Nigella sativa Tea Improved Serum Paraoxonase-1 Activity, Glycemic Control and Lipid Profile in Type 2 Diabetes Mellitus

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ABSTRACT

Background: Most studies have found that paraoxonase-1 (PON-1) activity is reduced in type 1 and type 2 diabetic patients. The activity of PON-1 was found to be inversely correlated with the development of atherosclerosis. Nigella sativa (N. sativa) has favorable effect as regard glycemic control and dyslipidemia. Objective: The aim of this work was to study the effect of N. sativa tea (hot water extract) on glycemic control, lipid profile and PON-1 in type 2 diabetes mellitus (T2DM). Subjects and Methods: Sixty six subjects were divided into two age, sex and culture matched groups. The first group included 41 patients with T2DM and the second group included 25 apparently healthy volunteers. The patients were chosen to be free of diabetic complications. All subjects received N. sativa tea (5gm/day) for 6 months. This was added to the oral antidiabetic drugs in the patients group. All subjects were submitted to thorough clinical workup and had the following laboratory investigations done: fasting (FBG) and postprandial (PPBG) blood glucose, glycosylated hemoglobin (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum bilirubin (total, direct and indirect), blood urea, serum creatinine, serum lipids (total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglycerides) and serum PON-1 activity. Blood sampling was done before and after 1, 2, 3 and 6 months of treatment. Results: By comparing the mean values of the 1st, 2nd, 3rd and 6th months with that before treatment in both groups, there were significant decreases of FBG, PPBG, AST, ALT, total serum bilirubin, blood urea, and serum creatinine. Again, by comparing the mean values of the 3rd and 6th months with that before treatment in both groups, there were significant decreases of HbA1c, serum total cholesterol, LDL-C and serum triglycerides, meanwhile HDL-C level showed a significant increase. In both groups, the mean values of serum PON-1 activity after 6 months of treatment were significantly increased when compared with that before treatment. Conclusion: N. sativa tea improved the glycemic control and the lipid profile and increased PON-1 activity in T2DM.

Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion and/or insulin action, which results in hyperglycemia with disturbances of carbohydrate, fat and protein metabolism (Heydari et al., 2010). Such insulin defect along with other mechanisms can damage many of the body’s systems (Agrawal et al., 2011). Type 2 diabetes mellitus (T2DM) results from an interaction between a genetic predisposition and environmental factors (Reimann et al., 2009). It is associated with a number of microvascular and macrovascular complications (Heydari et al., 2010). It is well established that DM is a strong risk factor of atherosclerosis. Not only does it stimulate the atherogenic progression but it also causes the destabilization of the atheromatous plaque leading to cardiovascular diseases (Mytas et al., 2009). Management of T2DM involves controlling weight and optimizing levels of blood glucose, blood pressure, lipids and reducing cardiovascular risk (American Diabetes Association, 2011).

Paraoxonase-1 (PON-1) is the common member of the PON gene family (Betanzos-Cabrera et al., 2011). It is synthesized by the liver (Précourt et al., 2011). In plasma, PON-1 is in close physical association with the high density lipoprotein cholesterol (HDL-c) (Abdin et al., 2010). The concentration of PON-1 is inversely correlated with the development of atherosclerosis. Moreover, its reduced activity is associated with hypercholesterolemia, DM and coronary vascular disease (Kasprzak et al., 2009). The mechanism by which
PON-1 is reduced in DM is poorly understood. Glycation can both inactivate PON-1 and increase lipid peroxidation in HDL-c (Deakin and James, 2004).

The use of plants as medicines dates from the earliest years of man’s evolution (Salem, 2005). Traditionally, *Nigella sativa* (*N. sativa*) plant has been in use in many Middle Eastern countries as a natural remedy for DM. Significant reduction in blood glucose level in humans following the use of the plant was reported by Bansal et al. (2010). This hypoglycemic effect of *N. sativa* can occur whether it is used as oil (Haq et al., 1999), powder (El-Shamy et al., 2000) or even in a plant mixture (El-Shabrawy and Nada, 1996). To our knowledge, no previous reports used *N. sativa* seeds in the form of hot water extract (*N. sativa* tea). There are also no reports about the effect of *N. sativa* on PON-1 activity.

The present work aimed to study the effect of *N. sativa* tea (hot water extract) on glycemic control, lipid profile and PON-1 activity in T2DM.

**Materials And Methods**

Sixty six subjects were included. They were consented in advance about the nature of the study. All subjects were submitted to thorough clinical workup before and during the experiment. They were divided into two age, sex and culture matched groups. The first group included 41 patients with T2DM. DM was diagnosed according to the ADA, 2012. The patients were chosen to be free of diabetic complications. They were recruited from the outpatient clinics of the National Research Centre and Ain Shams University Specialized Hospital, Cairo, Egypt. The second group included 25 apparently healthy volunteers.

All subjects received oral *N. sativa* tea (5gm/day) daily for 6 months. This was added to their oral antidiabetic drugs in the patients group. Mature *N. sativa* seeds were obtained from the Egyptian Ministry of Agriculture and packed at Atos Pharma factory, Belbes, Al-Sharqia, Egypt in filter packs.

Blood samples were collected from the subjects in the morning after an overnight fast. 5 ml of venous blood were collected using a sterile plastic syringe. 1 ml of venous blood was added to EDTA in a clean dry test tube to separate plasma. The EDTA-plasma has been used for HbA 1c determination (after periods of 3 and 6 months of treatment). To obtain serum, the other 4 ml of venous blood were left in a dry centrifuge tube for 2 hours in room temperature to clot. Serum was obtained by centrifugation at 4000 r.p.m. for 15 min. at room temperature. 1ml of serum was taken immediately after separation for the measurement of FBG. The rest of serum was divided into 2 clean dry epindorph tubes, one for lipid profile assessment and the second for PON-1 activity determination. All samples were stored at -20ºC. After two hours of breakfast, 1 ml of blood was collected for the measurement of PPBG.

Fasting and postprandial blood glucose (Trinder, 1969), glycohemoglobin (HbA1c) (Abraham et al., 1978), serum total cholesterol (Richmond, 1973 and Allain et al., 1974), HDL-c (Grove, 1979 and Burstein et al., 1980), LDL-c (Assmann et al., 1984), serum triglycerides (Fossati and Prencipe, 1982) were assayed colorimetrically. Paraoxonase-1 activity was determined according to Abbott *et al* (1995) using chemicals from Sigma-Aldrich Co.

**Statistical Analysis:**

All statistics are performed using SPSS 12 for Windows package (SPSS Inc., Chicago, IL, USA). The difference between groups was calculated using T-Test (Paired-Sample T-Test) and the data obtained in the present work are represented in tables as average (mean) ± standard deviation.

**Results:**

By comparing the mean values of the 1st, 2nd, 3rd and 6th months of treatment with *N. sativa* tea with that before treatment in both groups, statistical analysis showed the following results. In case of the normal group, there were significant decreases after the first (*p*≤0.05), the second (*p*≤0.01), the third (*p*≤0.001) and the sixth months (*p*≤0.001) of treatment. Meanwhile there were significant decreases (*p*≤0.001) of FBG in the diabetic patients after the 1st, 2nd, 3rd and 6th months of treatment. There were significant decreases (*p*≤0.001) of the mean values of PPBG after the 1st, 2nd, 3rd and 6th months of treatment compared with that before treatment in both the normal and diabetic groups. Statistical analysis showed significant decreases (*p*≤0.001) of the mean values of HbA1c % after the 3rd and 6th months of treatment with *N. sativa* tea when compared to that before treatment in both normal and diabetic groups (Table 1).

The mean values of serum total cholesterol, LDL-C and triglycerides showed significant decreases (*p*≤0.001) after the 3rd and 6th months of treatment with *N. sativa* tea when compared with that before treatment in both the normal and diabetic groups. The mean values of HDL-C of both the normal and diabetic groups exhibited significant increase (*p*≤0.001) after 3 and 6 months of treatment with *N. sativa* tea compared to the values before treatment. However, all the values of the normal subjects were within the normal ranges and the increases in the values recorded in diabetic patients were more pronounced (Table 2).
Daily treatment with *N. sativa* tea for 6 months revealed a significant ($p \leq 0.001$) increase in the mean value of serum PON-1 activity in both groups (Table 3).

<table>
<thead>
<tr>
<th>Time Group</th>
<th>Parameter</th>
<th>Before</th>
<th>After one month</th>
<th>T.V.</th>
<th>After six months</th>
<th>T.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>FBG</td>
<td>80.22</td>
<td>78.14</td>
<td>2.30</td>
<td>0.03</td>
<td>13.34</td>
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<tr>
<td></td>
<td>PON-1</td>
<td>101.3</td>
<td>14.12</td>
<td>5.06</td>
<td>0.000</td>
<td>92.20</td>
</tr>
<tr>
<td></td>
<td>HbA$_1c$</td>
<td>4.43</td>
<td>0.36</td>
<td>-</td>
<td>-</td>
<td>4.26</td>
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<tr>
<td>Diabetic</td>
<td>FBG</td>
<td>148.7</td>
<td>157.9</td>
<td>5.86</td>
<td>0.000</td>
<td>251.4</td>
</tr>
<tr>
<td></td>
<td>PON-1</td>
<td>251.4</td>
<td>266.09</td>
<td>6.82</td>
<td>0.000</td>
<td>174.27</td>
</tr>
<tr>
<td></td>
<td>HbA$_1c$</td>
<td>7.18</td>
<td>0.83</td>
<td>-</td>
<td>-</td>
<td>8.59</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±S.D.  

(P $< 0.05$): significant  
(P $< 0.01$): highly significant  
(P $< 0.001$): very highly significant

Table 2: Effect of *Nigella sativa* tea daily treatment (5gm/day) on serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and serum triglyceride levels (mg/dl) in normal subjects and diabetic patients after 3 & 6 months of treatment.

<table>
<thead>
<tr>
<th>Time Group</th>
<th>Parameter</th>
<th>Before</th>
<th>After three months</th>
<th>T.V.</th>
<th>After six months</th>
<th>T.V.</th>
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<tbody>
<tr>
<td>Normal</td>
<td>T. cholesterol</td>
<td>184.56</td>
<td>174.96</td>
<td>8.360</td>
<td>0.000</td>
<td>167.27</td>
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<td>HDL-C</td>
<td>46.96</td>
<td>50.83</td>
<td>-11.16</td>
<td>0.000</td>
<td>55.00</td>
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<tr>
<td></td>
<td>LDL-C</td>
<td>115.86</td>
<td>105.43</td>
<td>11.189</td>
<td>0.000</td>
<td>95.03</td>
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<tr>
<td></td>
<td>Triglyceride</td>
<td>83.14</td>
<td>76.20</td>
<td>10.185</td>
<td>0.000</td>
<td>70.92</td>
</tr>
<tr>
<td>Diabetic</td>
<td>T. cholesterol</td>
<td>243.85</td>
<td>217.92</td>
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<td>209.95</td>
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<td>HDL-C</td>
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<td>LDL-C</td>
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<td>Triglyceride</td>
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<td>7.942</td>
<td>0.000</td>
<td>114.17</td>
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</table>

Data are expressed as Mean±S.D.  

(P $< 0.05$): insignificant  
(P $< 0.01$): significant  
(P $< 0.001$): very highly significant

Table 3: Effect of *Nigella sativa* tea daily treatment (5gm/day) on serum paraoxonase-1 (PON-1) activity level (u/ml) in normal control subjects and diabetic patients after 6 months of treatment.

<table>
<thead>
<tr>
<th>Time Group</th>
<th>Parameter</th>
<th>Before</th>
<th>After six months</th>
<th>T.V.</th>
<th>P.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>PON-1 activity</td>
<td>632.20</td>
<td>664.60</td>
<td>-5.234</td>
<td>0.000</td>
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<tr>
<td>Diabetic</td>
<td>PON-1 activity</td>
<td>517.02</td>
<td>633.37</td>
<td>-16.489</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±S.D.  

(P $< 0.05$): insignificant  
(P $< 0.01$): highly significant  
(P $< 0.001$): very highly significant
Diabetes mellitus is a multifactorial disease, associated with a number of microvascular and macrovascular complications (Heydari et al., 2010). The treatment of DM includes weight loss, regular physical activity (American Diabetes Association, 2008), pharmacologic approaches (Karnib and Ziyadeh, 2010) and even using the natural remedy (Bamosa et al., 2010).

*Nigella sativa*, commonly named as black seed or black cumin. Traditionally, it has been in use in many Middle Eastern countries as a natural remedy for DM (Sankaranarayanan and Pari, 2011).

In the present work, *N. sativa* was supplemented in the form of tea (hot water extract) to all subjects for six months. In the patients group, it was supplemented to their OAD. In the diabetic group, *N. sativa* tea produced significant decreases in the levels of both FBG and PPBG. These significant decreases in the levels of FBG and PPBG were also obtained in the normal group. Six months treatment with *N. sativa* tea in the present work also showed significant decreases in the levels of HbA1c in both the normal and diabetic groups. In humans, few studies showed that *N. sativa* can reduce blood glucose level in T2DM and in normal controls (Bamosa et al., 2010). However, many studies have shown the antidiabetic effect of *N. sativa* in normal and in diabetic animal models (Meddah et al., 2009). Such studies used *N. sativa* in the form of a plant mixture (El-Shabrawy and Nada, 1996), powder (El-Shamy et al., 2000) or oil (Al-Hader et al., 1993). However, no one studied the effect of *N. sativa* as a tea. The significant improvement in the glycemic control in T2DM in this study, reflected by the significant decreases in the levels of FBG, PPBG and HbA1c after six months of using *N. sativa* tea, suggests the use of *N. sativa* tea as an adjuvant therapy for DM.

It is well established that DM is defined as a strong risk factor of atherosclerosis. Not only does it stimulate the atherogenic progression but it also causes the destabilization of the atheromatic plaque leading to severe ischemic events (Mytas et al., 2009). Many studies had examined the effect of different forms of *N. sativa* on lipids in different animal species. There were significant decreases in plasma triglyceride and cholesterol concentrations in the diabetic rats treated with TQ (Farah et al., 2010). There is also a significant reduction in cholesterol level in humans following the use of the plant (Bamosa et al., 1997).

Using *N. sativa* tea for six months in the present work showed very highly significant decreases in the levels of serum total cholesterol, serum LDL-c and triglyceride with a very highly significant increase in serum HDL-c in both normal control and diabetic groups. These results are in agreement with El-Shamy et al. (2000); Zaoui et al. (2002); Le et al. (2004); Khanam and Dewan (2009); Salem (2005) as they found that *N. sativa* in any of its forms such as powder, the fixed oil, the petroleum ether extract, the aqueous extract, the volatile oil even the crude *Nigella* can reduce the level of total serum cholesterol, LDL-c and triglyceride and can ameliorate the level of HDL-c. In addition to these studies, our work showed that *N. sativa* tea can ameliorate the lipid profile in DM.

Paraoxonase-1 belongs to the family of serum paraoxonases (Betanzos-Cabrera et al., 2011). PON-1 activity is reduced in several chronic diseases, including type1 and 2 diabetes mellitus, and hypercholesterolemia (Ikeda et al., 2009). Several studies suggest that a low-level plasma PON-1 activity is associated with an increased prevalence of atherosclerosis and could be an independent risk factor for coronary events (Mackness et al., 2003). PON-1 is a calcium-dependent esterase associated with HDL-c, HDL-c has been shown to prevent oxidative modification of LDL. So, PON-1 is believed to be in part responsible for the protective effect of HDL-c against LDL-c oxidation. Furthermore, serum PON-1 activity has been found to be lower in diseases where the HDL-c concentration is reduced such as DM. The antioxidant action of PON-1 is related to glycemic control in patients with or without diabetic complications (Abdin et al., 2010).

There is a lack of studies or available literatures that deal with the relation between PON-1 and *N. sativa*. In the present work, the six months treatment with *N. sativa* tea showed a very highly significant increase in the activity of PON-1 in both normal control and diabetic groups. It appeared that *N. sativa* has antiatherogenic characteristics.

The mechanism by which PON-1 is reduced in DM is poorly understood, but may be associated with hyperglycemia. Glycation can both inactivate PON-1 and increase lipid peroxidation in HDL-c. Glycated HDL-c also has a reduced ability to protect against oxidation (Deakin and James, 2004). This improvement in the activity of PON-1 with *N. sativa* tea in our patients may be due to the improvement in the glycemic control and in the level of HDL-c as the carrier and site of action of PON-1.

In conclusion, the six months treatment with *N. sativa* tea in addition to the usual OAD, diet and exercises showed improvement in glucose level, glycosylated hemoglobin, serum total cholesterol, HDL-c, LDL-c, triglyceride and PON-1. This improvement suggests the use of *N. sativa* tea in diabetic patients as an adjuvant therapy for DM.
References


