Vitamin E versus propolis

as an add-on therapy to sitagliptin/metformin on body mass index and glycemic control in type 2 diabetic patients

Vitamina E versus propóleo como terapia adicional a sitagliptina/metformina en el IMC y el control glucémico en pacientes diabéticos tipo 2

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Abstract

Diabetes mellitus is represented worldwide as a healthcare challenge. Various treatments have been used in an attempt to improve glycemic control and decrease the disease's devastating complications. The present study aimed to assess the effect on diabetes-relevant parameters of the combination therapy with sitagliptin plus metformin in type 2 diabetic patients using adjuvant vitamin E or propolis as add-on therapy. For this purpose, three serum samples were collected from patients; **first visit** (baseline) and 8-week vitamin E added to their course of therapy, then serum sample collected at **second visit** followed by a washout period of one week and propolis added to the list of patients' therapy for additional 8-weeks and serum collected at the end of the 8-weeks (**third visit**). The collected samples were frozen until analysis. The baseline and post-therapy samples were analyzed for fasting blood sugar (FBS), glycated hemoglobin (HBA1c), C-peptide, and insulin; and the results were analyzed statistically. The results confirmed that vitamin E and propolis have both improved measured parameters through significant reduction of FBS and HBA1c together with improved insulin sensitivity through significant reduction of insulin level and C-peptide compared to baseline levels. Moreover, propolis has shown a higher effect over vitamin E shown by its increased positive effects on measured parameters. The study concluded that adjuvant addition of either propolis or vitamin E could improve glycemic control and associated parameters of insulin and relevant C-peptide level.

Keywords: propolis, vitamin E, diabetes, sitagliptin, metformin.

Resumen

La diabetes mellitus se presenta a nivel mundial como un reto sanitario. Se han aplicado varios tratamientos en un intento de mejorar el control glucémico y disminuir las complicaciones devastadoras de la enfermedad. El presente estudio tuvo como objetivo evaluar el efecto que sobre los parámetros relevantes de diabetes que ejerce la terapia combinada con sitagliptina más metformina en pacientes con diabetes tipo 2, usando como adyuvante a la vitamina E o propóleo como terapia adicional. Para ello, se recogieron tres muestras de suero de pacientes; primera visita (basal) y la adición a la terapia de vitamina E por 8 semanas de terapia, luego en la segunda visita se recolecto una muestra de suero, seguida de un período de lavado de una semana, y luego se adicionó el propóleo como terapia por 8 semanas adicionales. al final de las 8 semanas (tercera visita) y recolecto una muestra de suero. Las muestras recolectadas se congelaron hasta su análisis. Las muestras de referencia y posteriores a la terapia se analizaron para glucosa en sangre en ayunas (FBS), hemoglobina glicosilada (HBA1c), péptido-C e insulina; y los resultados fueron analizados estadísticamente. Los resultados mostraron que la vitamina E y el propóleos mejoran los parámetros evaluados, ya que producen una reducción significativa de FBS y HBA1c, junto con una mejor sensibilidad a la insulina a través de una reducción significativa del nivel de insulina y péptido-C en comparación con los niveles de referencia; además, el propóleo mostró un mayor efecto que la vitamina E a través de sus efectos positivos sobre los parámetros evaluados. El estudio concluyó que la adición adyuvante de propóleo o vitamina E podría mejorar el control glucémico y los parámetros asociados de insulina y el nivel de péptido-C.

Palabras clave: propóleos, vitamina E, diabetes, sitagliptina, metformina.

Introduction

Diabetes mellitus, a hormonal metabolic illness, is a primary worldwide medical problem with serious implications that contribute to high morbidity and mortality with high costs for the country every year[1-4]. In 2015, it became reported that 415 million persons globally had diabetes, with 90-95 percent having type 2 diabetes mellitus[5]. T2DM is significantly connected with macro and microvascular problems, as well as complications such as heart disease, which are often associated with negative glucose tolerance[6]. Hyperglycemia induces the formation of reactive oxygen species (ROS) while decreasing antioxidant defenses through activation of many pathways of diabetic tissue damage, including intracellular AGE formation of advanced glycation end products and glycation of enzymes[7]. Thus, it is thought that by reducing ROS production may play a significant role in regulating diabetes complications[8]

Currently, a wide range of natural compounds are utilized to treat various types of systemic disorders. Propolis, a non-toxic resin-like material made by bees from the buds of poplar and cone-bearing trees. Bees use it to build hives, and it may contain beehive byproducts and to help in the construction and preservation of their colonies by eliminating diseases and covering the honeycomb from rain. Furthermore, because of its sticky properties, propolis inhibits foreign guests from invading the hive[9]. Propolis' biological action is primarily related to its flavonoids and hydroxycinnamic acid concentration[10]. The evidence indicates that the flavonoid content of propolis and its very complex chemical composition due to several phytogeographic characteristics that are inherent to the varying plant habitats and plant types that bees choose from to produce propolis. These characteristics include, vegetation, season, and the environmental conditions of the collection site, the location of collecting, the origin and kind of plant pollen, and the species of bees that generated it. Propolis is a natural product with many biological properties including hypoglycemic activity and modulating lipid profile, in addition to his antimicrobial, anticancer, antifungal, antiviral, and anti-inflammatory effects[10, 11].

It was shown that propolis hypoglycemic activity positively impacts diabetic complications, and also can improve antioxidant status by increasing enzymatic antioxidants concentrations and lowering blood glucose, which might reduce diabetes symptoms and result in improved diabetic management[12-14]. Due to the high cost of preventing and treating diabetes, we were prompted to conduct the current research to identify the effectiveness of propolis and vitamin E in controlling diabetes by comparing its effect to vitamin E, a well-known antioxidant medication.

Materials and methods

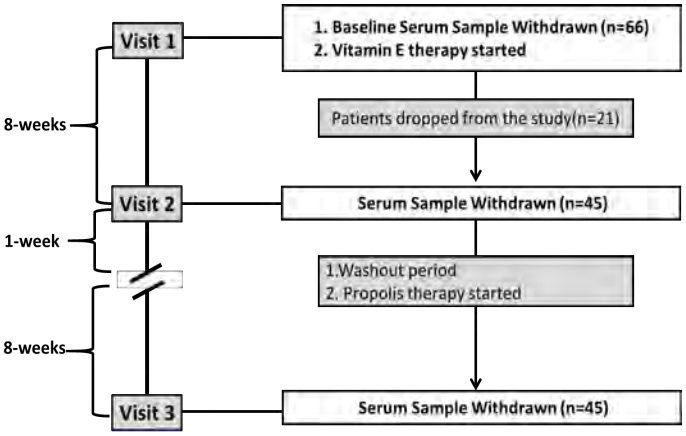
A total number of 45 type 2 diabetic patients were enrolled in the present study (Table 1). A consent form was taken from conjoined patients to confirm individuals' awareness and acceptance. Patients with other illnesses, smokers, alcoholics, lactating or pregnant women, and patients on drugs other than sitagliptin/metformin were excluded from the study. Starting from December 1st, 2020, to December 1st, 2021, a pre-post-sequential interventional study was conducted at the Diabetic and Endocrinology Civil Clinic and Diabetic Centre in Mosul, Iraq.

Table 1. Body Mass Index (BMI) of type 2 diabetic patients on sitagliptin/metformin therapy at the beginning of the study [n=45].

Parameter	Mean ± SD
BMI (Kg/m^2)	32.90 ± 3.56
Age (years)	54.48 ± 6.16

Included diabetic patients were those who are solely based on combination therapy of Sitagliptin/Metformin on a dosing schedule of 12-hours intervals basis, for at least 3 months on Sitagliptin/Metformin therapy. Serum samples were withdrawn from patients on their first visit and after 8-week of vitamin E treatment (second visit), then the patients were asked to keep a washout period of one week and start propolis for an additional 8 weeks followed by serum sample collection (third visit) as shown in the workflow diagram (Figure1).

Figure 1. Workflow diagram.



The details and origin of used conventional therapy and add-on therapy is shown in Table 2.

Table 2. Origin and supplier's details of used medication in the present study.

Medications	Trade Name	Suppliers	Dose
Sitagliptin/ Metformin	Stevia plus	Pioneer	50/500 (2 times daily)
Vitamin E	Vitamin E	Adrien Gagnon/ Canada	400 I.U/day
Propolis	Bee Propolis	Lake Avenue Nutrition	1000 mg/day

Serum was analyzed for the glycemic control parameters and insulin resistance including fasting blood sugar (FBS), glycated hemoglobin (HbA1c), insulin, C-peptide, and insulin resistance index. Serum glucose was measured by colorimetry using a kit supplied by ThermoFisher Scientific (EIAGLUC). The principle of the assay is based on the oxidation of glucose by glucose oxidase enzyme-producing colorless hydrogen peroxide which in presence of horseradish peroxidase is converted to the colored compound to be quantified spectrophotometrically at a wavelength of 560 nm.

HbA1c measured by colorimetric enzymatic method using kit supplied by Genrui Pa54 (China). Whole blood is hemolysed by a hemolysing solution. The protease enzyme separates glycated hemoglobin from non-glycated and the ratio between them was determined.

The principle of measurement of insulin was based on Sandwich ELISA Technique using a kit supplied by COBAS (12017547, Roche) for insulin and (98126) for C-peptide, which includes two incubation periods. The first incubation; insulin from a 20 µL sample, a biotinylated monoclonal insulin-specific antibody, and a monoclonal insulin-specific antibody labeled with a ruthenium complex form a sandwich complex. The second incubation period: after the addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via the interaction of biotin and streptavidin. Followed by aspiration of reaction mixture into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. The use of a voltage to the electrode then induces chemiluminescent emission which is quantified by a photomultiplier. Insulin resistance determined as Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) using the equation [HOMA-IR = (insulin x glucose) / 22.5 mg/dl].

The statistical analysis was conducted using Prism 6 (GraphPad, USA) software, and further analysis and histogram production were conducted on Excel. One-way ANOVA with posthoc test was conducted to determine the significant differences between groups, $p<0.05$/CI 95% was considered as significant.

Results

Compared to baseline levels (147.5±28.5), serum FBS level (mg/dL) was significantly ($p<0.05$) reduced after administration of either vitamin E (132.1±23.5) or propolis therapy (131.32±20.52). Additionally, significantly ($p<0.05$) lower levels of FBS were demonstrated in a propolis-treated group compared to the vitamin E-treated group (Figure 2).

Compared to baseline levels (7.468±0.995), The HbA1c level (%) was significantly ($p<0.05$) reduced after administration of either vitamin E (6.896±0.819) or propolis therapy (6.773±0.666). A non-significant difference in HbA1c level exists between propolis-treated compared to vitamin E-treated group (Figure 2).

Compared to baseline levels (11.10±7.27), serum insulin level (µIU/mL) was significantly ($p<0.05$) reduced after administration of either vitamin E (7.260±3.636) or propolis therapy (7.26 ± 3.64). Additionally, significantly ($p<0.05$) lower levels of insulin were demonstrated in a propolis-treated group compared to the vitamin E-treated group (Figure 2).

Compared to baseline levels (2.323±0.818), serum C-peptide level (ng/mL) was significantly ($p<0.05$) reduced after administration of either vitamin E (1.978±0.625) or propolis therapy (1.9513±0.6376). A non-significant difference in C-peptide level exists between propolis-treated compared to vitamin E-treated group (Figure 2).

Compared to baseline values (4.262±2.447), Insulin resistance values (mg/dL) were significantly ($p<0.05$) reduced after administration of either vitamin E (2.797±1.763) or propolis therapy (2.315±1.085). Additionally, significantly ($p<0.05$) lower Insulin resistance values were demonstrated in a propolis-treated group compared to vitamin E-treated group (Figure 2).

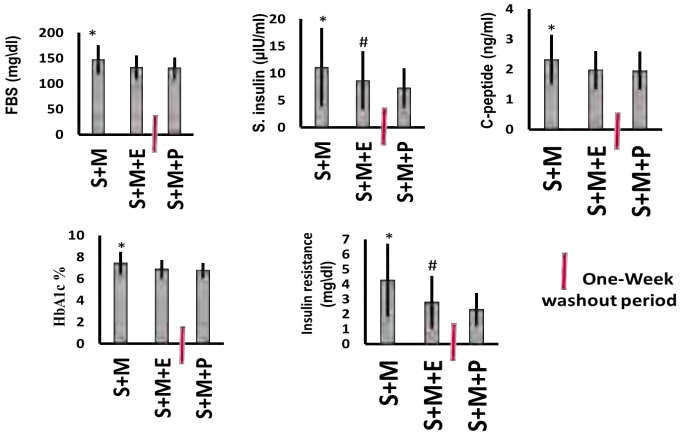


Figure 2. Vitamin E and propolis positively improved glycemic control in T2DM indicated by reduced insulin resistance. Data are expressed as mean ± SD, *$p<0.05$ control versus add-on therapy, #$p<0.05$ vitamin E compared to propolis. S=sitagliptin, M=Metformin, P=propolis, HBA1c=glycated hemoglobin, FBS=fasting blood sugar. The red bar indicates the one-week washout period.

Discussion

The current study demonstrated that the sequential addition of vitamin E and bees propolis to type 2 diabetic patients on a combination of sitagliptin and metformin therapy decreased serum glucose level and HBA1c. The study also reported that both vitamin E and propolis reduced insulin resistance through decreasing insulin concentration and associated C-peptide levels. Correspondingly, BMI was slightly improved in both groups Bees propolis induced a better therapeutic adjuvant approach compared to vitamin E when the studied parameters were compared. It is noteworthy to mention that the current study conducted on the same patients' sample as a sequential model involving one week wash-out period between vitamin E and propolis to avoid inter-individual variation of usual case-control studies.

The action of propolis depends on the complex composition of propolis from different compounds depending on the source from which they are obtained and also depends on the constituent component of the propolis. These variations in composition and the presence of different components in propolis make propolis superior over vitamin E as an intervention add-on therapy to patients' drugs. Propolis induce the improvement in diabetes parameters through the dualistic mode of action compared to vitamin E. In effect, vitamin E induces an improvement through its antioxidant effects, while propolis induces supra plus action through other effects in addition to their antioxidant effects, such as regenerative effects compared to vitamin E which has a limitation on antioxidant action[15, 16].

Some propolis-isolated compounds, such as the anti-inflammatory compound quercetin confirmed that they regenerate pancreatic tissues and restore or improve insulin secretion with subsequent improvement of glycemic control[15]. Quercetin has an action comparable to Propolis-isolated chromium known to improve peripheral insulin-receptor sensitivity via increasing the insulin receptor activation or phosphorylation, together with increasing insulin receptor number.

In a randomized double-blind controlled clinical trial, Zakerkish et al.[16] demonstrated that although the treatment with propolis did not induce significant difference in FBS and the mean HDL-C was significantly increased when compared to the placebo; however, the study demonstrated that propolis has beneficial effects on reducing post prandial blood glucose, serum insulin, insulin resistance, inflammatory cytokines, and reduced the HOMA-IR and HOMAβ scores. This action collectively might give a clue about the regenerative role of propolis on pancreatic function, nonetheless, these results contradict with our findings of the positive role of propolis on glycemic control and associated metabolic parameters and this could be explained in the context of variation of the source, origin, and composition of propolis used in these studies. A separate study conducted by Li et al., 2012[17], confirmed that insulin concentration was elevated significantly after propolis therapy and glycemic control improved correspondingly. A non-significant change has been reported by Fukuda et al., in his study on the role of propolis in reducing glucose or improving insulin levels[18].

Our study confirmed that vitamin E significantly improved insulin levels and reduced glycemic parameters. A meta-analysis study demonstrated that vitamin E administration in different studies revealed an improvement in HbA1c, patient weight, and fasting insulin level, with no changes associated with fasting glucose levels[19].

The limitation of this study is the small sample size, presence of different types of commercial propolis, short duration of the study. A larger sample size needs to be enrolled in the study and different type of propolis needs to be tested.

Conclusion

Propolis and Vitamin E improved the fasting insulin level following the two-month administration of propolis. Correspondingly, HbA1c, serum fasting glucose, body mass index, and insulin resistance was improved. Moreover, results indicated that propolis has shown potentiated effects in addition to that over vitamin E. This study encourages the addition of propolis or vitamin E to the patient's standard therapy in an attempt to reduce insulin dysregulation, improve insulin action, and boost glucose cellular utilization.

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Conflict of Interest: The authors declare that no conflict of interest exists for this research

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