Anethum graveolens L. supplementation has anti-inflammatory effect in type 2 diabetic patients

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This randomized, double-blind, placebo-controlled clinical trial designed to assess the effects of Anethum graveolens L. supplementation on the pro-inflammatory biomarkers (IL-6, TNF-α) and acute phase protein hs-CRP concentrations in type 2 diabetic patients. For 8 weeks, intervention group received 3.3 gm/day Anethum powder and placebo group received same amounts starch. At the onset and at the end of study collected 5 cc blood samples. The concentration of hs-CRP was measured by spectrophotometer method. IL-6 and TNF-α were assayed using ELISA method. Serum concentration of IL-6, TNF-α and hs-CRP decreased significantly at the end of study in intervention group (p<0.031, p<0.026, and p<0.045 respectively). Further clinical studies are needed to confirm the results.

Keywords: Anethum graveolens, Inflammatory biomarkers, Diabetic patients

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Diabetes mellitus (DM) is associated with many chronic disorders such as metabolic syndrome cardiovascular, cererbovascular and peripheral diseases. Blindness, end stage renal diseases and amputation are the most common complication of diabetes. There were approximately 194 million adults with diabetes mellitus aged between 29-79 yrs in 2003 worldwide and it is estimated to increase over 333 million by 2030¹. The prevalence of diabetes in Iran has been reported 7.7% in 2008².

Increasing the concentration of circulatory cytokines results in inflammation as a common feature of diabetic patients³,⁴. Inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF)-α and C-reactive protein (CRP) as an acute phase proteins, are produced by different cell types due to regulate the different organs’ metabolism⁵. Increasing the concentration of these cytokines in plasma by 2-3 fold, result in low-grade systemic inflammation⁶. According to the several studies, patients with type 2 diabetes and insulin resistance had high concentration of IL-6, TNF-α and CRP⁷-¹². High concentration of these inflammatory biomarkers can affect on insulin sensitivity through insulin signaling pathways¹³.

Although numerous medications have been identified to treat the inflammatory diseases, their long term use leads to more harmful side effects¹⁴. On the other hand, it was shown that, the use of herbs as complementary and alternative medicine has been prevailed in recent 20-25 years¹⁵. Anethum graveolens L. known as Dill, is one of the most common herbs with a long history of applying as a remedy and spices in foods¹⁶,¹⁷. This herb is an annual herb, belongs to the Apiaceae family¹⁸. It is growing in the Mediterranean region, Europe, central, southern Asia and widely cultured in the southeastern region of Iran¹⁷.

All segments of freshly plant including stem, leaves, seed and fruit are widely used for as condiment in foods and in various medicinal
productions\cite{16}. Anethum leaves are a source of mineral, protein and fiber\cite{19}. Anethoferon, carvone and limonene are the most important components of Anethum oil with a variety of biological roles\cite{20-24}. Myristicin, anethole, umbelliferone also, are other chemical constituents of this herb\cite{25-29}. Beside many beneficial effects of Anethum graveolens including anti-cancer, anti-spasmodic, anti-hypolipidemic and anti hypercholesterolemia, prevention of colic (in babies) and improving bad breath, galactagogue effects (in nursing mothers)\cite{27} and antimicrobial\cite{28,29}, a few experimental studies have been shown that Anethum extract can reduce inflammatory responses\cite{30,31}. Considering the deleterious complication of diabetes and due to the lack of studies regarding anti-inflammatory effects this herb in clinical field, this study designed to determine the effects of Anethum graveolens supplementation on some pro-inflammatory biomarkers (IL-6, TNF-\(\alpha\)) and acute phase protein hs-CRP in type 2 diabetic patients.

**Methods and materials**

**Study design**

This randomized, double-blind, placebo-controlled clinical trial was conducted on 60 type 2 diabetic patients. Subjects were recruited from diabetes association in Tabriz, Iran from January to March in 2012. The regional ethics committee of Tabriz University of Medical Sciences approved the research protocol by the number of 9043. Insulin therapy at the onset or during the study, smoking and use of alcohol, pregnant and breastfeeding women, consumption of Anethum and other herbal supplements, antioxidants, etc. during previous 3 months, presence the acute and chronic diseases including kidney, liver, cardiovascular and gastrointestinal diseases were exclusion criteria of study. After explanation of nature of intervention, finally a written informed consent was taken from 60 patients with ages between 18-65 yrs.

The sample size was determined based on data from previous study\cite{31}. By considering the confidence interval of 95\%, \(\alpha = 0.05\) and power of 80\%, using formula \(N = \frac{[(Z1-\alpha/2 + Z1-B)^2 \cdot (SD_1^2 + SD_2^2)]}{\Delta^2}\), 25 diabetic patients were computed per group. Regarding a possible loss in follow-up period, a margin of 20\% was determined, and finally 30 patients were allocated in each group. Figure 1 display how to recruit the patients at the beginning and during the study.

**Tablets preparation**

Fresh and green Anethum graveolens herbs (leaf, stem) were purchased from local market and after washing were dried to make powders. The powders were delivered to a pharmaceutical lab (Tabriz university of medical science, Iran) to prepare tablets containing 1.1 gm powder of Anethum in each. For the justification of prepared samples, Dr Delazar and Dr Nazzemiye as experts in pharmaceutical lab, controlled the samples. Starch was used to make placebo. The color and shape of Anethum and placebo tablets were similar together. A third person who not directly involved in the study was placed tablets in the same bottles. This person labeled the bottles with 2 cods which retained unknown for researchers until the end of intervention. To evaluate the compliance of patients, participants who consumed more than 90\% of tablets were included in statistical analysis and bottles containing tablets were given monthly.

**Treatment**

Eligible patients were randomly divided to intervention and placebo groups (n=30) based on a random block procedure produced by Random Allocation Software (RAS)\cite{32}. The intervention group received one tablet of Anethum after each meal (breakfast, lunch and dinner) and the placebo group received the same amount starch for 8 weeks. Participants were asked to continue their usual diet and medications according to physician prescription. During the study, 8 patients did not complete the study and data analysis were done on 52 patients (26 in each group) who carefully completed follow-up period and protocol of study.
Anthropometric and Biochemical assessments

Anthropometric indices measurements including weight, height, waist and hip circumference were measured at the baseline and after intervention. The body weights were measured without shoes and light clothing by a Seca scale (Seca, Hamburg, Germany). Heights were also measured using a stadiometer (Seca) without shoes. BMI was calculated as the weight in kilogram divided by the square of the height in meter. Waist and hip circumference was measured with a non-elastic tape.

Blood samples (5cc) were collected at the beginning and at the end of 8 weeks after 10-12 hrs fasting state and the serum samples were obtained by high-speed centrifugation at least 10 minute and were frozen immediately at −70°C until assay. The concentration of hs-CRP was measured by spectrophotometer method using commercial kit (Tehran, Iran). IL-6 and TNF-α were also was evaluated using ELISA method.

Statistical analysis

The data were analyzed by SPSS software (version 16.0; SPSS Inc, Chicago, IL). Normality of data was evaluated using the Kolmogorov-Smirnov test. Continuous variables were expressed as mean ± standard deviation (SD) and qualitative data were presented as frequency (percentage). Paired t-test was used to compare the differences within group before and after the supplementation. Analysis of covariance (ANCOVA) was used for the comparison of post treatment values of variables after adjusting for baseline values and confounding variables (age, gender, type of consumed hypoglycemic drug and use of hypolipidemic drugs) in both groups. For hs-CRP variable, because of non-normality, Mann-Whitney test was used to compare the intervention and placebo groups. In addition, Chi-square test was examined the differences in gender variable in both groups. P value less than 0.05 considered statistically significant.

Table 1—Demographic characteristics of participates in the onset of study (N=52)

<table>
<thead>
<tr>
<th>variables</th>
<th>Intervention group (N=26)</th>
<th>Placebo group (N=26)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) [Mean±SD]</td>
<td>53.11±7.23</td>
<td>53.11±7.93</td>
<td>1.00</td>
</tr>
<tr>
<td>Gender (N (%)) Male</td>
<td>11(42.3)</td>
<td>13(50.0)</td>
<td>0.582</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>74.13±12.83</td>
<td>79.38±16.97</td>
<td>0.214</td>
</tr>
<tr>
<td>BMI (Kg/m^2)</td>
<td>28.20±3.93</td>
<td>30.63±5.19</td>
<td>0.062</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>96.53±10.78</td>
<td>100.92±11.22</td>
<td>0.157</td>
</tr>
<tr>
<td>Hip(cm)</td>
<td>107.73±8.64</td>
<td>108.73±8.63</td>
<td>0.678</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>8.20±2.59</td>
<td>7.27±2.15</td>
<td>0.001*</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>20.47±4.57</td>
<td>19.11±5.60</td>
<td>0.026*</td>
</tr>
<tr>
<td>Hs-CRP (pg/ml)</td>
<td>3.78±3.42</td>
<td>2.73±3.95</td>
<td>0.345</td>
</tr>
</tbody>
</table>

Table 2—Results of inflammatory biomarkers (IL-6, TNF-α, Hs-CRP) of type 2 diabetic patients supplemented with Anethum Graveolens (N=52)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Intervention group</th>
<th>Placebo group</th>
<th>p^</th>
<th>p^a</th>
<th>p^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>8.20±2.59</td>
<td>7.27±2.15</td>
<td>7.59±2.86</td>
<td>0.427</td>
<td>0.031*</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>20.47±4.57</td>
<td>19.11±5.60</td>
<td>19.98±5.32</td>
<td>0.214</td>
<td>0.026*</td>
</tr>
<tr>
<td>Hs-CRP (pg/ml)</td>
<td>3.78±3.42</td>
<td>2.73±3.95</td>
<td>2.78±3.95</td>
<td>0.312</td>
<td>0.045*</td>
</tr>
</tbody>
</table>

*statistically significant a Paired t-test, b: ANCOVA test between two groups with adjusting for baseline values and confounding variables (age, gender, type of consumed hypoglycemic drug, use of hypolipidemic drugs)
Discussion

Low grade inflammation, a common feature in type 2 diabetic patients, play a major role in pathogenesis of its secondary complications such as atherothrombosis. In this study, 8 week supplementation of *Anethum graveolens* in type 2 diabetic patients resulted in a significant reduction in inflammatory biomarkers. Few in vivo studies were assessed anti-inflammatory effects of *Anethum*; Valadi et al. showed that hydroalcoholic extract of *Anethum graveolens* seed (100, 200, 400, 500 mg/kg) in mice resulted in significant reduction in inflammation especially in high applied dose (500 mg/kg). In addition, Naseri et al. supplemented 6 male rats (*Rattus rattus*) in 3 groups (Formalin, Dill-Oil and Diclofenac-gel) for eight days with 2 gm of Dill-Oil and 2 gm del, respectively. Results manifested a significant reduction in inflammation compared Formalin as inflammatory group (p<0.001). In some studies, anti-inflammatory effects of carvone and limonene have been proved.

Limonene and carvone are the major components of *Anethum*. Limonene can suppress the cyclooxygenase 1 and 2 pathways and reduce the releases of inflammatory mediators. It is determined that green vegetables such as *Anethum* are rich in antioxidant components including vitamin C, polyphenols and carotenoieds. Reactive oxygen species (ROS) are associated with the inflammatory response and frequently they contribute to the tissue damaging effects of inflammatory reactions. *Anethum* may neutralize ROS by antioxidant components. In addition, it was shown that oral administration of Myristicin markedly inhibited lipopolysaccharide and D-galactosamine induced increasing serum TNF-α concentration in mice. Considering the side effects of numerous medications for treatment of inflammation as the most distinctive characteristics of diabetic patients and the beneficial effects of *Anethum graveolens* in significant reduction of inflammatory biomarkers, it can be suggested to add this vegetable in dietary plan of diabetics.

Conclusion

To the best of our knowledge, this study was the first study, which assessed the anti-inflammatory effects of *Anethum graveolens* in type 2 diabetic patients. It can be consider as a strength and novelty of our study, however, future studies are needed to confirm our results. In conclusion, the results of present study showed that supplementation of type 2 diabetic patients with *Anethum graveolens* for 8 weeks improved inflammatory responses through a significant reduction in inflammatory biomarkers (IL-6, TNF-α and hs-CRP) in intervention group. Considering the beneficial effects of *Anethum* with no obvious reported side effects, it can be suggested to relieve disorders with chronic inflammation.

Acknowledgement

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