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Benefit of Lemon Verbena in Healthy Subjects; Targeting Diseases Associated with Oxidative Stress

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ABSTRACT

Medicinal plants are considered as natural sources of antioxidant compounds which may protect organisms against oxidative stresses. The present study was conducted to investigate the antioxidant potential of lemon verbena (*Lippia citriodora*). This clinical investigation was performed on a group of 43 healthy subjects. They were invited to use 3 mg of lemon verbena leaves as infusion daily at the morning and evening for two weeks. At the beginning and the end of investigation, blood samples were taken to study their lipid peroxidation levels and antioxidant activities beside the total thiol groups. At the end of experiment, data were subjected to the paired t-test analysis. After treatment, the total antioxidants of serum (1.95 ± 1.1 vs. $1.67 \pm 0.95 \mu\text{mol mL}^{-1}$) significantly increased while the lipid peroxidation reduced (12.23 ± 9.35 vs. $15.66 \pm 12.15 \text{ nmol mL}^{-1}$). The treatment had no significant effect on the total thiol groups. Lemon verbena is beneficial in improving body oxidant/antioxidant balance and thus its clinical efficacy remains to be tested in oxidative-stress-related diseases or conditions. Use of this herbal extract is recommended as a dietary supplement.

Key words: *Lippia citriodora*, Lemon verbena, clinical trial, oxidative stress, medicinal plants

INTRODUCTION

Lemon verbena is an aromatic plant of the family Verbenaceae. Although, the plant is native to South America and growing wild in Chile, Peru and Argentina. It is widely grown all over the world in tropical and subtropical regions for commercial and ornamental purposes now a days. In Iran, lemon verbena is cultivated in some parts of the country mainly for its medicinal properties. The plant produces a yellow to light green essential oil with special aroma like that of lemon. Although, citral, 1,8-cineol, limonene and geraniol have been reported as the most abundant components of its essential oil, limonene, neral, geraniol, 1-octone-3-ol and α -curcumene are known dominant in the samples got from Iran (Ghassemi-Dehkordi, 2002; Chevallier, 1996). Flavonoids such as salvigenine, eupatorine, luteoline and diosmetin, mucilage, tannin and some triterpenes are among the other constitutes of lemon verbena leaves.

Besides to its uses as a spice plant, lemon verbena is also used for treatment of dyspepsia, headaches, neuralgic pains, flatulence and vertigo, colds, etc., in traditional systems of medicine. So far, the insecticidal and antibacterial properties of essential oils extracted from leaves, which have a wide application in the perfume industry, have been reported (Ghassemi-Dehkordi, 2002).

Due to the presence of a single electron, free radicals are very reactive and therefore, have the potential to destruct different biomolecules such as proteins, lipids, amino acids and nucleic acids. To prevent cell injuries, therefore, two defensive systems including enzymatic and non-enzymatic have been evolved over the time against oxidants and oxidation reactions in organisms. These systems act with prevention of free radicals formation, repair of resulted injuries, excretion of damaged molecules and minimizing cell mutations (Abdollahi *et al.*, 2004; Malekirad *et al.*, 2005). Factors such as environmental pollutants disturb the balance between formation and elimination of free radicals and as a result, oxidative stress occurs which is considered as the causing agent of cancers, diabetes, aging and etc. (Rahimi *et al.*, 2005; Rezaie *et al.*, 2007; Abdollahi *et al.*, 2005). In the previous studies, the antioxidant potential of lemon verbena leaves have been reported (Funes *et al.*, 2009; Angela and Meireles, 2008; Pereira and Meireles, 2007) and therefore, the present study aimed to assess the potential of this plant in inhibiting oxidative stress on healthy peoples.

MATERIALS AND METHODS

Chemicals were purchased from Sigma-Aldrich (Tehran). A clinical investigation was performed on 43 healthy people subjects from Young Research Center of Arak after they were informed about the study and their consents were acquired. The included subjects did not use smoking, alcohol, drug, or antioxidants. A questionnaire was filled for each subject to record their diet during experiment. Five mL blood sample was taken at the beginning and the end of study from each individual who completed using 3 g of lemon verbena leaves daily at two occasions of morning and evening as infusion for two weeks. Afterwards, blood samples were centrifuged, their serums separated and analyzed for oxidative stress parameters.

For measuring total thiol groups, 1 mL of Tris buffer was added to 50 μ L of plasma and its absorbance (A1) was recorded at 412 nm against blank (Tris buffer). On the other hand, 20 microliter of 2,2-dithionitrobenzoic acid (DTNB) was added to each tube and left for 15 min in room temperature. Afterwards, the absorbance of all samples (A2) as well as the control (Tris buffer containing DTNB, B2) were measured. To measure the levels of thiol groups, the molar absorbance of $13600 \text{ Cm}^{-1} \text{ M}^{-1}$ was used and their levels were calculated (mM L^{-1}) according to the following equation:

$$(A2-A1-B) \times (1.07/0.05 \times 13.6) = (A2-A1-B) \times 1.57 \text{ mM}$$

To measure lipid peroxidation, the thiobarbituric acid method was used. As a result of free radicals attack, different aldehydes such as Malondialdehyde (MDA) are produced from lipids which react with thiobarbituric acid in acidic conditions and high temperatures. The resulted complex has the maximum absorbance at 532 nm. To evaluate peroxidation of lipids, serum proteins were precipitated at first with addition of 2.5 mL trichloroacetic acid to 0.5 mL serum and left for 10 min at room temperature. The mixture was subsequently centrifuged at 3000 rpm for 10 min, supernatant removed and precipitate washed with 0.5 M sulphuric acid. Afterwards, 2.5 mL 0.5 M sulphuric acid and 3 mL 0.2% thiobarbituric acid solution was added to each tube. After

Table 1: The effect of aqueous extract of lemon verbena on blood oxidative stress parameters

Oxidative stress parameter	Before treatment	After treatment	p-value
Total antioxidant power	1.670±0.95	1.950±1.1	0.003
Lipid peroxidation	15.660±12.15	12.230±9.35	0.044
Total thiol groups	0.157±0.137	0.158±0.127	0.964

preparation of 3 mL 0.2% TBA solution and 2.5 mL of each standard, all samples besides these solutions were incubated in a 100°C water bath for 30 min. Subsequently, 4 mL n-butanol was added to each cold tube and mixed well with vigorous vortexing. Finally, the mixture was centrifuged at 3500 rpm for 10 min and the absorbance of supernatant recorded at 532 nm.

To measure total antioxidant power, Ferric Reducing Ability (FRA) was used. The method is based on the ability of serum in reducing Fe^{+3} cations to Fe^{+2} at the presence of 2,4,6-tripyridyl-striazine (TPTZ). The Fe^{+2} -TPTZ complex has the maximum of absorbance in 593 nm. For determination of serum antioxidants, 25 mL phosphate buffer was mixed with 2.5 mL ferric chloride and 2.5 mL TPTZ to produce FRA solution. This solution should be prepared freshly and immediately used. Of prepared FRA solution, 1.5 mL was added to each tube and they were incubated at 37°C for 5 min. Then, 50 microliter of each serum sample was added to each tube and kept in 37°C for 5 min. Finally, the absorbance of all tubes was separately registered at 593 nm and their antioxidant content was determined according to the obtained standard curve.

All data were tested by paired t-test using Stats Direct 2.7.8.

RESULTS

Out of 43 studied individuals, who ranged in age from 15 to 44 years, 20 were men and 23 were women. According to the results, the total antioxidants of serum significantly ($p < 0.05$) increased after treatment of individuals with the aqueous extract of lemon verbena leaves ($1.95 \pm 1.1 \mu\text{mol mL}^{-1}$). Similarly, there was significant ($p < 0.05$) lower lipid peroxidation activity in the serum of individuals after treatment (12.23 ± 9.35 vs. $15.66 \pm 12.15 \text{ nmol mL}^{-1}$). However, treatment had no significant effect on the levels of total thiol groups (0.157 ± 0.137 and 0.158 ± 0.127 , before and after treatment, respectively) (Table 1).

DISCUSSION

In the present study, treatment with the aqueous extract of verbena lemon leaves improved oxidant/antioxidant balance of the body by increasing serum antioxidant power and reducing lipid peroxidation. These effects of verbena lemon leaves returns to antioxidant components of this herb that have been earlier reported in several studies (Ali *et al.*, 2008; Ono *et al.*, 2008; Quirantes-Pine *et al.*, 2009). The antioxidant components that are previously found in this extract include neral, α -pinene, β -pinene, β -caryophyllene, curcumene, β -caryophyllene, 1,8-cineole, citronelol, verbascoside and its derivatives, diglucuronide derivatives of apigenin, luteolin, eukovoside, gardoside, verbascoside, cistanoside F, theveside, campneoside I, chrysoeriol-7-diglucuronide, forsythoside A and acacetin-7-diglucuronide.

Our team have already proved role of oxidative stress in many debilitating diseases or conditions such as osteoporosis (Salari Sharif *et al.*, 2010), diabetes (Mehri *et al.*, 2011; Hasani-Ranjbar *et al.*, 2009, 2010a, b; Momtaz and Abdollahi, 2010; Rahimi *et al.*, 2005), islet transplantation (Mohseni-Salehi-Monfared *et al.*, 2009a), inflammatory bowel diseases (Rezaie *et al.*, 2007; Rastegarpanah *et al.*, 2011), preeclampsia (Rahimi *et al.*, 2009), pancreatitis

(Mohseni-Salehi-Monfared *et al.*, 2009b) and toxicity of metals (Mohammadirad and Abdollahi, 2011) or pesticides (Abdollahi *et al.*, 2004). Results of the present study are optimistic showing marked antioxidant activity of the lemon verbena extract when used in healthy subject. Our previous study indicated that cinnamon having the same antioxidant activity is useful in human oxidative stress conditions (Ranjbar *et al.*, 2007). Therefore, lemon verbena is expected to show the same positive effects in oxidative stresses conditions of different diseases. The next step of this preliminary study would be to test clinical efficacy of this natural product in above-mentioned oxidative-stress-related diseases and conditions. In the meantime, use of this herbal extract is recommended as a dietary supplement.

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