Research Article

Hypoglycemic Potential of Supplementation with a Vegetable and Legume Juice Formula in Type 2 Diabetic Patients

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Abstract

Background and Objective: Type 2 diabetes affects a large number of people all over the world; hence, it is worthy to supplement those patients with a food formula containing biologically active ingredients that can help to control the disease and its complications. The present study was designed to prepare a plant formula in the form of a juice taken during treatment to synergies and reduce the drug dose used and minimize disease complications. Materials and Methods: The formula is formed of spinach, broccoli, celery, green beans and chickpea. The total fat, fatty acids, polyphenol content and the antioxidant power of the formula were determined. The blood sugar tolerance curves of the volunteers were followed after breakfast alone, breakfast and treatment dose, also when this formula was given before breakfast. Results: The vegetable and legume juice could reduce postprandial glucose level when given before the breakfast and the effect was more or less similar to the effect of the treatment drug. The juice proved to contain some polyphenols and possess an antioxidant character. Analysis proved that it is rich in polyunsaturated fatty acids. Conclusion: It is recommended to use this juice for diabetic patients to reduce hyperglycemia and synergize the effect of the used drug. It can thus avoid health problems that occur to those patients when using drugs for long time and in massive amounts.

Key words: Diabetes, legumes, hyperglycemia, vegetables, antioxidants, polyphenols


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Data Availability: All relevant data are within the paper and its supporting information files.
INTRODUCTION

Type 2 diabetes is one of the health problems that affect a considerable number of people worldwide particularly obese persons and elder. The number of persons suffering from this disease is around 400 million and is expected to reach more that 600 million by the year\textsuperscript{1} 2040. This disease in particular is usually associated with a lot of systemic complications, due to its chronic nature, such as micro and macro-vascular disorders\textsuperscript{2}, dyslipidemia, diabetic foot ulcers, damage to the peripheral nerves, coronary artery disease, nephropathy and other complications such as retinal damage\textsuperscript{3}.

Certainly, such complications affect life quality decreasing the life expectancy\textsuperscript{4} and increasing health burden for governments and health care centers, especially in the developing world.

Postprandial blood glucose control in T2DM patients due to ingestion of starchy food is of great health concern worldwide. The rise of blood glucose level after a meal in T2DM patients presents a challenge for controlling meal-associated hyperglycemia. Several phenolic compounds have the potential to serve as a therapeutic solution against these conditions. It was stated that phenolics may exert a hypoglycemic effect through either the antioxidant character or the capacity to inhibit digestion of starch\textsuperscript{5}. Combination between phenolics and synthetic enzymes may help to reduce the dose of the synthetic enzymes necessary to control blood sugar\textsuperscript{6}. This in turn reduces the development of both cardiovascular as well as neurological complications\textsuperscript{7}.

Based on the functionality of phenolic compounds in various food systems and being safer for humans, it is possible to develop functional foods for type 2 diabetic patients. Preserving the native structure of the active molecules during functional food preparation is important\textsuperscript{8}.

Prescribing glucosidase inhibitors to control post-prandial hyperglycemia is a common therapeutic strategy, particularly in non-insulin-dependent diabetes mellitus. These are developed to target α-amylase and α-glucosidase, which are found in the intestinal tract that are crucial for carbohydrate digestion\textsuperscript{9}.

However, many of these synthetic glucosidase inhibitors have a documented variety of side effects. Gastrointestinal problems such as diarrhea and abdominal discomfort are some of the side effects that can cause diabetic patients not to follow therapeutic protocols\textsuperscript{10}.

Plant polyphenolic fractions were reported to inhibit the activity of α-amylase and α-glucosidase and help in a better blood glucose control\textsuperscript{11}.

Given the low cost and related safety of plant based supplements, they can be a more acceptable source of glucosidase inhibitors\textsuperscript{12}.

Phenolic compounds in plants can act as antioxidants. They can improve cell function, stimulate secretion of insulin, improve the metabolism of adipose tissue and counteract oxidative stress and inflammatory processes\textsuperscript{13}. Thus, previous studies proved that polyphenolic compounds have the capacity to minimize the complications associated with the incidence of diabetes such as cardiovascular disease, neuropathy, nephropathy and retinopathy\textsuperscript{14}.

There is a lot of evidence that incorporation of vegetables in the daily dietary pattern can protect from obesity and diabetes\textsuperscript{15}. It is worth mentioning that vegetables are an important constituent of Mediterranean diet, also it is a main constituent of vegetarian diets, thus participate to lower the risk of chronic diseases including type 2 diabetes\textsuperscript{16}. This action may be attributed to the antioxidants present in these sources such as polyphenols, carotenoids and vitamin C. In addition, they are rich in dietary fiber that protect against atherosclerosis and reduce sharp rise in blood sugar.

The purpose of this study was to formulate a dietary supplement for T2DM patients in the form of a juice containing nutrients or compounds that synergize the effect of the treatment drug, thus control blood sugar and minimize side effect of the disease and for the investigation of chemical characteristics of this formula. Thus offer new knowledge concerning the health benefits of these sources and the potential of using them to treat diabetics.

MATERIALS AND METHODS

The study was carried out at the National Research Centre, Cairo, Egypt, for a period of 3 years, 2014-2016 the duration of the project financed from the in house projects office. This study was made according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt.

The formula used in this study is composed of a blend of fresh green vegetables, prepared from 300 g spinach, 200 g broccoli (flower and stem), 50 g celery and from legumes 200 g green beans (Phaseolus vulgaris) and 50 g chickpea. These were purchased from the local market.

Vegetables were washed thoroughly in running water and juice was prepared (a total amount of 800 g of the blend), using philips/avance-collection-juicer/HR1871/00 model 2015. An amount of 300 mL of juice was given fresh to each patient before breakfast.
**Estimation of blood glucose response:** On the day of the experiment, the first blood glucose sample was collected to measure basal blood glucose levels (0 min). Post-prandial blood samples were taken at 30, 60, 90, 120 and 150 min. Capillary blood samples were obtained by a finger prick using a mono ejection Lancet device (Accu-Chek Softclix). The first drop of blood was placed onto the strip and readings were taken (within 5-10 sec) and recorded in mg dL⁻¹. Roche Accu-Chek Active blood glucose meter was used for blood glucose measurement. The glucose meter was obtained from Accu-Chek Egypt.

**Composition of the juice formula:** The relative fatty acids content in the juice formula were determined using gas liquid chromatography (GLC)⁷. The antioxidant capacity was estimated as a percentage DPPH radical scavenging activity⁸, EC50 (concentration of the substrate required to decrease the initial DPPH radical concentration by 50%). HPLC analysis of phenolic compounds was done according to Khoddami et al.⁹.

**Studied subjects:** A total of 36 eligible subjects with type 2 diabetes (sixteen women and twenty men) aged 40-50 years old and body mass index of 25±1.3 kg m⁻² were recruited into the study. Diabetic duration was of 6-10 years. Subjects controlled their diabetes by taking oral hypoglycemic agents besides their normal daily diet; Subjects had no evidence of any chronic illness including hepatic, renal, thyroid or cardiac dysfunction. Subjects were informed about all procedures and possible risks involved and they wrote consent to participate in the study at the National Research Centre. Subjects were divided into 3 equal groups:

- **Group 1:** Consumed a breakfast meal only
- **Group 2:** Consumed a breakfast meal and their usual oral antidiabetic drug metformin hydrochloride, locally sold as (Gidophase-CID Company) in a dose from 500-850 mg)
- **Group 3:** Consumed 300 mL of the green juice daily, 30 min before taking their breakfast meal. This regimen lasted for 4 weeks. On the day of the blood glucose measurement, they were instructed to fast overnight for 10 h

The breakfast meal consisted of bread, cottage cheese and eggs. The meal is designed to give 30% energy from fat, 15% from protein and 55% from carbohydrate. Subjects were allowed to drink 250-300 mL of water.

**Statistical analysis:** Data were statistically analyzed by the computer program SPSS software, version 19 for Windows. Independent two-sample t-test was used to compare between mean values of the analyzed parameters. Data was represented as Mean±SE. Values were considered significant at p<0.05, otherwise were considered non-significant.

**RESULTS**

**Fatty acid pattern in the formula:** Results showed that the vegetables blend is rich in the long chain polyunsaturated fatty acids, C24(1) nervonic acid (n-9) at the percentage of 49.9% relative to the total fatty acids contents. It also contain C18(1) oleic acid (n-9), 5.8%, C24(0) lignoceric acid, 4.9%, C20(0) behenic acid, 2.9% and C18(3) linolenic acid (n-3), 3.4%. The total fat content of the blend was found to be 2%.

Furthermore, values of the DPPH radical scavenging activity (%) of the juice amounted to 64.5%. The tannins content was 6.2 mg g⁻¹. Data in Table 1 show the values of different polyphenol compounds as determined by HPLC. As shown from results the juice formula contains variable amounts of polyphenols and possesses an antioxidant power, also contain considerable quantity of tannin.

**Postprandial absorption curves:** The postprandial curves obtained following ingestion of the green vegetables juice plus breakfast, breakfast plus oral hypoglycemic drug and breakfast only are shown in Fig. 1. As shown in the figure, the diabetic group 3 that consumed the legumes juice before their usual breakfast had a reduction in the postprandial glycemic spikes. As shown in Table 2, all 3 groups of diabetic patients had no significant differences between their fasting blood glucose where the mean and SEM of the 3 groups was as follows: Group (1) 121.3 ± 1.6, group (2) 118 ± 1.8 and group (3) 119.4 ± 2.7.

At 60 min, mean and SEM blood glucose values increased to 235 ± 3.3 for group 1 also group 2 values were elevated to 198 ± 2.7 but significantly lower than group 1. As for group 3

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Level (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocatechuic acid</td>
<td>-</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>23.0</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>3.2</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>-</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>11.2</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>-</td>
</tr>
<tr>
<td>Coumarin</td>
<td>48.3</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>24.4</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>2.8</td>
</tr>
<tr>
<td>Chrysin</td>
<td>45.8</td>
</tr>
</tbody>
</table>

Table 1: Phenolic compounds (µg g⁻¹) in Juicer formula
blood glucose was significantly lower than the other 2 groups. After 120 min postprandial, there was a significant difference between group 2 (166±2.8) and group 3 (181.3±3.9).

**DISCUSSION**

The obtained results clearly show that postprandial elevations in blood glucose levels in type 2 diabetic patients were significantly reduced by the ingestion of the vegetable juice formula before consumption of breakfast. Postprandial blood glucose control in T2DM patients due to ingestion of rapidly digested carbohydrates is of great health concern, because it results in a quick increase in the post-meal blood glucose level\(^2\). Regular consumption of rapidly digestible carbohydrates is known to be the main cause for the development of hyperglycemia that leads to incidence of chronic diseases\(^1\), induced by glycosylation of proteins\(^2\). It is thus clear that the vegetable juice formula could avoid the presence of rapidly digestible carbohydrates thus did not cause sharp hyperglycemia that lead to complication of the disease.

This substantial reduction in postprandial glycemia by the vegetable juice in those patients could be influenced by several factors such as slowing digestion of carbohydrates and delaying gastric emptying which leads to a reduction in glycemic excursions mainly due to dietary fiber in the vegetable juice\(^2\). These findings support the present results indicating control of the blood sugar rise after meals. The other possibility for the decrease of the post-prandial hyperglycemia is through plant-based enzyme inhibitors that cause an inhibition of the enzymes α-amylase and α-glucosidase, in the digestive tract leading to the retardation of glucose absorption. So they delay the rate of glucose absorption by preventing carbohydrate digestion and consequently damping the postprandial plasma glucose rise\(^2\). This agrees with the results obtained from this study containing mainly vegetables and fruits. It is important to mention here that plant-based enzyme inhibitors are known to be potentially safer than synthetic enzyme inhibitors\(^2\). The use of plant-based enzyme inhibitors is also encouraged because there is concern about the critical side effects of synthetic pharmaceutical agents to either avoid or decrease the adverse effects of currently used synthetic agents\(^1\).

Chickpeas contained in the juice formula may also be beneficial in controlling postprandial glucose level in type 2 diabetes because it has a low glycemic index through having higher levels of slowly digestible starch. It is worth mentioning that chickpeas were found to have a positive effect on the pathways related to improving insulin sensitivity\(^2\). It is also possible that some of the isoflavone components found in chickpea may be responsible for this effect\(^1\). Yang et al.\(^2\) found that chickpea have an insulin-sensitizing action in high-fat-fed rats, attributed to the possibility that some components of chickpea such as isoflavone improved insulin resistance (in the ITT) and reversed impaired glucose intolerance.
Green beans are rich source of vitamins A, C, K, folic acid and fiber. Previous studies showed that consumption of more plant foods, such as green beans in the diet decreases the risk of obesity, diabetes, heart disease and overall mortality\textsuperscript{10}. Diabetes sufferers generally have high blood glucose levels that come from an inability to produce enough insulin and/or effectively utilize the insulin.

Green beans are low in calories and carbohydrates. They were found to limit the spikes in glucose levels after meals. According to Martin\textsuperscript{33}, the green beans pods skin contains silica and certain hormone substances that are closely related to insulin. Green beans are full of antioxidants that neutralize harmful free radicals. They are an excellent source of vitamin A and C\textsuperscript{32}.

The results obtained (Table 1) show that the prepared juice contain considerable amount of polyphenols which are usually found in plant-based foods including vegetables and legumes\textsuperscript{33}. In general, it can be stated that dietary polyphenols can inhibit both salivary and pancreatic amylase activities and also alpha amylase, thus inhibiting glucose absorption. Also stimulation of insulin secretion and protection of pancreatic $\beta$-cells from gluco-toxicity. It was found that polyphenols could suppress glucose release from the liver and enhance glucose uptake in peripheral tissues by regulating intracellular signaling\textsuperscript{34}.

Polyphenols also are known to have antioxidant role\textsuperscript{35} and could stimulate the growth and activity of some beneficial bacteria in the digestive tract, thus leads to improved glucose tolerance\textsuperscript{66,37}. The findings in this study can be explained based on the above information, since the present juice formula contains considerable quantities of polyphenols. Experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes and more importantly in the development of diabetic complications\textsuperscript{14}. It has been revealed that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models\textsuperscript{59}, as well as reducing the severity of diabetic complications\textsuperscript{40} and this study is parallel to the present findings.

The juice formula used in this study containing broccoli, spinach, celery, green beans and chickpea proved to possess antioxidant power due to the presence of polyphenol, isoflavones together with other compounds with reducing power present in these vegetables such as vitamin C and $\beta$-carotene. Polyphenols may also possess hypoglycemic effect through inhibiting glucose absorption, stimulation of insulin secretion, reduction of hepatic glucose output and enhancing glucose uptake\textsuperscript{38}.

Celery contains fiber, vitamin C, folate, vitamin K, potassium, manganese, riboflavin, vitamin B6, magnesium and calcium. It also contains some antioxidants such as vitamin E, zinc and selenium\textsuperscript{41}.

Celery extract possess several nutraceutical properties such as being an antioxidant with hypolipidemic action, beside being hypoglycemic with antiplatelet aggregation effect\textsuperscript{42}.

Among vegetables used in this given formula is spinach which is known to contain considerable amount of vitamin C\textsuperscript{43}. Spinach leaves also contain cyanogenic glycosides and possess an antioxidant power\textsuperscript{44,45} which may contribute to its anti-diabetic character particularly type 2-diabetes as previously reported by Sah et al.\textsuperscript{46}. In addition spinach is rich in nitrates which enhance the production of nitric oxide in the body thus promote the general health state and in turn improve hyperglycemia in type2-diabetes\textsuperscript{47,48}.

Broccoli extract was found to lower blood sugar in type 2-diabetic patients. This was attributed to a compound called sulforaphane, a chemical found in broccoli sprouts\textsuperscript{49}. Sulforaphane was reported to modulate Nrf2, NF-$\kappa$B and PPAR$\gamma$ signaling and protect vascular, adipose, hepatic and/or pancreatic cells and tissues. It is a promoting therapy for type 2-diabetes\textsuperscript{46}. The above findings show that the selection of the vegetables and fruits used to prepare the present juice formula was well done to realize the target of prohibiting the sharp rise of blood sugar in diabetic patients after meals.

**CONCLUSION**

It is clear that the given plant juice formed from spinach, broccoli, celery, green beans and chickpea could lower postprandial glucose concentration in blood, thus could synergize and maximize the effect of the treatment drug. The effect was attributed to the presence of compounds with antioxidant and health value such as polyphenols, vitamins, minerals and polyunsaturated fatty acids. This juice formula is recommended to be taken by type 2-diabetic patients.

**ACKNOWLEDGMENT**

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**SIGNIFICANCE STATEMENT**

This research work discovered a new formula that help diabetics to reduce their blood sugar and minimize the dose
of the treatment drug, thus avoid health hazards and side effects of chemicals. This formula is also rich in natural vitamins, phytonutrients and minerals that are of high health concern to individuals. This formula is relatively new and can be added to the already studied sources by other authors. This study revealed that the vegetable and legume juice formula reduce postprandial blood glucose by slowing down digestion of carbohydrates, delaying gastric emptying and leading to retardation of glucose absorption. Thus, this formula of plant-based juice can be useful for type 2 diabetic patients. It is recommended to be consumed daily before breakfast for controlling postprandial blood glucose level and reduce anti-diabetic drug dose.

REFERENCES


