ORIGINAL ARTICLE

Comparison of Sour and Black Tea Consumption on the Serum Lipid Oxidizability in Diabetic Patients

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ABSTRACT

OBJECTIVE: Oxidative stress is a serious complication in diabetic patients. Black tea and also sour tea contained some flavonoids with antioxidant properties and may be helpful in prevention of lipid oxidation. The aim of this study was to compare the effects of sour tea and black tea on in-vitro copper induced serum lipids oxidizability in diabetic patients.

MATERIALS AND METHODS: In this sequential double-blind randomized controlled clinical trial, 60 diabetic patients were divided into case and control group randomly. Patients in case group used sour tea, and control subjects used black tea as the same program for 4 weeks. Fasting blood samples were taken at the beginning and end of the study for evaluation of serum lipid oxidizability. Lipid oxidation was followed by the formation of conjugated dienes, in diluted serum, after added Cu^{2+}.

RESULTS: There were no significant differences in serum lipid oxidation parameters between the case and control groups.

CONCLUSION: Our finding revealed that sour tea or black tea consumption does not affect the quantitative parameters of serum lipid oxidation.

KEYWORDS: Diabetic patients, Sour tea, Black tea, Serum lipid oxidizability.

INTRODUCTION

Diabetes Mellitus (DM) is a common metabolic disease caused by insulin deficiency or decreased responsiveness of some body cells to secreted insulin. Insulin deficiency results in persistence increased blood glucose level, which in turn causes development of some chronic complications in vital organs including kidney and cardiovascular system (1).

Cardiovascular disease is an important chronic complication and life treating health problem in diabetic patients (2). Although the underlying mechanism in diabetic patients chronic complications are poorly understood, protein glycosylation and increased oxidative stress condition are the two plausible candidates (3,4). Elevated levels of plasma lipids and increased lipid peroxidation condition are closely implicated in the development and progression of atherosclerosis and development of cardiovascular disease in general population and also in diabetic patients (5,6). One of the most effective approaches for treating diabetes is to decrease the plasma levels of glucose and prevent of oxidative stress by diet supplementation in diabetic patients with

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natural herbal products (7,8).

Various species of tea contains many chemical constituents including some flavinoids with antioxidant properties (9,10). There are many reports about the antioxidant effects of black tea on animal model and humans (11-14). Some studies also have shown anti-hyperlipidemic and antioxidant effects of the sour tea on animal model, in-vitro studies and some human disease (15,16), but there is little available data about the antioxidant effects of the infusions of these herbal on whole serum lipid oxidizability in humans including diabetic patients. Therefore, the present study was designed and conducted to compare the antioxidant effects of consuming infusions sour tea and black tea on Cu-induced serum lipid oxidation by monitoring the formation of conjugated dienes in a group of diabetic patients who referred to Yazd Diabetes Research Center, Iran.

MATERIALS AND METHODS

Subjects and Study Design: This sequential double-blind randomized controlled trial was conducted on 60 type 2 diabetic patients in Yazd Diabetes Research Centre in 2007. Inclusion criteria were having type 2 diabetes Mellitus for more than 5 years and not taking anti-oxidant drugs. Exclusion criteria were having allergy to tea, preferring not to drink tea or suffering from other diseases which needed taking medicine for.

After obtaining informed consent from all patients, they were randomly assigned into one of the two groups. The patients in case group were given sour tea sachets and the patients in control group were given similar sachets, in shape and weight of black tea. Instructions for preparation and usage of black and sour tea were given. The patients were instructed to use one glass of the decoction (two spoonful of blended tea in one glass of boiling water boiled for 20-30 minutes) two times a day. On the first day of the study an overnight fasting blood sample was taken for determination of serum lipids, lipoprotein lipids, glucose, glycated hemoglobin (HbA1c) and assessing the serum lipid oxidizability. Evaluation of serum lipid oxidizability was repeated at the end of the study.

Study Duration and Tea Preparation Directions: According to various studies, drinking tea was continued for 1 month, two times a day, one in the morning and the second one in the afternoon, between the main meals. The patients were asked to pour one spoonful of tea in one glass of hot water (240 ml) and drink it after 20-30 minutes with one cube of sugar (5 gr). They were prohibited from drinking any other type or amount of tea during the study and their medications and diet were kept unchanged.

The sour tea was obtained from local market and verified by agricultural experts. The black tea was selected from one of certified domestic brands.

Measures and Biochemical Analyses: Overnight fasting blood samples were obtained before and after intervention. Serum separated from the clots after complete coagulation (1h in room temperature) by low speed centrifugation (15 min at 2000 g), and serum lipids, lipids lipoproteins and glucose concentration were determined and serum lipid oxidizability was evaluated. Serum Total Cholesterol (TC) and triglycerides (TG) concentrations were determined by enzymatic methods, cholesterol oxidase and glycerol oxidase, respectively. High Density Lipoprotein-Cholesterol (HDL-C) concentration was determined with dextran-sulfate-magnesium chloride precipitation of betalipoproteins, followed by the same enzymatic method for TC. Low Density Lipoprotein-Cholesterol (LDL-C) was calculated using Friedewald formula when the TG levels were less than 400 mg/dl (17). Glucose measured by glucose oxidase and HbA1c was determined by ion exchange chromatography. Copper-induced serum lipid peroxidation was estimated in a 60-fold diluted serum in 20 mM phosphate buffer containing 150 mM NaCl and 720 µM citrate, pH = 7.4. The lipid oxidation procedure was conducted at 37ºC and was initiated by addition of $CuCl_2$

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to give a final concentration of 60 μM. The kinetics of conjugated dienes formation were monitored spectrophotometrically (Perkin-Elmer UV.VIS Double beam spectrophotometer 505S) by measuring absorbance in a 1-cm quartz cuvette at 245 nm every 10 min for 300 min. Microsoft Excel software was used for plotting of the kinetic curves of the accumulation of lipid peroxide products (change of absorbance at 245 nm versus time in min), and a number of quantitative oxidation parameters including lag-time (the interval between the addition of $CuCl_2$ to the serum and the beginning of extensive oxidation), maximal rate of oxidation (V-max), maximal amount of lipid peroxide products accumulation (OD-max), and the time needed to gain maximal rate of oxidation (T-max) were evaluated (18). Before the processing of samples, method of serum lipid oxidation were optimized and an inter-individual coefficient of variations (CV) of 6% (for lag-time, n = 10), 7.4% (for OD-max, n = 10) and 7.5% (for V-max, n = 10) were obtained.

Statistical Analysis: All values reported are mean ± SD. Differences between sour tea and black tea group were assessed by student's T-test and paired T-test. P-value < 0.05 was considered as statistically significant. All P-values were two-tailed. All the tests were performed using SPSS Version 11 (SPSS Inc., Chicago, IL, USA).

Ethical Considerations: An informed consent was obtained from each patient. They could quit the study freely, whenever they liked. All the patients were continuing their medical treatment and no interruptions were made. The sour tea, as described in this study did not have any adverse affect on the patients; hence, it was thought to be useful for them. The Ethics Committee of Shahid Sadoughi University of Medical Sciences approved the study.

RESULTS

There were 60 patients in both groups at the beginning of the study, of whom 53 patients concluded the study. Three patients from case group and four patients from control group stepped out due to going on trips, illness or other reasons. These 53 patients included 45 female (84.9%) and 8 male (15.1%). Despite the majority of female gender among the patients, the sex distributions in these two groups did not show statistically significant difference; as in the ST group there were 22.2% male and 77.8% female patients and in BT group 7.7% were male and 92.3% female.

Eighty three percent of patients were on oral anti-hyperglycemic agents, 13.2% on insulin therapy and 20.8% on diet only, as their treatment. Regarding the treatment method, there was no statistically significant difference among the two groups. Compliance to tea consuming in both groups was around 92-95%.

The mean of quantitative variables of the study including weight, age, diabetes duration, BMI, fasting blood glucose, HbA1c, TC, LDL-C, HDL-C, and TG before the starting of the intervention are shown in table 1.

Table 1- Mean of quantitative variables in two study groups at the beginning of the study.

Variable	Sour Tea Group (n = 27)		Black Tea Group (n = 26)		Student's T-test P-Value
	Mean	SD	Mean	SD	
Weight (Kg)	70.44	11.31	69.90	10.11	0.8
Age (years)	55.37	8.6	50.42	8.56	0.04
Duration (years)	9.81	5.81	10.7	5.1	0.05
BMI (Kg/m2)	28.28	3.8	28.35	4.8	0.09
FBS (mg/dl)	213.22	55.8	196.4	73.5	0.3
HbA1c (%)	10.36	1.7	10.00	2.5	0.3
Total Cholesterol(mg/dl)	236.2	58.1	221.8	52.2	0.2
LDL_C (mg/dl)	137.5	53.1	123.9	55.4	0.2
HDL_C(mg/dl)	48.20	10.1	46.03	15.01	0.6
TG (mg/dl)	246.1	84.9	247.5	84.7	0.9

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The Kinetics analysis of conjugated diene production revealed that sour tea or black tea consumption does not affect the quantitative parameters of serum lipid oxidation. The mean of serum lipid oxidation parameters in two groups before and after intervention are shown in table 2 and table 3, respectively. In each group serum lipid oxidation parameters were compared before and after intervention. Serum lipid oxidation parameters before and after intervention in case and control groups are compared in table 4 and table 5, respectively.

Table 2- Mean of quantitative serum oxidation parameters in two study groups at the beginning of the study.

Variable	Sour Tea Group (n=27)		Black Tea Group (n=26)		Student's T-test P-Value
	Mean	SD	Mean	SD	
Lag time	35.2	8.3	38.2	15.2	0.52
V-max (OD/Min)	1.84	0.59	1.94	0.69	0.6
OD-max	0.27	0.06	0.29	0.07	0.23
T-max	86.7	19.7	106	39.5	0.04

Lag-time = the time needed (in min) to initiation of lipid oxidation products accumulation during the lipid oxidation course after addition of $CuCl_2$, **OD-max** = maximal amount of lipids peroxide products accumulation during the lipid oxidation course, **V-max** = maximal rate of oxidation during the lipid oxidation course, **T-max** = time needed (in min) to gained the maximal rate of lipid peroxide products accumulation during the lipid oxidation course.

Table 3- Mean of quantitative serum lipid oxidation parameters in two groups at the end of study.

Variable	Sour Tea Group (n=27)		Black Tea Group (n=26)		Student's T-test P-Value
	Mean	SD	Mean	SD	
Lag time	33.7	6.6	36.7	14.8	0.4
V-max (OD/Min)	1.83	0.556	1.84	0.574	0.9
OD-max	0.27	0.06	0.28	0.07	0.5
T-max	85	23.4	89	31.7	0.6

Table 4- Mean of quantitative serum oxidation parameters in sour tea group, before and after the intervention.

Variable	Before (n=27)		After (n=27)		Student's Ttest P-Value
	Mean	SD	Mean	SD	
Lag time	33.7	8.3	34.5	6.6	0.7
V-max (OD/Min)	1.84	0.59	1.87	0.57	0.85
OD-max	0.267	0.06	0.272	0.06	0.7
T-max	84.5	19.7	89	23.4	0.4

Table 5- Mean of quantitative serum lipid oxidation parameters in black tea group, before and after the intervention.

Variable	Before (n = 26)		After (n = 26)		Student's T-test P-Value
	Mean	SD	Mean	SD	
Lag time	37.2	12.2	43	11.8	0.25
V-max (OD/Min)	1.84	0.68	1.94	0.57	0.5
OD-max	0.278	0.08	0.298	0.07	0.3
T-max	89.5	39.5	106	31.7	0.13

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DISCUSSION

Out of 60 patients who entered the study, 53 (88.4%) concluded it. Compliance to tea consumption was 92-95% in both groups which shows satisfactory co-operation. Sex and treatment method distribution of patient into two groups did not show significant difference. At the beginning of the study, mean of BMI, fasting blood glucose, HbA1c, Total cholesterol, LDL-C, HDL-C and TG were not significantly different in the two groups (Table 1). This data shows the random allocation of patients into two groups was acceptable; especially when mean of fasting blood glucose, lipids, and lipoproteins were not significantly different in the two groups at the beginning of the study.

The results indicated no significance change in serum lipid oxidation parameters in two groups before and after intervention. Therefore, we did not observed any change in susceptibility of serum lipid from diabetic patients to oxidation by this intervention. Since all the patients used black tea before participating in this study, there was not a basic change in control group in the course of this study.

Black tea contains some flavonoids with antioxidant properties (19), and there are many reports about its effects on oxidative status in animal model and humans (11-14). Although Mac Anlis et al. (20) and VanhetHof et al. (21) did not show any significant effect of short term black tea consumption on isolated LDL oxidation profile in humans, Ohmori et al. in a study showed antioxidant activity of various types of tea including black tea against purified human LDL oxidation (7). Hodgson et al. evaluated the acute effects of green and black tea on lipoprptein oxidation in human whole serum, and they showed mild protective effect of black tea on lipoprotein oxidation in human serum (12). These and some other animal studies (22,23) confirmed the protective effects of black tea consumption at least in chronic consumption against lipids and lipoprotein oxidation as isolated or in whole human serum.

Our study population used black tea chronically in their normal life time, and this life style habits probably has some protective effects on their serum lipids against oxidative modification. Since there was no basic change in tea consumption in our control group, this is a reasonable explanation for any changes of serum lipid oxidation properties after intervention in control group.

Our results also indicated no significant change in serum lipid oxidation parameters after sour tea consumption in case group. Sour tea also contains some flavonoids with antioxidant properties (24). Change et al. in an in-vitro study examined the effects of sour tea extract on LDL oxidation and they suggested some protective effect from sour tea extract against in-vitro LDL oxidation (25). Hirunpanich et al. also evaluated the effects of an aqueous extract from dried calyx of sour tea on in-vitro isolated rat LDL oxidation parameters (26). They found a dose dependent protective effect of aqueous extract of sour tea against isolated rat LDL to oxidation. We did not find any report about the effects of sour tea consumption on serum lipid oxidizability in healthy and various diseases in human, but our results in this study indicated similar effects of sour tea with black tea consumption on serum lipid oxidation parameters in diabetic patients.

CONCLUSION

All together these results showed that neither black nor sour tea has effect on serum lipid oxidizability in diabetic patients. All of patients in case and control groups in this study used chronically black tea with some antioxidant properties. Therefore, the results indicate that use of sour tea has the same effect on serum lipid oxidation profile in diabetic patients as black tea.

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