The Effect of Hydro Alcoholic Nettle (Urtica dioica) Extract on Oxidative Stress in Patients with Type 2 Diabetes: A Randomized Double-blind Clinical Trial

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Abstract: Diabetes type 2 is a metabolic disorder that characterized by hyperglycemia and insulin resistance. Hyperglycemia and impairment of oxidant/antioxidant balance, can increase oxidative stress and increase risk of cardiovascular disease. In the present study, Effects of hydro alcoholic extract of Nettle on oxidative stress in type 2 diabetes were evaluated. Fifty patients (27 men, 23 women) with type 2 diabetes patients were studied. They received 100 mg kg⁻¹ of nettle extract of body weight hydro alcoholic for 8 weeks. At the baseline and end of 8th weeks of intervention blood levels of oxidative stress markers were measured. Data was analyzed by SPSS version 18, p<0.05 was considered significant for all variables. After 8 weeks, Total Antioxidant Capacity (TAC) and Superoxidant Dismutase (SOD) showed a significant increase in the intervention group compared to the control group (p<0.05). The findings showed that the hydro alcoholic extract of nettle has increasing effects on TAC and SOD in patients with type 2 diabetes without no changes in Malondialdehyde (MDA) and Glutathione Peroxides (GFX) after eight weeks intervention.

Key words: Nettle, free radical, complications, antioxidant, type 2 diabetes

INTRODUCTION

Free radicals are atoms or molecules with unpaired electron, so they are active and can damage different tissues in the body. In healthy person there is a balance between antioxidant enzymes and free radical species in the body. The imbalance causes oxidative stress (Atalay and Laaksonen, 2002). Diabetes is one of the common diseases that are associated with increased oxidative stress (Abdel-Hamid et al., 2008). Hyperglycemia is one of the predisposing factor for oxidative stress (Rasheed et al., 2008), increasing oxidative stress can increase risk factors of cardiovascular incidence and other complications in patients with diabetes mellitus (Vincent et al., 2004; Malekirad et al., 2011), so every intervention with minimum side effect that reduce glucose level and oxidative stress markers can be effective for diabetes complication prevention (Rains and Jain, 2011).

In response to high interest of patients with diabetes for using alternative medicine, studies on antidiabetic herbs are increasing (Egede et al., 2002; Karim et al., 2011). *Urtica dioica* (Nettle) is one of the medical herbs. Several studies showed beneficial effects of nettle in different disease such as rheumatoid arthritis (Nourmohammadi et al., 2010), diabetes (Namazi et al., 2011a), atherosclerosis (Chrubasika et al., 2007; Namazi et al., 2011b) stomachache.

Some studies have shown antioxidant effects of nettle (Ozen et al., 2003; Kanter et al., 2005; Yener et al., 2009; Golalipour and Khor, 2007; Bitiren et al., 2010; Mahmoud et al., 2006; Odakeya et al., 2007). It seems that the effects of Nettle on oxidative stress markers in patients with type 2 diabetes have not studied, yet. So, the aim of this study was to investigate the effects of hydro alcoholic extract of Nettle on oxidative stress in patients with type 2 diabetes.

MATERIALS AND METHODS

A Randomized Double-blinded clinical trial was done on 50 patients (27 men, 23 women) with type 2 diabetes (T2DM) in Clinic of Diabetes in Sina Hospital of Tabriz (Tabriz is one of the city in North-west of Iran). The inclusion criteria for the trial were as follows: Both genders over the age of 30 years old, HbA1C levels equal...
or less than 10%, common diabetes drugs usage (Metformin and Glitinclamide), patients with triglyceride levels less than 400 mg dL⁻¹. The exclusion criteria included patients with cardiovascular, renal, liver or thyroid diseases, infections, allergies, argina and the regular use of NSAIIDs (Non-Steroid Anti Inflammatory Drugs), warfarin, alcohol, herbal tea, dietary supplements and insulin injection.

Patients were informed about purpose of the study, each patient that is satisfied with participate in the study, signed an informed consent form, they were advised to continue their diet and physical activity habits without any changes during intervention.

After adjusting the patients by age, sex and duration of diabetes, they were randomly divided into intervention and control groups, they received 100 mg kg⁻¹ of nettle extract or placebo in 3 portions a day. They dissolved each portion in 1 glass of water and drank after each 3 main meals for 8 weeks. Patients were contacted every week with telephone, they were asked for any compliance about nettle extract usage. Each two weeks, patients were asked to return any used bottles of extract and received new bottles. Biochemical, dietary record and physical activity were assessed at the beginning and end of the study. Eventually, forty five patients completed the study.

This research was approved by the Ethics committee and Human Studies review board of Iran-Tabriz University of Medical Sciences.

**Extract specifications:** Stinging Nettle certified by the Pharmacogenecy department in Iran-Gorgan University of Medical Science. Aerial parts of Nettle dried and powdered, extract was prepared with percolation method and ethanol (60°) was used. Final hydro alcoholic extract of Nettle contained 45% ethanol, 53% water and 2.7 g of dry matter in 1 L of extract. Water and alcohol percent in placebo was equal to Water and alcohol percent in Nettle extract, chlorophyll color added to placebo. There was not any difference in color between extract and placebo.

Participants were instructed to complete 24 h dietary recall for three days (2 week days and 1 weekend day) at baseline and the end of study. These records were used to calculate the habitual dietary energy and nutrient intake. International Physical Activity (IPA) questionnaires (Hagstromers et al., 2006) were filled out by researcher with face to face interview at the baseline and the end of study. Weight and height were measured by standard method, then BMI (Body Mass Index) was calculated by divided weight (kg) to square of height (m).

**Biochemical measurements:** Five milliter of blood was taken from forearm vein after an overnight fast (12-14h) at the beginning and end of the study. Two milliter of blood was collected in heparinized tubes for measurement of GPX activity in erythrocyte and TAC. TAC measured by Ferric Reducing Antioxidant Capacity (FRAP) method (Benzie and Strain, 1999).

Rest of blood sample was collected in tube containing EDTA, for measurement of erythrocytes SOD. SOD and GPX were measured by commercially kit (Ransel and Randox, UK) and by colorimetric method on an Autoanalyser. MDA level was used as the (Idonije et al., 2011) indicator of lipid peroxidation and were determined via reaction with thiobarbituric acid (TBA) (Ahmed et al., 2006).

**Statistical analysis:** Data are analysed as Mean±Standard Deviation (SD). Kolmogorov-Smirnov test was used to determine normality of the data. Data with Abnormal distribution were converted to normal distribution by calculating logarithmic ratio. Then data at the end of study were compared to their own baseline values by Paired t-test. Comparison quantitative and qualitative variables between two groups was performed by Student’s t-test and Exact fisher test, respectively. SPSS version 18 (IBM Inc, USA) was used for data statistical analyses. Assessment of dietary intake was done with Nutritionist IV software. The p<0.05 was considered significance for all variables.

**RESULTS**

The mean values of sex, age and duration of diabetes at the baseline, did not show any statistical significant differences because of adjusting before dividing patients into two groups. Physical activity level showed no significant differences in two groups at the baseline (p>0.05) (Table 1).

Comparison of two groups by BMI index showed that in case and control groups 9 and 11% of patient had normal BMI, 66 and 51% were overweight, respectively (Fig. 1). At the baseline, there were not any significant differences between groups. Also, there were not observed any statistical significant changes in BMI during the intervention.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention (n = 24)</th>
<th>Control (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)†</td>
<td>54.4±6.38</td>
<td>53.1±6.78</td>
</tr>
<tr>
<td>Sex (%)</td>
<td>Man 54.2</td>
<td>47.6</td>
</tr>
<tr>
<td></td>
<td>Woman 45.8</td>
<td>52.4</td>
</tr>
<tr>
<td>Duration of diabetes (Years)†</td>
<td>8.2±5.04</td>
<td>8.7±4.52</td>
</tr>
<tr>
<td>Smoking (%)‡</td>
<td>55.6</td>
<td>47.4</td>
</tr>
<tr>
<td>Diet obe (%)</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Physical activity (%)‡</td>
<td>Sitting 7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Walking 81</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Moderate 12</td>
<td>15</td>
</tr>
</tbody>
</table>

* Mean±SD, † Independent samples t-test for comparison of two groups at the baseline, ‡ Exact Fisher test
Table 2: Comparison of Dietary Intake in two groups at the baseline and the end

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>1697.8±465.6*</td>
<td>1996.7±243.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>265.6±61.5</td>
<td>281.3±27.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>63.1±17.9</td>
<td>63.3±14.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>66.8±20.2</td>
<td>62.1±14.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Zn (mg)</td>
<td>4.9±1.7</td>
<td>4.9±1.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Cr (mg)</td>
<td>0.03±0.01</td>
<td>0.02±0.01</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>154.9±108.5</td>
<td>71.3±42.8</td>
<td>0.01*</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>2.4±1.9</td>
<td>3.7±2.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin A (RE)</td>
<td>755.1±580.5</td>
<td>413.5±356.2</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

* Mean±SD; †: Independent samples t-test for comparison of two groups. p<0.05 considered as significant difference between two groups.

Table 3: Comparison of antioxidant status and MDA in two groups at the baseline and the end

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (umol mL⁻¹)</td>
<td>2.82±0.87</td>
<td>2.78±0.73</td>
<td>0.04</td>
</tr>
<tr>
<td>SOD (U mg⁻¹ Hb)</td>
<td>1200±111</td>
<td>1500±154</td>
<td>0.02*</td>
</tr>
<tr>
<td>GPX (U g⁻¹ Hb)</td>
<td>26.52±1.06</td>
<td>26.37±1.11</td>
<td>0.92</td>
</tr>
<tr>
<td>TAC (UmM Fe⁺⁻³ L⁻¹)</td>
<td>0.51±0.1</td>
<td>1.51±0.34</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Mean±SD; †: ANCOVA, p<0.05 considered as Significant difference between two groups.

**DISCUSSION**

This study showed that hydro alcoholic extract of Nettle increased TAC and increased SOD, in type 2 diabetes patients after 8 weeks. But it could not change GPX level and lipid peroxidation indicator (MDA). Results suggested that hydro alcoholic extract of Nettle can protect body against free radicals and diabetes complications.

Golalipour and Kho (2007) concluded that the hydroalcoholic extract of nettle, by the present antioxidant compounds scavenged free radicals, so could regenerate pancreatic beta cells. So, they suggested that nettle has protective effect against oxidative stress in hyperglycaemia rats.

Yener et al. (2009) studied on antioxidant effects of nettle seeds on rats, they showed that nettle has protective effect on liver against Aflatoxin, so they declared that nettle seed may have antioxidant properties. Gulcin et al. (2004) showed that water extract of nettle in 50, 100 and 250 µg mL⁻¹ have stronger antioxidant effects compared to alpha tocopherol in linoleic acid peroxidation. All of these reviews are in support of the results of present study.

Toldy et al. (2005) showed that 30 mg kg⁻¹ nettle decreased ROS (Reactive Oxygen Species). Another study showed that the hydroalcoholic extract of nettle, decreased brain peroxidation more than 50% and had inhibition effect on xanthine oxidase about 30%.

Ozen and Korkmaz (2003) with experiments on rats concluded that hydroalcoholic extract of nettle has significant effect on antioxidant enzymes such as catalase,
SOD, GPX and glutathione Reductase. Present study confirmed by Ozen and Korkmaz (2003) study about decreasing effects of nettle on SOD. On the other hand Ozen results about effects of nettle on GPX does not confirm the present study result. Dose, duration of diabetes, amount and solvent (Samsam-shariat, 1980) type may cause these differences in results of studies.

Studies have shown that flavonoids and carotenoids properties, in alcohol solvent showed more Antioxidant characteristics than water solvent (Annegowda et al., 2010). So, 45% ethanol in the present extract may caused suitable background in improving antioxidant status.

Poly phenol compounds, are the most important part of the flavonoids family (Boots et al., 2008), the polyphenols that are found in nettle can be pointed to tannin, anthocyanin, chlorogenic acid and cafe oil malaic these compounds can play antioxidant activity. Quercetin (3, 3, 4, 5, 7-penta hydroxy flavone), is dedicated to the greatest extent of flavonoids in nettle. These properties may be one of the factor that caused antioxidant characteristic of nettle in patients with type 2 diabetes (Pourmorad et al., 2006).

More studies are suggested for determination of antioxidant effects of nettle in patients with diabetes by longer time intervention and larger sample size.

CONCLUSION

Hydro alcoholic extract of Nettle increased TAC and increased SOD Levels in type 2 diabetes patients after 8 weeks intervention. So, it seems that hydro alcoholic extract of Nettle can play a protective role from CVD in patients with type 2 diabetes by improving Antioxidant status.

ACKNOWLEDGMENTS

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REFERENCES


