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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 (GPX) 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 Khori, 2007; Bitiren *et al*., 2010; Mahmoud *et al*., 2006; Odukoya *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

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or less than 10%, common diabetes drugs usage (Metformin and Glibenclamide), patients with triglyceride levels less than 400 mg dL^{-1}. The exclusion criteria included patients with cardiovascular, renal, liver or thyroid diseases, infections, allergies, angina and the regular use of NSAIDs (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 Pharmacogenosy 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, 55% 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 (Hagstromera *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 mililiter of blood was taken from forearm vein after an overnight fast (12-14 h) at the beginning and end of the study. Two mililiter 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 1: Characteristic of patients in intervention and control groups at baseline

Variable	Intervention (n = 24)	Control (n = 21)
Age (years)†	54.48±6.38	53.16±7.76*
Sex (%)		
Man	54.2	47.6
Woman	45.8	52.4
Duration of diabetes (Years)†	8.25±5.04	8.79±4.52
Smoking (%)◊	55.6	47.4
Diet obey (%)◊	15	11
Physical activity (%)◊		
Sitting	7	5
Walking	81	83
Moderate	12	15

*: 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

	Intervention		Control		
Variable	Beginning	End	Beginning	End	p-value
Energy (kcal)	1897.8±463.6*	1996.7±243.3	1881.4±230.5	2044.1±241.3	0.8
Carbohydrate (g)	265.6±61.5	281.3±27.7	261±38.0	290.7±25.5	0.1
Protein (g)	63.1±17.0	63.3±14.2	55.3±9.5	56.9±8.2	0.3
Total Fat (g)	68.0±20.2	62.1±14.4	67.5±7.2	61.0±7.2	0.9
Zn (mg)	4.9±1.7	4.9±1.7	4.0±1.6	6.2±5.9	0.2
Cr (mg)	0.03±0.01	0.02±0.01	0.03±0.02	0.03±0.01	0.5
Vitamin C (mg)	154.9±108.5	71.3±42.8	71.3±42.8	71.1±42.9	0.01†
Vitamin E (mg)	2.4±1.9	3.7±2.1†	2.9±2.5	1.9±1.3	0.2
Vitamin A (RE)	755.1±580.5	413.5±356.2	195.9±175.4	402.1±223.8	0.03†

*: 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

	Intervention		Control		
Variable	Beginning	End	Beginning	End	p-value
MDA (nmol mL^{-1})	2.82±0.87	2.78±0.73†	3.01±0.77	2.90±0.80	0.84
SOD (U mg^{-1} Hb)	1200±111	1500±154	1141±90	1100±95	0.02†
GPX (U g^{-1} Hb)	26.52±1.06	26.37±1.11†	27.30±1.17	27.32±1.10	0.92
TAC (UmM Fe^{2+} L^{-1})	0.51±0.01	1.51±0.34	0.90±0.19	0.95±0.21	0.02†

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

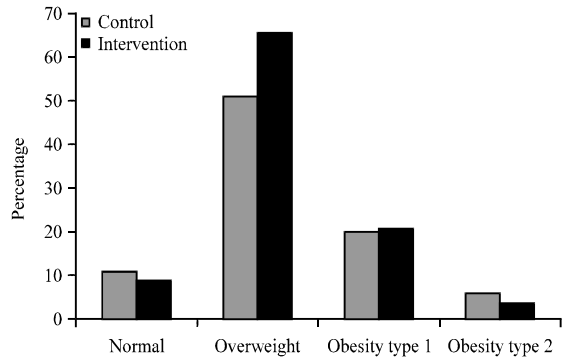

Fig. 1: Comparison of patients with Body Mass Index (BMI) in two groups at the baseline

Dietary intake showed significant difference in vitamin A and C levels between two groups at the baseline. For neutralizing their confounder effects, laboratory data were compared after adjusting two groups in vitamin A and C levels (Table 2).

Paired t-test showed that there were no changes in dietary intake and physical activity level during the study in both groups.

The antioxidant parameters and MDA showed significant differences at the baseline in two study groups (Table 3). Independent t-test showed statistical significant differences in SOD (1200±111 at the baseline, 1500±154 U mg^{-1} Hb at the end; $p = 0.02$) and TAC (1.51±0.34 at the baseline, 0.51±0.01 U m M Fe^{+2} L^{-1} at the end; $p = 0.02$) in intervention group compare to control group. But no statistically significant differences between two groups in MDA and GPX were seen ($p>0.05$).

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 peroxidant indicator (MDA). Results suggested that hydro alcoholic extract of Nettle can protect body against free radicals and diabetes complications.

Golalipour and Khori (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 hyperglycemia 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 compare 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 antioxidative status.

Poly phenol compounds, are the most important part of the flavonoids family (Boots *et al*., 2008), the polyphenols that are found in nettles can be pointed to tannin, anthocyanin, chlorogenic acid and cafe oil malic.these compounds can play antioxidant activity. Quercitin (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 antioxidative characteristic of nettle in patients with type 2 diabetes (Pourmorad *et al*., 2006).

More studies are suggested for determination of antioxidative 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.

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