Antioxidative stress potential of *Cinnamomum zeylanicum* in humans: a comparative cross-sectional clinical study

Akram Ranjbar, Sara Ghasmeinezhad, Hosnieh Zamani, Ali Akbar Malekirad, Akram Baiaty, Azadeh Mohammadirad & Mohammad Abdollahi†

†Author for correspondence Tehran University of Medical Sciences, Laboratory of Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Pharmaceutical Sciences Research Center, Tehran 14155 6451, Iran Tel.: +98 216 695 9104 Fax: +98 216 695 9104 mohammad@sina.tums.ac.ir

Background: *Cinnamomum zeylanicum* is an important spice and aromatic crop used in folk medicine. Cinnamon is usually regarded as the bark of the *C. zeylanicum* tree. **Objective:** The objective of this study was to determine the antioxidative stress capacity of cinnamon in humans when administered in a regular and controlled manner. **Methods:** A total of 54 normal subjects were divided into three groups, receiving water, regular tea or cinnamon tea for 2 weeks. Blood samples were obtained before and after treatment and analyzed for lipid peroxidation levels, total antioxidant power and total thiol groups by standard methods. **Results:** The results indicated increased total antioxidant power and total thiols but a decrease in lipid peroxidation levels in individuals who received regular or cinnamon tea compared with controls. The extent of increase in total antioxidant power and decrease in lipid peroxidation levels were more evident in individuals who received cinnamon tea compared with those who received regular tea. **Conclusion:** Cinnamon has a marked antioxidant potential and may be beneficial in alleviating the complications of many illnesses related to oxidative stress in humans.

Keywords: antioxidant power, cinnamon lipid peroxidation, oxidative stress, tea

Background

Free radicals are very reactive atoms or molecules as they possess single electrons. The free radical formation in a living system leads to oxidative damage of macromolecules, such as DNA, proteins and lipids. Oxidative stress is defined most simply as the imbalance between the production of free radicals capable of causing peroxidation of the lipid layer of cells and the body's antioxidant defense. The balance between the production of free radicals and antioxidant defense in the body has important health implications. If there are too many free radicals or too few antioxidants for protection, a condition of oxidative stress develops, which may cause chronic and permanent damage [1].

Most spices and vegetables with potential antioxidant activity in biologic systems have been used in folk medicine [2]. Other than the biologic system, lipid oxidation is one of the major changes that occurs during the processing, distribution, storage and final preparation of food. Oxidation could be prevented by adding synthetic or natural antioxidants to the diet while the safety of synthetic ones is in question [3].

Antioxidant activities of volatile extracts isolated from cinnamon were evaluated by various *in vitro* assays [4]. The contents of glutathione (GSH) and lipid-conjugated dienes were studied in rats fed a high-fat diet along with cinnamon or cardamon, and it was reported that cinnamon stimulates the activity of antioxidant enzymes [5]. In addition, the effect of cinnamate, a phenolic compound found in cinnamon bark and other plant materials, on lipid metabolism and antioxidant enzyme activities in rats fed a high cholesterol diet has been studied and indicated that cinnamon suppresses lipid peroxidation via the enhancement of hepatic antioxidant enzyme activities [6].

Cinnamon tea is a common drink in Iran and is available commercially. No controlled study was performed on the antioxidant potential of cinnamon in humans. To address this, a comparative cross-sectional study was designed and the blood of different study subjects was analyzed for total antioxidant power, lipid peroxidation and the concentration of total thiol groups, which are important indicators of oxidative stress parameters in the body [1,7–11].

Material & methods

Subjects

A comparative cross-sectional study was designed with 54 subjects. Subjects were female students selected from Arak University of Medical Science in the age range of 18 to 25 years, and were divided into three groups with 18 in each. The first group of volunteers drank 100 mg/30 ml of cinnamon with tea daily for 2 weeks. The second group of individuals from the same university consumed the same amount of tea without cinnamon for the same length of

Future Drugs Ltd

time. The third student group were assigned as controls and received only water. They were all age-matched and lived in the same urban area. All participants were provided with specific written information about the aims of the study before written consent was obtained, in accordance with the Declaration of Helsinki. Prior to blood collection, all subjects completed a structured questionnaire specifying date of birth, smoking and dietary habits, work-related exposure to hazardous agents, consumption of vitamin supplements and other antioxidants, and use of therapeutic drugs. Vegetarians and those who had used vitamin supplements, antioxidants or any therapeutic drugs were excluded. Since exercise, smoking, polyunsaturated fat intake and dietary antioxidant vitamin intake can effect oxidative status, subjects were given specific guidelines to follow throughout the study. They were instructed not to take any multivitamin supplements or traditional herbs during the study. In addition, it was recommended that the subjects engage in low-impact exercise for approximately 30 min/day.

Chemicals

Dithiobis-2-nitrobenzoic acid (DTNB), Tris base, tetraethoxypropane (malonedialdehyde [MDA]), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol and 2,4,6-tripyridyl-*S*-triazine (TPTZ) were used in this study.

Sampling & laboratory measurements

In this clinical trial, blood samples were collected before and 2 weeks after participation in the study and received the abovementioned treatments. After centrifugation of blood at 3000 g for 30 min at 4°C, the plasma supernatant fluid was separated and stored at -80°C until analyzed further. Processing and scoring samples of groups were performed blind and concurrently in the laboratory. At the end of the study, the data from the questionnaire and measured parameters were linked to the code number for data analysis.

Measurement of plasma total antioxidant power

Antioxidant power of plasma was determined by measuring the subjects' ability to reduce Fe^{3+} to Fe^{2+}, established by the ferric-reducing ability of plasma (FRAP) test described previously [12]. In this test, the medium is exposed to Fe^{3+} and the antioxidants present in medium start to produce Fe^{2+} as an antioxidant activity. The reagent included 300 mmol/l acetate buffer, pH 3.6, and 16 ml $C_2H_4O_2$/l of buffer solution (10 mmol/l TPTZ in 40 mmol/l HCl, 20 mmol/l $FeCl_3.6H_2O$). The working FRAP reagent was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3.6H_2O$ solution. The 10 µl of H_2O-diluted sample was then added to 300 µl freshly prepared reagent warmed at 37°C. The complex between Fe^{2+} and TPTZ produces a blue colour with an absorbance at 593 nm.

Measurement of lipid peroxidation

MDA is the end product of the oxidation of polyunsaturated fatty acids and its concentration in the medium is an established measure of the extent of lipid peroxidation [13]. In this test, the reaction of MDA with TBA forms a complex, which is determined spectrophotometrically. Lipid peroxidation in samples can then be assessed in terms of the TBA reactive substances (TBARS) produced. The samples were diluted by buffered saline (1:5) and 800 µl of TCA (28% w/v) was added to 400 µl of this mixture and centrifuged at 3000 g for 30 min. Then, 600 µl of the supernatant was added to 150 µl of TBA (1% w/v). Next, the mixture was incubated for 15 min in a boiling water bath and 4 ml n-butanol was added. Finally, the solution was centrifuged, cooled and the absorption of the supernatant was recorded in 532 nm by a UV-160-A Shimadzu double-beam spectrophotometer. The calibration curve of a 1,1,3,3-tetraethoxypropan standard solution was used to determine the concentrations of TBA–MDA adducts in samples.

Measurement of plasma total thiol groups

Total thiol groups of plasma were evaluated spectrophotometerically at 412 nm using DTNB as the reagent [14].

Statistical analysis

A detailed multiple variable database was formed. All data were collected either as dichotomous variables (e.g., age, weight, consume cinnamon) or as continuous variables (e.g., laboratory measurements). All data were analyzed with SPSS version 11. A two-sample t-test was used for statistical comparisons after plotting and testing for equal variances, and p-values greater than 0.05 were considered to be nonsignificant. Data were expressed as mean ± standard error.

Table 1. Demographic characteristics of study subjects (n = 54).

Characteristic	Yes (%)	No (%)
Female gender	54 (100)	0
18–25 years of age	54 (100)	0
Smoking	0	54 (100)
Sport	15 (27.7)	39 (72.2)
History of disease	0	54 (100)

Results

The characteristics of the subjects are shown in Table 1. Table 2 shows the oxidative stress parameters before and after the intervention in the study groups. Both regular and cinnamon tea were significantly effective in the reduction of lipid peroxidation and increasing total antioxidant power and total thiols in comparison with controls. Administration of regular tea and cinnamon tea significantly decreased plasma TBARS (15 and 38%, respectively). The total antioxidant power of plasma was increased significantly by regular and cinnamon tea (9 and 21%, respectively). Total plasma thiols were increased significantly in plasma of regular tea and cinnamon tea drinkers (18 and 22%, respectively). When groups of regular and cinnamon tea consumers were compared, the extent of increase in plasma TBARS for the cinnamon tea group was significantly higher than that of the regular tea group. The extent of the increase in plasma total antioxidant power for cinnamon tea consumers was more than that of the regular tea group.

The control values for plasma TBARS, total antioxidant power and total thiols were 17.93 ± 9.4 nmol/ml, 2.25 ± 0.52 µmol/ml and 0.59 ± 0.12 mM, respectively.

Discussion

The main aim of this study was to measure the oxidative stress status of individuals who consume cinnamon tea. The present results indicate the antioxidative stress potential of cinnamon. Specifically, regular administration of tea is effective in terms of oxidative stress parameters when compared with control subjects; however, this is not a new finding and has been indicated previously. The important finding of this study is that the extent of the increase in total antioxidant power and the decrease in lipid peroxidation levels were more evident in individuals who received cinnamon tea in comparison with those who received regular tea. *C. zeylanicum* is an important spice and aromatic crop, having wide applications in flavoring, perfumery, beverages and medicines [15]. *Trans*-cinnamaldehyde, the principal component of cinnamon flavor, has significant antimicrobial effects [16]. Even mosquito larvicidal activity and the strongest antifungal activities have been reported for cinnamaldehyde congeners [17,18]. Cinnamon extracts are used regularly as food antioxidants together with improving food palatability [3]. Cinnamon tea is a mixture of regular tea and cinnamon that is used in Iran. The antioxidant activity of this dietary spice confirms that along with its influence on the flavor of food, it possesses potential health benefits by inhibiting lipid peroxidation. Animal studies indicate that dietary cinnamate inhibits hepatic HMG-CoA-reductase activity, resulting in lower hepatic cholesterol content as well as suppressing lipid peroxidation via the enhancement of hepatic antioxidant enzyme activities [2,6]. There is evidence that intake of 1, 3 or 6 g of cinnamon/day reduces serum glucose,

Table 2. Oxidative stress parameters.

	Cinnamon + tea (A)			Tea (B)			Control		
	Before	*After*	*p*	*Before*	*After*	*p*	*Normal value*	*p (Control vs A)*	*p (Control vs B)*
Lipid peroxidation (nmol/ml)	16.74 ± 8.9	10.39 ± 6.1	0.01	17.26 ± 8.7	14.7 ± 8.5	0.01	17.93 ± 9.4	0.01	0.01
Total antioxidant power (µmol/ml)	2.30 ± 0.36	2.78 ± 0.38	0.01	2.31 ± 0.51	2.52 ± 0.75	0.02	2.25 ± 0.52	0.01	0.01
Total thiols (mM)	0.58 ± 0.12	0.71 ± 0.17	0.03	0.62 ± 0.14	0.73 ± 0.12	0.04	0.59 ± 0.12	0.02	0.01

Highlights

- Cinnamon is an important spice and aromatic crop having wide applications in flavoring, perfumery, beverages, and medicines. Cinnamon tea that is a mixture of regular tea and cinnamon is traditionally used in Iran.
- The present investigation for the first time shows that in individuals who drink cinnamon tea, their blood lipid peroxides decrease and total antioxidant capacity and total thiol molecules increase much more than those who drink regular tea.
- It has been established that free radicals play marked role in the pathophysiology of many deliberating human diseases and thus cinnamon tea with its high anti-free radical potential is recommended to be used in these patients.

triglyceride, low-density lipoprotein cholesterol and total cholesterol in people with type II diabetes [19].

It is important to note that the antioxidant potential of tea has been well indicated previously and that is not a new finding. Supporting this finding, it has been reported recently that supplementation with black tea extract protects animals against pesticide-induced liver damage through increasing the antioxidant potential of hepatocytes against free radicals [20].

Conclusion

In conclusion, the present investigation shows for the first time that cinnamon extract exhibits significant antioxidant activity in humans. Thus, cinnamon, which is used as a flavoring agent in food or tea, acts as a potent antioxidant. Therefore cinnamon tea is recommended to be used in individuals who have oxidative stress-related illnesses.

Bibliography

Papers of special note have been highlighted as of interest (•) or of considerable interest (••) to readers.

1. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaiee A. Pesticides and oxidative stress: a review. *Med. Sci. Monit.* 10(6), RA141–RA147 (2004).

• **Describes the production of free radicals in humans including how they damage organs and their functions.**

2. Shobana S, Naidu KA. Antioxidant activity of selected Indian spices. *Prostaglandins Leukot. Essent. Fatty Acids* 62(2), 107–110 (2000).
3. Mancini-Filho J, Van-Koiij A, Mancini DA, Cozzolino FF, Torres RP. Antioxidant activity of cinnamon (*C. zeylanicum*, Breyne) extracts. *Boll. Chim. Farm.* 137(11), 443–447 (1998).
4. Lee KG, Shibamoto T. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *J. Agric. Food Chem.* 50(17), 4947–4952 (2002).

• **Describes herbal extracts and spices that have antioxidant potential *in vitro*.**

5. Dhuley JN. Antioxidant effects of cinnamon (*Cinnamomum verum*) bark and greater cardamom (*Amomum subulatum*) seeds in rats fed high fat diet. *Indian J. Exp. Biol.* 37(3), 238–242 (1999).
6. Lee JS, Jeon SM, Park EM *et al*. Cinnamate supplementation enhances hepatic lipid metabolism and antioxidant defense systems in high cholesterol-fed rats. *J. Med. Food* 6(3), 183–191 (2003).
7. Radfar M, Larijani B, Hadjibabaie M, Rajabipour B, Mojtahedi A, Abdollahi M. Effects of pentoxifylline on oxidative stress and levels of EGF and NO in blood of diabetic type-2 patients: a randomized, double-blind placebo-controlled clinical trial. *Biomed. Pharmacoth.* 59(6), 302–306 (2005).
8. Astaneie F, Afshari M, Mojtahedi A *et al*. Total antioxidant capacity and levels of epidermal growth factor and nitric oxide in blood and saliva of insulin-dependent diabetic patients. *Arch. Med. Res.* 36(4), 376–381 (2005).
9. Malekirad AA, Ranjbar A, Rahzani K *et al*. Oxidative stress in radiology staff. *Environ. Toxicol. Pharmacol.* 20, 215–218 (2005).
10. Ranjbar A, Solhi H, Mashayekhi FJ, Susanabdi A, Rezaie A, Abdollahi M. Oxidative stress in acute human poisoning with organophosphorus insecticides: a case control study. *Environ. Toxicol. Pharmacol.* 20, 88–91 (2005).
11. Jahanshahi G, Motavasel V, Rezaie A, Hashtroudi AA, Daryani NE, Abdollahi M. Alterations in antioxidant power and levels of epidermal growth factor and nitric oxide in saliva of patients with inflammatory bowel diseases. *Dig. Dis. Sci.* 49 (11–12), 1752–1757 (2004).
12. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.* 292, 15–27 (1999).
13. Satho K. Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. *Clin. Chem. Acta* 90, 37–43 (1978).
14. Hu ML, Dillard CJ. Plasma SH and GSH measurement. *Methods Enzymol.* 233, 385–387 (1994).
15. Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. Volatile constituents from *C. zeylanicum* fruit stalks and their antioxidant activities. *J. Agric. Food Chem.* 51(15), 4344–4348 (2003).

• **Describes the specific antioxidant composition of cinnamon.**

16. Friedman M, Henika PR, Levin CE, Mandrell RE. Antibacterial activities of plant essential oils and their components against *Escherica coli* 0157:H7 and *Salmonella enterica* in apple juice. *J. Agric. Food Chem.* 52(19), 6042–6048 (2004).
17. Wang SY, Chen PF, Chang ST. Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. *Bioresour. Technol.* 96(7), 813–818 (2005).
18. Cheng SS, Liu JY, Tsai KH, Chen WJ, Chang ST. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophloeum* provenances. *J. Agric. Food Chem.* 52(14), 4395–4400 (2004).
19. Khan A, Safdar M, Ali Khan MM, Khattak KN, Anderson RA. Cinnamon improves glucose and lipids of people with Type 2 diabetes. *Diabetes Care* 26(12), 3215–3218 (2003).
20. Khan SM. Protective effect of black tea extract on the levels of lipid peroxidation and antioxidant enzymes in liver of mice with pesticide-induced liver injury. *Cell Biochem. Funct.* (2005) (Epub ahead of print).

Affiliations

Akram Ranjbar
Arak University of Medical Sciences, Faculty of Paramedical Science, Arak, Iran

Sara Ghasmeinezhad
Arak University of Medical Sciences, Faculty of Paramedical Science, Arak, Iran

Hosnieh Zamani
Arak University of Medical Sciences, Faculty of Medical Sciences, Arak, Iran

Ali Akbar Malekirad
Esfahan Payam Noor University of Sciences, Esfahan, Iran

Akram Baiaty
Isfahan University of Medical Sciences, Faculty of Nursing and Midwifery Sciences, Isfahan, Iran

Azadeh Mohammadirad
Tehran University of Medical Sciences, Faculty of Pharmacy, Pharmaceutical Sciences Research Center, Tehran, Iran

Mohammad Abdollahi
Tehran University of Medical Sciences, Laboratory of Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Pharmaceutical Sciences Research Center, Tehran 14155 6451, Iran
Tel.: +98 216 695 9104
Fax: +98 216 695 9104
mohammad@sina.tums.ac.ir