Broccoli sprouts powder could improve serum triglyceride and oxidized LDL/LDL-cholesterol ratio in type 2 diabetic patients: A randomized double-blind placebo-controlled clinical trial

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Abstract

Background and aims: In this study, broccoli sprout powder (BSP), a good source of bioactive components, was used as supplementary treatment in type 2 diabetic patients.

Methods: This randomized clinical trial included 81 patients with type 2 diabetes. Participants were randomly assigned to consume 10 g/d BSP (group A), 5 g/d BSP (group B), or the placebo (group C), each for 4 weeks. Fasting blood glucose (FBS), total cholesterol (TC), triglyceride concentration (TG), LDL-C, HDL-C, and oxidized-LDL were measured at baseline and 4 weeks after treatment. The ratios of OX-LDL/LDL, atherogenic index of plasma (AIP; log TG/HDL), TC/HDL and LDL/HDL were calculated as cardiovascular risk factors parameters, at baseline and 4 weeks after treatment.

Results: Seventy-two patients completed the study; n = 23, 26 and 23 for groups A, B and C, respectively. After 4 weeks, BSP in dose of 10 g/d, significantly decreased serum triglycerides, OX-LDL/LDL ratio and AIP (p < 0.05 for treatment effect). HDL-C concentration was significantly higher (p < 0.01 for treatment) in group A as compared with group B and placebo.

Conclusions: BSP as supplementary treatment in type 2 diabetes could have favorable effects on lipid profiles and OX-LDL/LDL ratio, as risk factors for cardiovascular disease.

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1. Introduction

Type 2 diabetes is associated with dyslipidemia and increased lipid peroxidation, key factors promoting atherosclerosis in these patients [1,2]. Despite availability of multiple interventions, studies show a rising trend in the occurrence of the diabetes complications [3]. Several nutraceuticals such as antioxidant phytochemicals used in clinical trials had favorable effects in the prevention of pathogenesis of diabetes mellitus and its complications [4,5]. Broccoli sprouts are rich source of health promoting compounds including glucosinolates and isothiocyanates [6-8]. Animal studies have reported that young broccoli sprout improved lipid profiles [9,10]. A key bioactive component in broccoli sprout is sulforaphane (1-isothiocyanate-4-methylsulfinylbutane) which is the most potent inducer of the endogenous antioxidant defense [11,12]. Recently a phase 1 study revealed that consuming fresh broccoli sprouts for one week increased HDL-C, and decreased total cholesterol, LDL-C and some biomarkers of oxidative stress [13]. Short duration of intervention, small sample size and lack of control group were some limitations of this study. The effects of broccoli sprout on lipid profile abnormalities and oxidized LDL/LDL-C ratio as cardiovascular disease risk factors in diabetic patients, has not yet been determined. We therefore conducted this trial to investigate the effect of broccoli sprouts powder on fasting blood glucose, oxidized-LDL/LDL-C ratio, lipid profiles and other lipid related parameters such as atherogenic index of plasma, in type 2 diabetic patients.

2. Materials and methods

2.1. Patients and study design

This is a parallel, randomized, double-blind and placebo controlled clinical trial, conducted between March and July 2010. Ethics approval was obtained from the ethical committee of the Research Institute for Endocrine Science of the Shahid Beheshti University of Medical Sciences. The trial has been registered in the Iranian Registry of Clinical Trials at http://www.irct.ir with the following identification: IRTC138901181640N2. The results have been reported according to Consolidated Standards of Reporting Trials guidelines [14]. Diabetic patient who were referred to the Iran Diabetes Society and endocrine clinic of Taleghani Medical Center, were screened for inclusion in the study. Patients aged 18-60 years, with a clinical diagnosis of type 2 diabetes for at least one year were recruited. Patients were excluded from the trial if they had severe impairment of cardiac, hepatic or renal function, gestation or lactation and if they used insulin injection or consumed estrogen, vitamin K-antagonists or antioxidant supplements. Eighty-one patients, initially eligible, were randomized to three groups, A (BSP 10 g/d, n = 27), B (BSP 5 g/d, n = 29) and C (placebo, n = 25), using computer-generated random number table. Randomization was performed by stratification for body mass index (BMI ≤ 25 kg/m², 25 < BMI ≤ 30 kg/m² and BMI > 30), with the use of sealed envelopes Group allocation was blinded to the investigator and participants. In order to assess dietary changes during the study period three-day dietary recalls, including 2 weekdays and 1 weekend day, was collected at baseline and again after 4 weeks from the subjects. Since the Iranian Food Composition Table (FCT), with limited data on nutrient content of raw foods and beverages, is incomplete, the U.S. Department of Agriculture (USDA) FCT was used to calculate energy and nutrient intakes [15]. However, the Iranian FCT [16] was used for some national foods that are not listed in the USDA FCT. An Excel program was designed to analyze the nutrients of each food item. In Excel sheet all nutrients of each food item was formulated based on 1 g of it.

Weight was measured with digital scale (Seca 707, Hamburg, Germany) while the subjects were minimally clothed without shoes. Height was measured to the nearest 0.5 cm, in a standing position without shoes, using a tape meter. Body mass index was calculated at baseline and 4 weeks later.

2.2. Interventions

Broccoli sprouts powder was purchased from Cyvex Nutrition Company (CA, USA). At baseline, subjects received 28 packets containing either 5 or 10 g of broccoli sprouts powder in the BSP groups whereas controls were given 5 g corn starch powder colored with chlorophyll. All packets were identically packaged to be indistinguishable. Patients were recommended to consume one packet daily for 4 weeks, preferably with a beverage after meals to reduce gastrointestinal complications; also they were asked to maintain their regular diet and lifestyle, during the study period. Patients were contacted every week to evaluate compliance to the intervention and to enquire regarding possible side effects. Patients were excluded from the analysis if they consumed < 80% of the packets or had changed their medication or reported severe side effects. Based on patients compliance, allergic reactions such as rash, or gastrointestinal events includes diarrhea and vomiting, or unusual headache, was considered as sever side effects.

2.3. Biochemical analysis

At baseline and again 4 weeks after intervention, the 12-h fasting blood samples were collected into tubes containing 0.1% EDTA and were centrifuged at 4 °C and 500 g for 10 min to separate plasma. Fasting plasma glucose was measured by the enzymatic colorimetric method using a glucose oxidation kit (Pars Azmun Company, Tehran, Iran). Serum total cholesterol and triglycerides level were measured by enzymatic colorimetric analysis with cholesterol esterase and cholesterol oxidase and glycerol phosphate oxidase, respectively (Pars Azmun Company, Tehran, Iran). High density lipoprotein cholesterol was measured by the immunoturbidimetry method after precipitation of apo B containing lipoproteins with phosphotungstic acid (Pars Azmun Company, Tehran, Iran). The monoclonal antibody was used to quantify the concentration of serum oxidized LDL using the ELISA kit (Mecodia Company, Uppsala, Sweden). LDL-C was calculated from serum total cholesterol, triglycerides and HDL-C, according to the Friedewald equation [17]. Inter- and Intra-assay
The coefficient of variations of all assays were < 5%. The ratios of OX-LDL/LDL, atherogenic index of plasma (AIP; log TG/HDL), TC/HDL and LDL/HDL ratio were calculated as cardiovascular risk factors parameters, at baseline and 4 weeks after treatment.

2.4. Statistical methods

The sample size was designed to detect a 25 mg/dl difference among groups in total cholesterol with 95% CI and 90% power. The sample size with regard to the possible loss of the samples was calculated as 25 patients in each group. Statistical analysis was performed with SPSS (version 16.0; SPSS, Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to test for a normal distribution. Differences between the three groups at baseline were tested with one-way ANOVA. Student’s paired t test was used to compare baseline and 4-week values in each group. To compare means of the variables after 4 weeks and obtain the main effect of treatment (10 g/d BSP, 5 g/d BSP or placebo), the general linear model (ANCOVA) was used with 4-week values as dependent variables, baseline values as covariates and treatment group as a fixed factor. When the analysis indicated a significant effect of treatment, the groups were compared pair-wise by the Bonferroni. The percent change for each variable was also calculated by the formula: 

\[ \text{Percent change} = \left( \frac{\text{4-week values}}{\text{baseline values}} \right) - 100 \]

A \( p \)-Value < 0.05 was considered significant.

3. Results

Of eighty-one randomized patients, seventy-two completed the study [10 g/d BSP (n = 23), 5 g/d BSP (n = 26), placebo (n = 23)] were included in the analysis (Fig. 1). No significant differences between groups were seen for age, sex, weight, and body mass index, duration of diabetes and antidiabetic drugs; nor were there any significant differences in the clinical variables.
measured between the patients in the 3 treatment groups at baseline (Table 1). Mean dietary intakes of participants at baseline and after 4-weeks of intervention are presented in Table 2. There was no significant difference between the groups in total energy and nutrient intakes, as estimated by 3-day dietary recalls. Baseline and 4-weeks biochemical values of participants in the three groups, and the treatment effects of BSP on FBS, lipid profiles, OX-LDL/LDL ratio and AIP are presented in Table 3. Fig. 2 presents the mean percent changes of variables in the three groups after 4 weeks intervention. FBS, TC, and LDL-C significantly decreased in group A and B after intervention period, but there was no significant difference in these variables in the fourth week, between three groups.

BSP treatment resulted in significant decrease in serum TG and OX-LDL/LDL ratio and AIP (p < 0.05 for treatment effect) in group A as compared to group B and control. At the end of the study, HDL-C concentration was significantly higher (p < 0.01 for treatment effect) in group A as compared with group B and placebo.

### Table 2 - Dietary intakes of the study participants at baseline and after 4 weeks of intervention in the three groups.

<table>
<thead>
<tr>
<th></th>
<th>10 g/d BSP (n, 21)</th>
<th>5 g/d BSP (n, 22)</th>
<th>Placebo (n, 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 4 Weeks</td>
<td>Baseline 4 Weeks</td>
<td>Baseline 4 Weeks</td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>1883 ± 572</td>
<td>1895 ± 576</td>
<td>1850 ± 680</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>231 ± 76</td>
<td>231 ± 70</td>
<td>250 ± 91</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>67.2 ± 17.5</td>
<td>68.1 ± 16.3</td>
<td>62.6 ± 19.3</td>
</tr>
<tr>
<td>Total fat (g/d)</td>
<td>66.1 ± 23.3</td>
<td>67.2 ± 24.5</td>
<td>66.6 ± 28.1</td>
</tr>
<tr>
<td>Saturated fat (g/d)</td>
<td>24.9 ± 8.8</td>
<td>24.5 ± 7.3</td>
<td>21.1 ± 8.6</td>
</tr>
<tr>
<td>Mono-unsaturated fat (g/d)</td>
<td>17.3 ± 8.7</td>
<td>17.1 ± 8.0</td>
<td>16.3 ± 9.5</td>
</tr>
<tr>
<td>Poly-unsaturated fat (g/d)</td>
<td>10.3 ± 5.7</td>
<td>7.9 ± 3.8</td>
<td>10.9 ± 7.3</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>75.1 ± 45.1</td>
<td>81.4 ± 44.9</td>
<td>96.3 ± 49.0</td>
</tr>
<tr>
<td>Vitamin E (mg/d)</td>
<td>10.7 ± 7.1</td>
<td>11.5 ± 8.6</td>
<td>8.9 ± 4.9</td>
</tr>
<tr>
<td>Total fiber (g/d)</td>
<td>31.7 ± 14.6</td>
<td>28.7 ± 17.0</td>
<td>28.5 ± 13.7</td>
</tr>
</tbody>
</table>

Data are mean ± SD. There were no significant differences in dietary intakes between the three groups (analysis of variance), and no significant differences in 4-week dietary intakes as compared with baseline in the three groups (student’s paired t-test).

### Table 3 - Biochemical values of patients after 4 weeks in BSP and placebo groups.

<table>
<thead>
<tr>
<th></th>
<th>(A) 10 g/d BSP (n = 23)</th>
<th>(B) 5 g/d BSP (n = 26)</th>
<th>(C) Placebo (n = 22)</th>
<th>P for treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 4 Weeks</td>
<td>Baseline 4 Weeks</td>
<td>Baseline 4 Weeks</td>
<td></td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>198 ± 109</td>
<td>154 ± 74</td>
<td>146 ± 55</td>
<td>126 ± 51</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>196 ± 43.6</td>
<td>177 ± 47.3</td>
<td>175 ± 28.9</td>
<td>150 ± 27.1</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>174 ± 93</td>
<td>135 ± 66</td>
<td>144 ± 54</td>
<td>131 ± 53</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>113 ± 38.2</td>
<td>100 ± 35.6</td>
<td>98 ± 26.7</td>
<td>82 ± 23.1</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>50.5 ± 13.5</td>
<td>48.8 ± 13.5</td>
<td>47.6 ± 9.7</td>
<td>41.7 ± 8.1</td>
</tr>
<tr>
<td>OX-LDL/LDL-C ratio</td>
<td>3.16 ± 1.30</td>
<td>2.63 ± 1.06</td>
<td>3.33 ± 1.36</td>
<td>3.35 ± 0.85</td>
</tr>
<tr>
<td>AIP</td>
<td>0.049 ± 0.02</td>
<td>0.041 ± 0.01</td>
<td>0.046 ± 0.01</td>
<td>0.047 ± 0.02</td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>4.14 ± 1.33</td>
<td>3.92 ± 1.23</td>
<td>3.78 ± 0.87</td>
<td>3.70 ± 0.82</td>
</tr>
<tr>
<td>LDL-C/HDL-C ratio</td>
<td>2.37 ± 0.86</td>
<td>2.22 ± 0.86</td>
<td>2.13 ± 0.67</td>
<td>2.02 ± 0.59</td>
</tr>
</tbody>
</table>

FBS, fasting blood glucose; TC, total cholesterol; TG, triglyceride concentration; LDL-C, low density lipoprotein; HDL-C, high density lipoprotein; OX-LDL, oxidized-low density lipoprotein; AIP, atherogenic index of plasma (conversion factors from mg/dl to mmol/l: glucose, 0.05551; triglycerides, 0.01129; total cholesterol, 0.02586; LDL-C, 0.02586; HDL-C, 0.02586).

* All values are mean ± SE.

Calculating significance using a general linear model with 4-week values as dependent variables, baseline values as covariates and treatment group as a fixed factor.

** Significant different from baseline values (student paired t-test) p < 0.05.

*** Significant differences between groups (Bonferroni pairwise comparisons in general linear model) p < 0.05.

4. Discussion

In the current study, supplementation with 10 g/d BSP in type 2 diabetic patients for 4 weeks had favorable effects on most typical diabetic dyslipidemia and lipid related parameters, as risk factors for cardiovascular disease in these patients.

Hypertriglyceridemia is a common form of dyslipidemia that is frequently associated with coronary heart disease (CHD) [18]. One mmol/l increase in serum triglycerides has been independently related with 14% and 37% increased risk of CHD in men and women, respectively [19]. In type 2 diabetic patients, hypertriglyceridemia is also known as a potent and independent predictor of CHD and decrease in triglyceride levels is a main target of lipid lowering therapy in diabetes [20]. In addition to statin therapy, the combination of fibrates, niacin or omega-3 fatty acids, has often be used to treat hypertriglyceridemia in type 2 diabetic patients [21]. The effectiveness of fibrates in decrease of triglycerides had been reported 36% in a meta-analysis [22]. Four-year treatment with atrovasstatin leads to a 19% decrease in serum triglycerides.
accompanied with decrease in major cardiovascular events and risk of stroke in type 2 diabetic patients [23]. Hence an 18.7% decrease in serum TG in our study, through SBP supplementation may be clinically valuable.

Low HDL-cholesterol is an independent cardiovascular risk factor and management of HDL-cholesterol may contribute to the optimization of cardiovascular risk in Type 2 diabetes [20]. In our study, there were significant reduction in HDL-C levels in 5 g/d BSP group and controls, whereas HDL-C was unchanged in 10 g/d BSP group. The reason for this is unclear; however significant higher HDL level in 10 g/d BSP treatment group in 4th week as compared with control, may suggest beneficial effects of BSP on HDL-C in type 2 diabetic patients.

Although there were significant decrease in fasting blood glucose, total cholesterol and LDL-C in BSP groups after 4 weeks, but there was no significant difference in these variables in the fourth week, between three groups.

Elevation in oxidized LDL/LDL ratio, an accurate estimation of in vivo LDL oxidation, has been reported in type 2 diabetic patients [24]. Oxidized LDL/LDL-C ratio has been shown to be a significant and independent marker of cardiovascular risk factor [25]. Supplementation with 10 g/d SBP induced 13.5% decrease in OX-LDL/LDL ratio which may associate with decrease risk of atherogenic complication development in diabetic patients.

Fifty-tow percent decrease in the atherogenic index of plasma in dose of 10 g/d BSP as compared with placebo was another significant finding in the present study. Atherogenic index of plasma as defined logarithm of the TG/HDL-C ratio, directly related to lipoprotein particle size and the risk of atherosclerosis [26-28].

Few studies have investigated the effect of broccoli sprouts on lipid profiles. In an animal experiment, administration of broccoli sprouts extract in rats, decreased levels of serum total cholesterol, LDL-C and triglycerides, the atherogenic index of plasma, cardiac risk factor (cholesterol/HDL-C) and increased HDL-C [9]. In humans, data remain inconsistent; recently a phase 1 study showed that 100 g/d fresh broccoli sprouts intake improve lipid metabolism after a one week intervention period [11]. In contrast in a double-blind clinical trial, ingestion of 10 g/d broccoli sprouts powder during 4 weeks intervention in patients with hypertension, had no effects on LDL-C, total cholesterol and HDL-C [29].

The mechanism effects of broccoli sprout on lipid metabolism is not exactly clear; but in vitro study showed that phytonutrient compounds in broccoli such as isothiocyanates, bind with bile acids and reduce fat absorption [30]. Broccoli sprouts extract also inhibited lipoprotein lipase activity in adipose tissue [9]. In vitro and animal studies demonstrated that indol-3-carbinol, which is produced by the breakdown of the glucosinolate glucobrassicin in broccoli, decreased gene expression and activity key lipogenic enzymes including diacylglycerol acyltransferases, fatty acid synthase, and acyl-CoA-cholesterol acyltransferase [31,32]. In addition, indol glucosinolates reduced apolipoprotein B secretion as a primary apolipoprotein of low-density lipoproteins [33].

The effect of BSP on oxidized LDL/LDL ratio in our study could be related with a key bioactive component in broccoli sprouts, sulforaphane, which is the most potent inducer endogenous antioxidant defense [11,12]. Administration of 100 g/d fresh broccoli sprouts in healthy subjects reduced urinary 8-isoprostane and plasma phosphatidylcholine hydroperoxide and promoted the reduced form of coenzyme Q10/ coenzyme Q9 ratio as an effective inhibitor of oxidative damage in LDL particles [13,34]. Also ingestion of 200 mg/d dried broccoli sprouts in rats increased glutathione as a critical antioxidant for scavenging peroxides and other lipid derived oxidant [35].

The doses used in this study provided 225 μmol and 112 μmol sulforaphane isothiocyanates daily per 10 g and 5 g BSP doses, respectively. Significant effects on lipid profiles, OX-LDL/LDL ratio, and other lipid related parameters were seen only with the higher dose. Like others [11,36] we also observed mild gastrointestinal events mainly flatulence via administration of broccoli sprouts, in 33% (n = 9) and 17% (n = 5) of total patients in 10 and 5 g/d BSP groups, respectively.

![Fig. 2 – Mean differences of variables compared with baseline values in three groups (*significant difference within the groups using paired t-test, p < 0.05). FBS, fasting blood glucose; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein; HDL-C, high density lipoprotein; OX-LDL, oxidized-low density lipoprotein; AIP, atherogenic index of plasma.](image-url)
The present study has a few limitations. Study duration was 4 weeks and only one sample was obtained following intervention. The doses were limited and therefore dose–response analysis and determination of optimum dose were not possible.

In conclusion, administration of BSP as supplementary treatment in type 2 diabetes could have favorable effects on lipid profiles and OX-LDL/LDL ratio, as risk factors for cardiovascular disease. Further studies with longer duration and various doses may shed more light on the importance of the therapeutic effects of BSP in diabetic patients.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.diabres.2012.01.009.

REFERENCES


