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On the Benefit of Cinnamomum zeylanicum for Radiology Unit Staff

¹Ali Fani, ²Ali Akbar Malekirad, ³Iman Fani, ²Habib Allahnazem, ¹Kobra Rahzani, ⁴Akram Ranjbar, ⁴Sanaz Vosough-Ghanbari and ⁴Mohammad Abdollahi

The aim of this study was to determine the anti-oxidative stress capacity of Cinnamomum zeylanicum (CZ) when administered in a controlled manner to radiology unit staff which are exposed to persistent low-dose radiation. A group of 27 radiology unit staff were invited to drink CZ (0.5 g powder mixed with 300 mL boiled water) twice daily for 2 weeks. Blood samples before and after entering the study was measured for lipid peroxidation level (LPO), total antioxidant capacity (TAC) and total thiol molecules (TTM). The results indicated a significant increase in plasma TAC (p = 0.0001) and TTM (p = 0.05) and a significant reduction in plasma LPO (p = 0.044). CZ has marked antioxidant potency and can alleviate complications of illnesses related to oxidative stress in radiology unit staff. CZ is recommended to be used as a dietary supplement for radiation protection.

Key words: Cinnamomum zeylanicum, radiology unit staff, lipid peroxidation, total antioxidant capacity, total thiol molecules

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Mohammad Abdollahi Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, Iran

Tel/Fax: +98 21 66959104



¹School of Medicine, Arak University of Medical Science, Arak, Iran ²Department of Biology, Payam-e-Noor University, Iran ³Isfahan University of Medical Sciences, Isfahan, Iran ⁴Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

INTRODUCTION

Oxidative stress can be defined most simply as the imbalance between the production of Reactive Oxygen Species (ROS) capable of causing oxidative damage including peroxidation of the lipid layer of cells and the body's antioxidant defense. Many evidences suggest that accumulation of ROS and the consequent oxidative stress is known to be involved in pathogenesis of some diseases (Rahimi et al., 2005; Abdollahi et al., 2005; Rezaie et al., 2007; Sadegh Soltan-Sharifi et al., 2007), conditions (Astaneie et al., 2005; Hadidi et al., 2006; Kajbaf et al., 2007), exposure to xenobiotics (Malekirad et al., 2005a; Ranjbar et al., 2005; Shadnia et al., 2005) and ionizing radiations (Robbins and Zhao, 2004). The ability of the body cells to counteract ROS depends on the capacity of the cell antioxidant capacity. If the endogenous antioxidant mechanisms cannot control increased cellular ROS then oxidative stress would happen. It has been shown that endogenous antioxidants themselves might be attacked by ROS, contributing to overall cell damage (Abdollahi et al., 2004).

Ionizing radiation is used in the diagnosis and treatment of human diseases but chronic exposure to it would be hazardous due to its mutagenic and carcinogenic effects. Cellular DNA is believed as the primary target for the biological and lethal effects of ionizing radiation most probably through induction of oxidative stress. The primary radiation-induced free radicals include hydroxyl (OH*) and superoxide (O2-) that give rise to other ROS. Chronic existence of excessive amounts of free radicals may also lead to several unrecoverable effects such as fibrosis, necrosis, atrophy and vascular damage. The relation of ionizing radiation and acute oxidative stress in animals has been reported by many papers but those considering the chronic oxidative stress are too scarce (Robbins and Zhao, 2004; Spitz et al., 2004).

Recent study in radiology staff indicated existence of oxidative stress as evidenced by increased Cellular Lipid Peroxidation (LPO) and reduced Total Antioxidant Capacity (TAC) (Malekirad *et al.*, 2005b). In addition, human studies support that workers occupationally exposed to ionizing radiation show increased cancer incidence (leukemia and solid cancers) and DNA breakage frequencies (Chung *et al.*, 1996; Bonassi *et al.*, 1997; Balakrishnan and Rao, 1999; Rozgaj *et al.*, 1999; Cardoso *et al.*, 2001; Maffie *et al.*, 2004).

Cinnamon zeylanicum (CZ) as dried inner bark of the tree from Lauraceae family is native to Sri Lanka and India but is cultivated extensively in the tropical regions of the world. CZ leaf and bark are used as spice and also in the

production of essential oils. The leaves have a hot taste and emit a spicy odor when crushed. CZ provides a variety of oils with different aroma characteristics and composition to the flavor industry. The root bark was reported to have camphor as the main constituent, but does not seem to have commercial value, unlike the leaf and stem bark oils (Chevallier, 2000). Antioxidant activities of volatile extracts isolated from CZ were evaluated by various in vitro assays (Lee and Shibamoto, 2002; Jayaprakasha et al., 2002, 2003, 2006). The contents of glutathione and lipid-conjugated dienes were studied in rats fed a high-fat diet along with CZ or cardamon and it was reported that CZ stimulates the activity of antioxidant enzymes (Dhuley, 1999). Earlier before/after clinical trial on healthy subjects showed that administration of exhibit significant antioxidant activity CZ extract (Ranjbar et al., 2006).

Biological modifiers targeting oxidative damage for radioprotection have been studied for decades with limited success. Hence, there is a need for better and more potent compounds, especially on herbal origin to boost antioxidant defense.

In the present study, a before/after clinical trial study was performed to explore benefit of CZ in radiology unit staff by evaluation of blood TAC, LPO and Total Thiol Molecules (TTM) as main oxidative stress biomarkers.

MATERIALS AND METHODS

Materials: Dithiobis-2-nitrobenzoic acid (DTNB), Tris base and 1,1,3,3'—tetraethoxypropane (malondialdehyde [MDA]) from (Sigma, UK), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol from (Merck, Tehran) and 2,4,6-tripyridyl-S-triazine (TPTZ) from (Fluka, Italy), were used in this study.

Subjects: A total of 27 technical staff, working in the radiology center of a University hospital in Arak were included in the study in 2006. The subjects were exposed to low-dose ionizing radiation (x-ray) not less than 2 years. None of these workers were professionally exposed to any hazardous agent other than ionizing radiation and did not have any radiology diagnostic or therapeutic intervention in the last 12 months. All participants were provided with specific written information about the aims of the study before written consents were obtained, in accordance with the Declaration of Helsinki. Prior to blood collection, each individual was extensively interviewed by a specialized physician who filled in a structured questionnaire specifying gender, date of birth, smoking status, dietary habits, alcohol consumption,

previous exposure to diagnostic x-ray as a patient, use of drugs, consumption of vitamin supplements or antioxidants. Since exercise, smoking, polyunsaturated fat intake and dietary antioxidant vitamin intake can affect 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. For several reasons, the responsible organizations did not provide us with the annual radiation exposure of the subjects recorded from their chest badges.

The included subjects were administered CZ powder (0.5 g) dissolved in 300 mL boiling water twice daily for 2 weeks at 7.5 am and 2 pm every day. CZ was originally from Iran that is purchased in the market as powder form. The dose of CZ was selected on the basis of our recent study and literature information. Regarding safety of CZ and results of earlier study (Ranjbar *et al.*, 2006), the dose of 1 g day⁻¹ was chosen. A supervisor carefully checked to make sure that the volunteers were taking CZ properly.

Plasma preparation: In this clinical trial, blood samples were collected from all subjects before administration of CZ and 12 h after the last dose of 2 weeks treatment with CZ. Five milliliter heparinized blood were obtained and centrifuged at 3000 g for 30 min at 4°C to separate plasma. The plasma samples were stored at -80°C until analyzed.

TAC assay: Antioxidant capacity of plasma was determined by measuring their ability to reduce Fe^{3+} to Fe^{2+} . 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^{-1} acetate buffer, pH 3.6 and 16 mL $C_2H_4O_2$ L^{-1} of buffer solution, 10 mmol L^{-1} TPTZ in 40 mmol L^{-1} HCl, 20 mmol L^{-1} FeCl₃ (6H₂O). Working TAC reagent was prepared as required by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3$ -6H₂O solution. Ten microliters of H_2 O-diluted sample was then added to 300 μL freshly prepared reagent warmed at 37°C. The complex between Fe^{2+} and TPTZ gives a blue color with absorbance at 593 nm.

LPO assay: The method is based on the reaction of MDA as the end product of the oxidation of polyunsaturated fatty acids and its concentration in the medium is an established measure of LPO extent. In this test, the reaction of MDA with TBA creates a complex which is determined spectrophotometrically while LPO in samples are assessed in terms of TBA reactive substances (TBARS) produced. Briefly, the samples were diluted by buffered saline (1:5) and 800 µL of TCA (28% w/v) was

added to 400 μ L of this mixture and centrifuged in 3000 g for 30 min. Then, the precipitation was dissolved in sulfuric acid and 600 μ L of the mixture was added to 150 μ L of TBA (1% w/v). The mixture was then incubated for 15 min in a boiling water bath. Following incubation, 4 mL of n-butanol was added, the solution centrifuged, cooled and the absorption of the supernatant was recorded in 532 nm using a UV-160-A Shimadzu double beam spectrophotometer. The calibration curve of a 1,1,3,3- tetraethoxypropane standard solutions was used to determine the concentrations of TBA-MDA adducts in samples.

TTM assay: A volume of plasma (0.20 mL) was mixed in a 10 mL test tube with 0.6 mL of Tris-EDTA buffer (Tris base 0.25 M, EDTA 20 mM, pH 8.2) followed by the addition of 40 mL of 10 mM of DTNB in methanol. The final volume of the reaction mixture was made up to 4.0 mL by adding 3.16 mL of methanol. The test tube was capped and the color was developed for 15-20 min, followed by centrifugation at 3000 g for 10 min at ambient temperature. The absorbance of the supernatant was measured at 412 nm.

Data analysis: A paired t-test was used for statistical comparisons of biochemical parameters. p-values lesser than 0.05 were considered significant. Data were expressed as mean±standard error.

RESULTS AND DISCUSSION

A total of 27 subjects including 14 males and 13 females with mean age of 36.1 year and mean employment history of 13.5 years were enrolled into study. Plasma LPO was decreased significantly (p=0.04) after 2 weeks (Fig. 1). Administration of CZ significantly increased plasma TAC (p=0.001, Fig. 2). Plasma TTM was significantly increased after treatment with CZ (p=0.05, Fig. 3).

In this study, the effect of CZ on oxidative stress status was investigated in radiology unit staff. The results indicated that CZ increases body TTM and TAC and reduces LPO in radiology staff. Regarding relation of ionizing radiation and oxidative stress, it is not surprising to conclude that chronic use of CZ would protect human cells from oxidative damage. Genotoxicity is one of the key outcomes of oxidative stress (Abdollahi *et al.*, 2004; Shadnia *et al.*, 2005) and explains the higher rate of cancer and chromosomal aberrations in radiology unit personnel (Chung *et al.*, 1996; Bonassi *et al.*, 1997; Balakrishnan and Rao, 1999; Rozgaj *et al.*, 1999; Cardoso *et al.*, 2001; Maffie *et al.*, 2004).

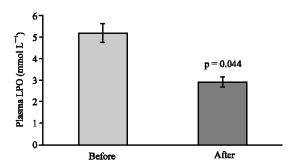


Fig. 1: Changes in plasma Lipid Peroxidation (LPO) before and after treatment with CZ. Cinnamomum zeylanicum (CZ) was administered as powder (0.5 g) in 300 mL boiling water twice daily for 2 weeks at 7.5 am and 2 pm every day. Data are mean±SE of 27 technical staff, working in the radiology center. The change between before and after values is significant with p = 0.044

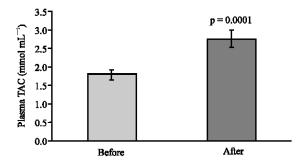


Fig. 2: Changes in plasma Total Antioxidant Capacity (TAC) before and after treatment with CZ. Cinnamomum zeylanicum (CZ) was administered as powder (0.5 g) in 300 mL boil water twice daily for 2 weeks at 7.5 am and 2 pm every day. Data are mean±SE of 27 technical staff, working in the radiology center. The change between before and after values is significant with p = 0.0001

Antioxidants are often added to foods to prevent the radical chain reactions of oxidation and they act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process. Many herbs and spices have been shown to impart antioxidant effects in food; the active principles are phenolics (Scwarz et al., 2001; Tanabe et al., 2002). A wide variety of phenolic substances derived from herbs and spices possess potent antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic and anti-tumor activities, which contribute to their chemopreventive potential (Surh, 2002; Marongiu et al., 2007; Dehghan et al., 2007). It has been reported the possible use of CZ as antioxidant

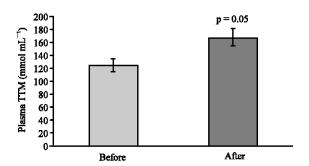


Fig. 3: Changes in plasma Total Thiol Molecules (TTM) before and after treatment with CZ. Cinnamomum zeylanicum (CZ) was administered as powder (0.5 g) in 300 mL boil water twice daily for 2 weeks at 7.5 am and 2 pm every day. Data are mean±SE of 27 technical staff, working in the radiology center. The change between before and after values is significant with p = 0.05

in cookies (Badei et al., 2002). It has been proved that irradiation of CZ did not affect its antioxidant potential (Kitazuru et al., 2004). The water extract of CZ contains maximum phenolics and it showed highest degree of antioxidant activity. CZ water extract has superlative antimutagenic activity against the mutagenicity of the direct acting mutagen sodium azide. The investigation showed that the fruit of CZ, an under-utilized and unconventional part of the plant, contains a good amount of phenolic antioxidants to counteract the damaging effects of free radicals and may protect against mutagenesis (Jayaprakasha et al., 2007). In addition, animal studies indicated that dietary CZ inhibits hepatic HMG COA-reductase activity resulting in lower hepatic cholesterol content as well as suppressing LPO via the enhancement of hepatic antioxidant enzyme activities (Shobana and Naidu, 2000; Lee et al., 2003).

Supporting the present study, the efficacy of CZ in operating room personnel has been indicated (Ranjbar *et al.*, 2007) by the reduction of cellular LPO and affecting body TAC. Also in agreement with present conclusion, the rationale for using multiple antioxidants in protecting humans against low doses of ionizing radiation has been proposed by Prasad (2005).

CONCLUSION

Results of this study suggest that CZ can provide biological protection against radiation damage in humans while it is non-toxic and cost-effective. Regarding marked antioxidant potency of CZ it is believed to be beneficial on illnesses related to oxidative stress in radiology unit staff. CZ is recommended to be used as a dietary supplement for radiation protection.

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