta.edu.eg

Abbreviations: GLUT4, glucose transporter4; GSK-3, glycogen synthase kinase-3; HDL-C, high-density lipoprotein-cholesterol; IAPP, islet amyloid polypeptide; LDL-C, low-density lipoprotein-cholesterol; OPCs, oligomeric proanthocyanidins; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; STZ, streptozotocin; T2D, type 2 diabetes mellitus; VLDL, very low-density lipoprotein-cholesterol

Key words: diabetes mellitus, proanthocyanidin, GLUT4, PI3K, antihyperlipidemic, cinnamon

Introduction

Diabetes mellitus is one of the largest epidemics worldwide that is recognized and classified as a cluster of heterogeneous metabolic disorders characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both. Diabetes mellitus occurs due to the destruction of pancreatic β-cells with consequent insulin deficiency by autoimmune disease or due to abnormalities that lead to a resistance to insulin actions (1).

Metabolic abnormalities in carbohydrates, fats and proteins in diabetes occur due to deficient insulin action at target tissues. It has been demonstrated that oxygen free radicals are produced due to hyperglycemia and can cause various oxidative stress-induced complications of diabetes, such as nephropathy, retinopathy and neuropathy (2). The accumulation of lipid peroxidation products termed as advanced glycosylation end products and damaged DNA eventually leads to the development of pathological diabetic complications (3).

Amylin, or islet amyloid polypeptide (IAPP), is an endocrine peptide hormone co-localized, co-secreted and co-packaged along with insulin by pancreatic β-cells that is associated with the type 2 diabetes mellitus (T2D) disease progression (4). Amylin is co-secreted with insulin in response to caloric intake. It plays a crucial role in maintaining glucose homeostasis by suppressing glucagon release, delaying the gastric emptying rate and stimulating the satiety center in the brain to limit caloric intake. Amylin serum concentrations in patients with type 1 diabetes were at a lower baseline with the absence of amylin response to caloric intake. At the same time, patients with T2D requiring insulin also have a diminished amylin response to caloric intake, possibly related to the degree of β-cell impairment (5).

It is well established that insulin enhances glucose uptake by promoting the translocation of glucose transporter 4 (GLUT4), the major insulin-regulated glucose transporter in skeletal muscle and adipose tissue, from the intracellular storage vesicles to the plasma membrane. Due to the absence or insufficient sensitivity to insulin in diabetic patients, GLUT4 expression is decreased, leading to resistance to insulin-stimulated glucose transport that profoundly contributes to disease pathophysiology. Phosphatidylinositol 3-kinase (PI3K) is a

crucial component of the insulin-signaling cascade, essential for the metabolic effects of insulin on glucose transport and GLUT4 translocation (6,7).

Glycogen synthase kinase-3 (GSK-3) regulates a number of metabolic and signaling proteins. Activated GSK-3 inactivates the glycogen synthase enzyme, which is responsible for converting glucose to glycogen for storage. Insulin can bind to GSK-3 receptors, relieve this inhibition and activate the PI3K/Akt pathway. However, the overexpression of GSK-3 results in impaired glucose tolerance and decreased glycogen synthase activity, and consequently, in glycogen synthesis. Furthermore, it has been reported that GSK-3 overexpression attenuates insulin signaling, which further supports the role of GSK-3 in diabetes (8,9).

Several limitations have been reported regarding the use of conventional oral hypoglycemic drugs in the treatment of diabetes due to adverse effects and high rates of secondary failure. These adverse effects have led diabetic patients to use natural products as an alternative source of anti-diabetic agents with a degree of efficiency without side-effects (10). Cinnamon is one of the popular spices containing several potent antioxidant polyphenolic compounds traditionally used in the treatment of various chronic diseases, such as cardiovascular diseases and diabetes (11).

Oligomeric proanthocyanidins (OPCs) are oligomeric flavonoids found in the aqueous extract of cinnamon that are responsible for the majority of the antioxidant properties of cinnamon; they have been proven to regulate insulin signaling and the expression of blood sugar transportation genes in adipocytes (12,13). There is increasing evidence to indicate that OPCs are effective both as a prophylactic or dietary treatment for certain fatal diseases such as cancer, cardiovascular, or metabolic syndromes due to their antioxidant and anti-inflammatory activities. It was previously reported that OPCs are effective in lowering the lipid index and blocking inflammatory responses by preventing lipid peroxidation and adjusting the lipid catabolic process (14,15). Furthermore, it has been found that OPC extracts can protect β-cells of the pancreas via the attenuation of oxidative stress, thereby increasing the sensitivity and secretion of insulin, as well as affecting certain enzyme activities in the metabolic process (16).

The present study was designed to investigate the potential role of cinnamon bark extract OPCs in controlling streptozotocin (STZ)-induced hyperglycemia and to clarify the underlying mechanisms.

Materials and methods

Experimental design. The present study was conducted following the institutional guidelines for care and use of laboratory animals approved by the local Ethics Committee of the Faculty of Pharmacy, Tanta University, Tanta, Egypt (approval no. 18112014). The study complied with the standards of animal care the European Community Directive (86/609/EEC) and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, 8th edition. The study included 60 adult male albino rats, weighing 150-200 g. The rats were purchased from the National Research Center, Giza, Egypt. Following 2 weeks of acclimatization under identical environmental conditions (22±2˚C with 55±5% controlled humidity and a 12-h dark/light cycle) and free access to a standard pellet diet and water *ad libitum*, the rats were weighed and randomly assigned to six groups (n=10/group) as follows:

Diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (45 mg/kg; Sigma-Aldrich; Merck KGaA) following overnight fasting (17). The rats were weighed and injected with STZ dissolved in a disodium citrate buffer (0.1 M, pH 4.5). The rats were provided withy 5% glucose solution as drinking water in the first 24 h following the STZ injection to counteract drug-induced hypoglycemia. After 3 days, blood samples were withdrawn from the retro-orbital venous plexus under isoflurane anesthesia (3.5% for induction and 2.5% for maintenance), and serum was separated by centrifugation at 1,600 x g/15 min to determine the glucose level.

Only rats with fasting blood glucose levels >250 mg/dl were considered diabetic rats and were selected for further experiments. Drug treatment commenced 7 days after the STZ injection (when hyperglycemia was confirmed). The diabetic rats were weighed and then randomly divided into four subgroups (n=10 for each group) as follows: i) The diabetic control group; ii) the OPC diabetic group: Diabetic rats treated with proanthocyanidin (eBioChem; 300 mg/kg/day, orally), as previously described (18) for 21 consecutive days; iii) the OPC diabetic + wortmannin group: Diabetic rats were treated with proanthocyanidin (300 mg/kg/day, orally for 21 consecutive days) and were then treated with a single i.p dose of a PI3K inhibitor (wortmannin) at 1 mg/kg (Acros Organics; Thermo Scientific Chemicals) 24 h after the final OPC dose (19); iv) the wortmannin control group: Diabetic rats were treated with a single dose of wortmannin (1 mg/kg) on day 22. In addition, there were two other groups: v) The normal control group (n=10): Non-diabetic rats received only citrate buffer; and vi) the proanthocyanidin control group (OPC control; n=10): Non-diabetic rats received the vehicle and proanthocyanidin (300 mg/kg, orally). Proanthocyanidin was dissolved in water, and wortmannin was dissolved in DMSO (S.D. Fine Chem Ltd.). The rats were weighed three times per week to monitor their body weight and general well-being. In the present study, any rat experiencing a weight loss of >10% or more received supportive treatment with insulin [6 U/kg, subcutaneous (s.c.) administration] and saline (1 ml 0.9% saline was administered via i.p. injection once a day). Any animal experiencing a weight loss >20% compared to the weight on day 1 was immediately euthanized as a humane endpoint. Of note, 2 rats out of the 10 rats in the diabetic control group were euthanized on days 18 and 19 due to reaching the humane endpoint of 20% weight loss.

At 24 h after the final administration of either drug, the rats were anesthetized by isoflurane (El-Nasr Pharmaceutical Chemicals Co.). All rats were anesthetized on day 23 with isoflurane (3.5% for induction and 2.5% for maintenance). Subsequently, large amounts of blood were rapidly withdrawn from the inferior vena cava of the rats and the rats were then sacrificed by the immediate removal of the heart. The blood was centrifuged, and serum was stored at -20˚C until used for the analysis of glucose, insulin, total cholesterol, triglyceride, LDL and HDL levels. After the rats were sacrificed, the liver, pancreas and the same part of the quadriceps femoris muscle (skeletal muscles) of all rats was carefully excised, washed with saline, and frozen at -80˚C for use in subsequent biochemical analysis.

Table I. Glucose and insulin concentrations in the different study groups.

Groups/parameter	Glucose (mg/dl)	Insulin (μU/l)
Normal control	88.15±6.61	34.29±3.96
OPC control	84.52±5.73	30.95±2.18
Diabetic control	376.37±29.66[a]	12.37±0.34[a]
Wortmannin control	565.49±32.86	9.42±0.34
OPC diabetic	143.01±6.36[a,b,c]	20.24±0.47[a,b,c]
OPC diabetic + wortmannin	300.07±25.52[a,c,e,f]	16.48±0.32[a,d,f]

Data are presented as the mean ± SEM, n=8-10 rats per group. [a]Significant difference vs. normal control (P<0.001); [b]significant difference vs. OPC control (P<0.001); [c]significant difference vs. diabetic control (P<0.001); [d]significant difference vs. diabetic control (P<0.01); [e]significant difference vs. OPC diabetic group (P<0.001); [f]significant difference vs. wortmannin control (P<0.001). OPC, oligomeric proanthocyanidin.

Biochemical analysis of serum. Insulin was measured in serum using rat insulin enzyme-linked immunosorbent assay (ELISA) kits (cat. no. ml-68036; eBioChem). The insulin concentration was determined according to manufacturer's protocol and expressed as mU/l. The glucose level was measured according to the method of Trinder (20) using commercial colorimetric kits (cat. no. 139203; Greiner Diagnostic GmbH), the absorbance (UV2-100 UV-visible spectrophotometer, ATi Unicam®) was measured at 510 nm, and the results are expressed as mg/dl. Serum cholesterol and triglyceride levels were measured according to the methods described in the studies by Savoldi *et al* (21) and Nagele *et al* (22), using commercial colorimetric kits (cat. nos. 118004, 183003 respectively; Greiner Diagnostic GmbH). The levels of high-density lipoprotein (HDL)-cholesterol (HDL-C) were measured as previously described by Austin *et al* (23) following the precipitation of very-low-density lipoprotein (VLDL)-cholesterol and low-density lipoprotein (LDL)-cholesterol (LDL-C) by phosphotungstate in the presence of magnesium ions (cat. no. 150103; Greiner Diagnostic GmbH). LDL-C was calculated using the Friedewald equation as follows: LDL-C=total cholesterol-HDL-C-triglycerides/5.

Determination of PI3K and GLUT4 levels. PI3K and GLUT4 levels were measured in rat muscle tissues using rat ELISA kits (cat. nos. ml003142 and ml254159 respectively; eBioChem) according to the protocol provided by the manufacturer and expressed as pg/ml and μg/l, respectively.

Determination of GSK-3 levels. GSK levels were measured in liver tissues using a rat ELISA kit (cat. no. ml620015; eBioChem) according to the protocol provided by the manufacturer and expressed as pg/l.

Determination of amylin levels. Amylin levels were measured in blood and pancreatic tissues using an ELISA kit (cat. no. ml003191; eBioChem) according to the protocol provided by the manufacturer and expressed as ng/l.

Statistical analysis. Data analysis was performed using the statistical package for social science (SPSS) software version 21.0 (IBM Corp.). All data are presented as the mean ± SEM. Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA). Differences between groups were identified using post-hoc Tukey test. P-values <0.05 were considered to indicate statistically significant differences.

Results

Serum glucose levels. The serum glucose level was significantly increased (P<0.001) in the diabetic control group (4.3-fold increase) compared with the normal control group. The OPC diabetic group exhibited a significant decrease (-62%) compared with the diabetic control group (P<0.001). The OPC diabetic + wortmannin group exhibited a significant (P<0.001) decrease (-20.27 and -46.94%, respectively) compared with the diabetic control and wortmannin control; however, this group exhibited a significant increase (P<0.001) in the serum glucose level (2.1-fold increase) compared with OPC diabetic group (Table I).

Serum insulin levels. The diabetic control group exhibited a significant decrease (P<0.001) in the insulin level (-63.92%) compared with the normal control group. The OPC diabetic group exhibited a significant decrease (P<0.001) in the serum insulin level (-34.6%) compared with OPC control, and a significant increase in the serum insulin level (+63.62%) (P<0.001) compared with the diabetic control. The OPC diabetic + wortmannin group exhibited a significant increase (P<0.001) in the serum insulin level (+74.95%) compared with the wortmannin control group (Table I).

Lipid profiles. The diabetic control group exhibited a significant increase (P<0.001) in cholesterol, triglyceride and LDL-C levels, and a significant decrease (P<0.001) in HDL-C levels compared with the normal control group. The OPC diabetic group exhibited a significant decrease (P<0.001) in cholesterol, triglyceride and LDL-C levels, and a significant increase (P<0.001) in HDL-C levels compared with the diabetic control. On the other hand, the OPC diabetic group exhibited a significant decrease (P<0.001) in cholesterol and HDL-C levels compared with the OPC control group. The OPC diabetic + wortmannin group exhibited a significant decrease in cholesterol and HDL-C levels, and a significant increase in triglyceride levels compared with the OPC control group.

Table II. Effect of OPC on lipid profiles.

Groups/parameter	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal control	55.93±1.67	29.61±1.81	15.29±1.16	34.72±2.51
OPC control	58.77±0.81	27.02±1.68	13.84±1.11	39.52±1.79
Diabetic control	76.95±4.33[a]	100.85±12.12[a]	47.14±5.18[a]	9.64±1.69[a]
Wortmannin control	70.52±3.09	89.91±5.39	41.98±2.61	10.56±1.52
OPC diabetic	40.47±1.21[a,b,c]	41.85±1.25[a,b,c]	12.83±0.57[a,b,c]	19.27±1.19[a,b,c]
OPC diabetic + wortmannin	45.41±1.8[a,c,d,e]	52.23±1.73[a,c,e,f]	15.07±0.99[c,e]	19.89±1.45[a,c,e]

Data are presented as the mean ± SEM, n=8-10 rats per each group. [a]Significant difference vs. normal control (P<0.001); [b]significant difference vs. OPC control (P<0.001); [c]significant difference vs. diabetic control (P<0.001); [d]significant difference vs. OPC diabetic group (P<0.01); [e]significant difference vs. wortmannin control (P<0.001); [f]significant difference vs. OPC diabetic group (P<0.001). OPC, oligomeric proanthocyanidin; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

Furthermore, the OPC diabetic + wortmannin group exhibited a significant decrease (P<0.001) in cholesterol, triglyceride and LDL-C levels, and a significant increase (P<0.001) in HDL-C levels compared with both the diabetic control and wortmannin control group (Table II).

Effect of OPC on muscular PI3K and GLUT4 levels in the studied groups. Compared with the normal control group, the diabetic control group exhibited a significant decrease (P<0.001) in PI3K and GLUT4 levels (-33.93 and -53.64%, respectively). The OPC diabetic group exhibited a significant decrease (P<0.001) in the PI3K level (-19.95%) compared with the OPC control group and a significant increase (P<0.001) (+18.25%) compared with the diabetic control group. Furthermore, the OPC diabetic group exhibited a significant decrease (P<0.001) in the GLUT4 level (-15 and -58%, respectively) compared with the OPC control and diabetic control group. The OPC diabetic + wortmannin group exhibited a significant increase (P<0.001) in the PI3K level (+10.69 and +23.42%, respectively), as well as a significant increase (P<0.001) in the GLUT4 level (+34.3 and +77.44%, respectively) compared with the diabetic and wortmannin control groups (Fig. 1A).

Effect of OPC on liver GSK-3 in the studied groups. The diabetic control group exhibited a significant increase in the liver GSK-3 level compared with the normal control group. The OPC diabetic group exhibited a significant decrease (P<0.001) in the GSK-3 level (-17.53 and -48.08%, respectively) compared with the OPC control and diabetic control groups. The OPC diabetic + wortmannin group exhibited a significant decrease (P<0.001) in the GSK-3 level (-32.64, -56.78 and -53.76%, respectively) compared with the OPC, diabetic and wortmannin control groups (Fig. 1B).

Effect of OPC on serum and pancreatic amylin levels in the studied groups. The diabetic control group exhibited a significant decrease (P<0.001) in the serum amylin level (-63.78%) compared with the normal control group. Both the OPC diabetic and OPC diabetic + wortmannin groups exhibited an increase (P<0.001) in the serum amylin level (+49.95 and +44.81%, respectively) compared with the diabetic control group (Fig. 1C).

Compared with the normal control and wortmannin control groups, the diabetic control group exhibited a significant decrease (P<0.001) in the amylin level in pancreatic tissue (-53.87 and -33.49%, respectively). The OPC diabetic group exhibited a significant decrease (P<0.001) in the pancreatic amylin level (-10.63%) compared with the OPC control group and a significant increase (P<0.001) in the pancreatic amylin level (+92.33%) compared with the diabetic control group. The OPC diabetic + wortmannin group exhibited a significant increase (P<0.001) in the pancreatic amylin level (+77.97 and +18.36%, respectively) compared with the diabetic control and wortmannin control group and a significant decrease (P<0.001; -17.31 and -7.46%, respectively) compared with the OPC control and OPC diabetic groups (Fig. 1C).

Discussion

Diabetes mellitus is one of the most prevalent chronic disease. It develops due to metabolic dysregulation, impaired insulin production, or insensitivity of insulin receptors (24). It is characterized by hyperglycemia, abnormal lipid profile and inappropriate consumption of glucose in addition to increased reactive oxygen species (ROS) production and altered insulin signaling and ROS-induced cellular damage that leads to severe microvascular and macrovascular secondary complications (25).

However, the use of oral anti-hyperglycemic agents for glycemic control has several limitations and severe adverse effects; this has led diabetic patients to use natural products with valuable therapeutic efficacy without side-effects (26). The present study investigated the anti-diabetic and anti-hyperlipidemic properties of proanthocyanidins in rats with STZ-induced diabetes and aimed to elucidate the underlying molecular mechanisms of proanthocyanidins regarding its effects on glucose, lipid metabolism and insulin signaling.

In the present study, T2D was induced in rats by an i.p. injection of STZ to mimic the metabolic characteristics of T2D. The findings revealed a significant increase in blood glucose, TC, TG and LDL-C levels, along with a significant decrease in insulin level and HDL-C levels in the diabetic control group, as previously reported (17).

The dysregulation of lipid metabolism is common among diabetic patients where the excessive production of free

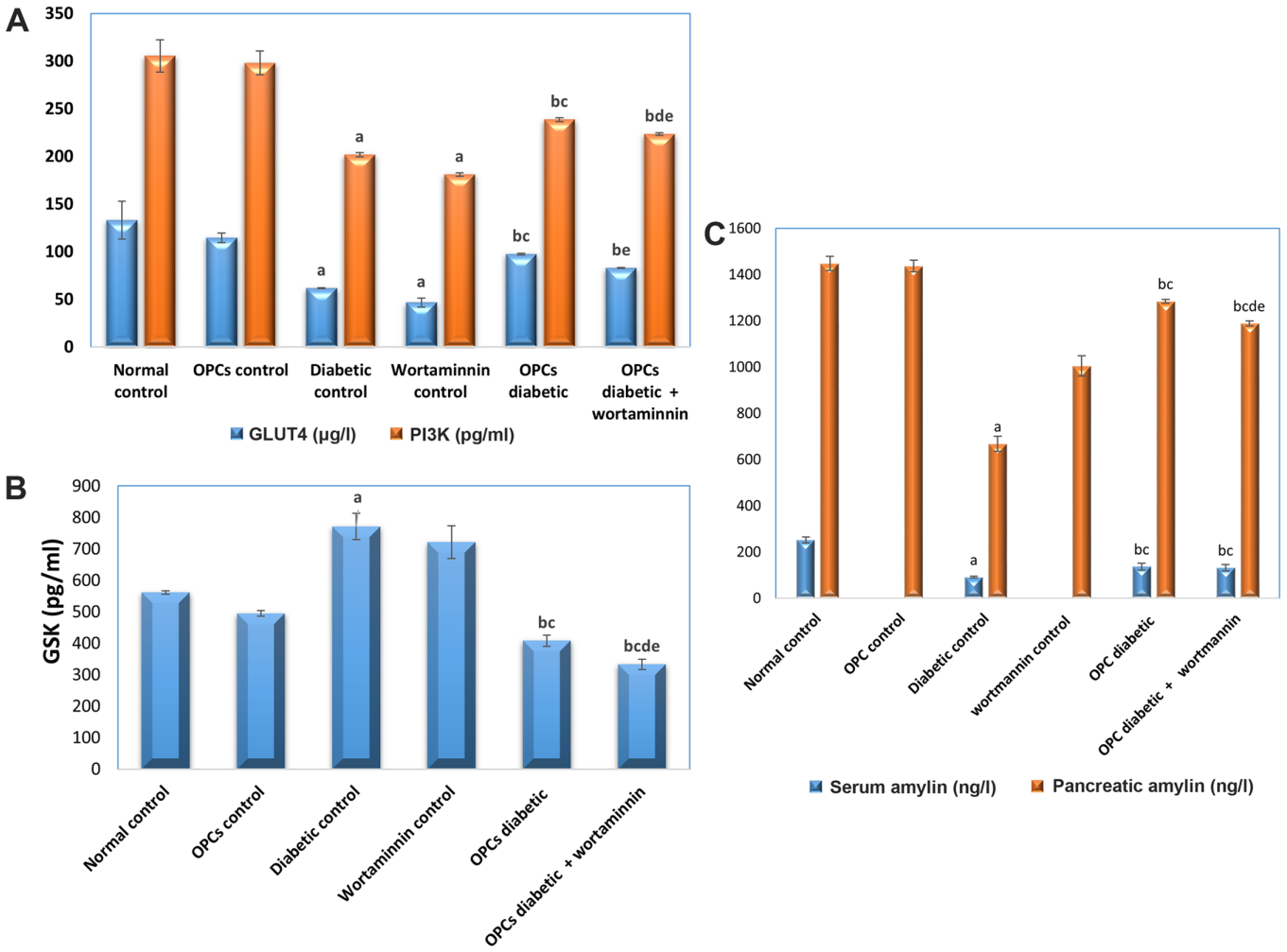

Figure 1. Effect on (A) muscular PI3K and GLUT4 (B) liver GSK (C) serum and pancreatic amylin levels in the different studied groups. Data are presented as the mean ± SEM, n=8-10 rats per group. The small letters on the top of the bars in the figure indicate significant differences as follows: a, significant difference vs. normal control (P<0.001); b, significant difference vs. OPC control (P<0.001); c, significant difference vs. diabetic control (P<0.001); d, significant difference vs. OPC diabetic group (P<0.001); e, significant difference vs. wortmannin control (P<0.001). OPC, oligomeric proanthocyanidin; PI3K, phosphatidylinositol 3-kinase; GLUT4, glucose transporter 4; GSK-3, glycogen synthase kinase-3.

fatty acids and the induction of endocrine factors leads to a decrease in the biological activity of insulin and insulin sensitivity. Therefore, a disruption in lipid profiles is considered a predictor of T2D development (27). The findings of the present study demonstrated a significant increase in TC, TG and LDL-C levels, along with a significant decrease in HDL-C levels in the diabetic control group. By contrast, an improved lipid profile was observed in the OPC diabetic group, which exhibited a significant decrease in TC, TG, LDL-C, and a significant increase in HDL-C compared with the normal control, OOC control and diabetic control groups.

The physiological activities of insulin are exerted through the post-insulin receptor cascade after binding insulin to its receptors on the surface of cell membranes of target cells. The PI3K/Akt signaling pathway is the dominant regulator where the activation of PI3K, an essential protein involved in glucose metabolism, increases the levels of phosphorylated Akt, which activates downstream signaling molecules, such as GSK-3β and GLUT4. Therefore, the PI3K/Akt signaling pathway mediates glucose uptake and intracellular glycogen synthesis in skeletal muscle tissues, glucose dysplasia and glucose output in the liver. The inhibition of the PI3K signaling pathway blocks glucose transportation to the plasma membrane (28). It has been previously reported that the dysregulation of the PI3K/Akt signaling pathway can lead to impaired glucose and lipid metabolism and eventually, to insulin resistance (29).

Wortmannin is a potent inhibitor of PI3K, which suppresses the PI3K/Akt signaling pathway. It has also been previously reported that the insulin-mediated induction of GSK-3β and insulin receptor substrate-1 is suppressed by wortmannin (30). In the present study, wortmannin was used to investigate the molecular mechanisms of OPCs as anti-diabetic agents in insulin signaling in the presence or absence of wortmannin, where the levels of glucose metabolism-associated proteins, including GSK-3β, PI3K and GLUT4 were evaluated.

The present study demonstrated that the OPC + wortmannin group exhibited a significant increase in HDL-C levels, and a decrease in glucose, TG, TC and LDL-C levels compared with the wortmannin control group. The

wortmannin control group exhibited a significant increase in glucose, TG, TC and LDL-C levels, and a decrease in HDL-C levels compared with the normal control group. The OPC + wortmannin group rats exhibited a significant increase in PI3K and GLUT4 levels compared with the wortmannin control group.

The data of the present study also demonstrated that the diabetic control group exhibited a significant increase in liver GSK-3 level compared with the normal control group. The OPC diabetic group exhibited a significant decrease in the liver GSK-3 level compared with OPC control and diabetic control groups. The OPC diabetic + wortmannin group exhibited a significant decrease compared to the OPC control, diabetic control and wortmannin control groups.

The mechanisms underlying the effects of OPC extract on the insulin signaling pathway were also investigated. The results revealed that OPC exerted its hypoglycemic effects through the activation of the PI3K/Akt pathway and the stimulation of GLUT4 translocation, which in turn enhanced glucose transport and cellular uptake, as well as lipid metabolism in addition to reduced glycogenesis (GSK-3β), which is in line with the findings of previous studies (31-33).

Amylin is co-secreted with insulin by pancreatic β-cells and contributes to blood glucose homeostasis along with insulin through complex neuronal, as well as endocrine pathways. The present study revealed a significant decrease in the amylin level in the diabetic group vs. the normal control group; however, the OPC diabetic groups with or without wortmannin treatment exhibited a significant increase in amylin levels compared to the diabetic group. These results are supported by the findings of previous studies that demonstrated that the induction of diabetes by STZ in rats resulted in the loss of the ability to secrete amylin (34,35).

The present study had some limitations. First, the effect of proanthocyanidins on the phosphorylation of Akt need to be investigated. Second, the present study only evaluated the expression of components on insulin signal pathway, but did not evaluate their activities. Therefore, additional studies are required to determine the underlying mechanism responsible for the anti-diabetic action of OPC extract on the insulin signaling pathway.

In conclusion, the findings of the present study provided a biochemical basis for the beneficial use of OPCs for glycemic control in diabetics. In the present study, treatment with OPC showed anti-diabetic action by significantly decreasing the blood glucose level and increasing the insulin level through the regulation of the PI3K signaling pathway, increasing insulin receptor downstream proteins, and decreasing glycogenesis (GSK-3). Moreover, OPC also exerted anti-hyperlipidemic effects by decreasing lipid profile levels. These results thus suggest that OPC as a cinnamon extract has potential for use in diabetic patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NEEA contributed to the conception and the design of the study, supervised the practical work, and revised and edited the manuscript. EGK was involved in the conception and design of the study, revised the manuscript and analyzed the data. NHA conducted the experiments for the study. AOI was involved in the writing of the manuscript, and in the statistical analysis of the data. NHA and AOI verify the authenticity of the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted following the institutional guidelines for care and use of laboratory animals approved by the local Ethics Committee of the Faculty of Pharmacy, Tanta University, Tanta, Egypt (approval no. 18112014).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Petersmann A, Nauck M, Müller-Wieland D, Kerner W, Müller UA, Landgraf R, Freckmann G and Heinemann L: Definition, classification and diagnosis of diabetes mellitus. Exp Clin Endocrinol Diabetes 126: 406-410, 2018.
2. Burgos-Morón E, Abad-Jiménez Z, Marañón AM, Iannantuoni F, Escribano-López I, López-Domènech S, Salom C, Jover A, Mora V, Roldan I, *et al*: Relationship between oxidative stress, er stress, and inflammation in type 2 diabetes: The battle continues. J Clin Med 8: 1385, 2019.
3. Moldogazieva NT, Mokhosoev IM, Mel'nikova TI, Porozov YB and Terentiev AA: Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in aging and age-related diseases. Oxid Med Cell Longev 2019: 3085756, 2019.
4. Asthana S, Mallick B, Alexandrescu AT and Jha S: IAPP in type II diabetes: Basic research on structure, molecular interactions, and disease mechanisms suggests potential intervention strategies. Biochim Biophys Acta Biomembr 1860: 1765-1782, 2018.
5. Hieronymus L and Griffin S: Role of amylin in type 1 and type 2 diabetes. Diabetes Educ 41 (Suppl 1): 47S-56S, 2015.
6. Takenaka N, Araki N and Satoh T: Involvement of the protein kinase Akt2 in insulin-stimulated Rac1 activation leading to glucose uptake in mouse skeletal muscle. PLoS One 14: e0212219, 2019.
7. Chadt A and Al-Hasani H: Glucose transporters in adipose tissue, liver, and skeletal muscle in metabolic health and disease. Pflugers Arch 472: 1273-1298, 2020.
8. Pearce NJ, Arch JR, Clapham JC, Coghlan MP, Corcoran SL, Lister CA, Llano A, Moore GB, Murphy GJ, Smith SA, *et al*: Development of glucose intolerance in male transgenic mice overexpressing human glycogen synthase kinase-3beta on a muscle-specific promoter. Metabolism 53: 1322-1330, 2004.
9. MacAulay K and Woodgett JR: Targeting glycogen synthase kinase-3 (GSK-3) in the treatment of Type 2 diabetes. Expert Opin Ther Targets 12: 1265-1274, 2008.

10. Salehi B, Ata A, V Anil Kumar N, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega A, Abdulmajid Ayatollahi S, Tsouh Fokou PV, Kobarfard F, Amiruddin Zakaria Z, *et al*: Antidiabetic potential of medicinal plants and their active components. Biomolecules 9: 551, 2019.
11. Dorri M, Hashemitabar S and Hosseinzadeh H: Cinnamon (Cinnamomum zeylanicum) as an antidote or a protective agent against natural or chemical toxicities: A review. Drug Chem Toxicol 41: 338-351, 2018.
12. Cao H, Ou J, Chen L, Zhang Y, Szkudelski T, Delmas D, Daglia M and Xiao J: Dietary polyphenols and type 2 diabetes: Human study and clinical trial. Crit Rev Food Sci Nutr 59: 3371-3379, 2019.
13. Sun C, Zhao C, Guven EC, Paoli P, Simal-Gandara J, Ramkumar KM, Wang S, Buleu F, Pah A, Turi V, *et al*: Dietary polyphenols as antidiabetic agents: Advances and opportunities. Food Frontiers 1: 18-44, 2020.
14. Wang TK, Xu S, Li S and Zhang Y: Proanthocyanidins should be a candidate in the treatment of cancer, cardiovascular diseases and lipid metabolic disorder. Molecules 25: 5971, 2020.
15. Koutsos A, Riccadonna S, Ulaszewska MM, Franceschi P, Trošt K, Galvin A, Braune T, Fava F, Perenzoni D, Mattivi F, *et al*: Two apples a day lower serum cholesterol and improve cardiometabolic biomarkers in mildly hypercholesterolemic adults: A randomized, controlled, crossover trial. Am J Clin Nutr 111: 307-318, 2020.
16. Yokozawa T, Cho EJ, Park CH and Kim JH: Protective effect of proanthocyanidin against diabetic oxidative stress. Evid Based Complement Alternat Med 2012: 623879, 2012.
17. Mabhida SE, Johnson R, Ndlovu M, Louw J, Opoku A and Mosa RA: Molecular basis of the anti-hyperglycemic activity of RA-3 in hyperlipidemic and streptozotocin-induced type 2 diabetes in rats. Diabetol Metab Syndr 11: 27, 2019.
18. Cheung DY, Kim JI, Park SH and Kim JK: Proanthocyanidin from grape seed extracts protects indomethacin-induced small intestinal mucosal injury. Gastroenterol Res Pract 2014: 618068, 2014.
19. Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y and Sato Y: Cinnamon extract (traditional herb) potentiates in vivo insulin-regulated glucose utilization via enhancing insulin signaling in rats. Diabetes Res Clin Pract 62: 139-148, 2003.
20. Trinder P: Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J Clin Pathol 22: 158-161, 1969.
21. Savoldi R, Prandini BD and Donisi C: Enzymatic determination of total serum cholesterol by 4-aminophenazone-phenol: Manual and automatic method (author's translation). Quad Sclavo Diagn 12: 238-247, 1976 (In Italian).
22. Nagele U, Hagele EO, Sauer G, Wiedemann E, Lehmann P, Wahlefeld AW and Gruber W: Reagent for the enzymatic determination of serum total triglycerides with improved lipolytic efficiency. J Clin Chem Clin Biochem 22: 165-174, 1984.
23. Austin GE, Maznicki E and Sgoutas D: Comparison of phosphotungstate and dextran sulfate-Mg^{2+} precipitation procedure for determination of high density lipoprotein cholesterol. Clin Biochem 17: 166-169, 1984.
24. Silva JAD, Souza ECF, Echazú Böschemeier AG, Costa CCMD, Bezerra HS and Feitosa EELC: Diagnosis of diabetes mellitus and living with a chronic condition: Participatory study. BMC Public Health 18: 699, 2018.
25. Jiang S, Young JL, Wang K, Qian Y and Cai L: Diabetic-induced alterations in hepatic glucose and lipid metabolism: The role of type 1 and type 2 diabetes mellitus (Review). Mol Med Rep 22: 603-611, 2020.
26. Zonszein J and Groop PH: Strategies for diabetes management: Using newer oral combination therapies early in the disease. Diabetes Ther 7: 621-639, 2016.
27. I S Sobczak A, A Blindauer C and J Stewart A: Changes in plasma free fatty acids associated with type-2 diabetes. Nutrients 11: 2022, 2019.
28. Huang X, Liu G, Guo J and Su Z: The PI3K/AKT pathway in obesity and type 2 diabetes. Int J Biol Sci 14: 1483-1496, 2018.
29. Yang H, Cao Q, Xiong X, Zhao P, Shen D, Zhang Y and Zhang N: Fluoxetine regulates glucose and lipid metabolism via the PI3K-AKT signaling pathway in diabetic rats. Mol Med Rep 22: 3073-3080, 2020.
30. Maffei A, Lembo G and Carnevale D: PI3Kinases in diabetes mellitus and its related complications. Int J Mol Sci 19: 4098, 2018.
31. Yogalakshmi B, Bhuvaneswari S, Sreeja S and Anuradha CV: Grape seed proanthocyanidins and metformin act by different mechanisms to promote insulin signaling in rats fed high calorie diet. J Cell Commun Signal 8: 13-22, 2014.
32. Santos HO and da Silva GAR: To what extent does cinnamon administration improve the glycemic and lipid profiles? Clin Nutr ESPEN 27: 1-9, 2018.
33. Sun Q, Jia N, Li X, Yang J and Chen G: Grape seed proanthocyanidins ameliorate neuronal oxidative damage by inhibiting GSK-3β-dependent mitochondrial permeability transition pore opening in an experimental model of sporadic Alzheimer's disease. Aging (Albany NY) 11: 4107-4124, 2019.
34. Ogawa A, Harris V, McCorkle SK, Unger RH and Luskey KL: Amylin secretion from the rat pancreas and its selective loss after streptozotocin treatment. J Clin Invest 85: 973-976, 1990.
35. Zhang XX, Pan YH, Huang YM and Zhao HL: Neuroendocrine hormone amylin in diabetes. World J Diabetes 7: 189-197, 2016.