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Effects of *Melissa officinalis* L. on Oxidative Status and Biochemical Parameters in Occupationally Exposed Workers to Aluminum: A Before after Clinical Trial

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Abstract: This study was designed to evaluate potential of *Melissa officinalis* L. (Lemon balm) infusion on the improvement of oxidative stress markers in workers occupationally exposed to aluminum (Al). In this before-after clinical trial, 30 Al workers were asked to drink Lemon balm infusion which was prepared like a tea bag twice per day (1.5 g/100 mL) for 30 days. Blood samples before and after entering the study were measured for lipid peroxidation (LPO), Total Antioxidant Capacity (TAC), Total Thiol Molecules (TTM), liver enzyme and some blood parameters. Use of Lemon balm infusion resulted in a significant increase in plasma levels of TTM and TAC and a significant decrease in triglyceride, cholesterol and aspartate transaminase (AST). LPO was not different before and after treatment. The conclusion is that infusions of Lemon balm improves oxidative stress condition in Al workers when used as a dietary supplement.

Key words: Lemon balm, oxidative stress, aluminum, toxicity, workers

INTRODUCTION

Aluminium (Al) is a frequently found metal in the environment while most of people are not aware of the sources of Al. In fact, Al can be found in almost everywhere in food, drinking water, cosmetics, toothpaste and as adjuvant in different parenteral preparation and pharmaceutical agents. It has been shown that users of Al-containing antacids and buffered aspirin may have increased body Al (Krewski *et al*., 2007). Workers in the industries related to Al, are usually in a chronic exposure to Al more than that expected coming from normal daily diet. Our previous study showed that Al production workers who are occupationally exposed to Al have an oxidative stress situation that is evident in their blood (Ranjbar *et al*., 2008). As reviewed recently by Mohammadirad and Abdollahi (2011), most of toxicities of Al in human being are mediated through disturbing the balance between body oxidant and antioxidant. The most common condition related to Al exposure is Alzheimer's Disease (AD). Al is known to induce or worsen AD by its oxidant effects (Garcia *et al*., 2010). With the same mechanism of action, Al is known as a risk factor for Parkinson's disease (Sanchez-Iglesias *et al*., 2009). Since oxidant/antioxidant imbalance is involved in the pathogenesis of many diseases (Abdollahi *et al*., 2004) thus it would not be surprising to find strong links between Al exposure and many deliberating diseases other than AD and Parkinson.

Melissa officinalis L. (Lemon balm) belongs to the family Lamiaceae that grows in the Central and Southern Europe, Asia and Northern Iran. In Iran this plant is called locally as Badranjbooye, Varangboo and Faranjmoshk. Lemon balm contains a rich source of natural antioxidants and effective in many oxidant-related disorders (Hasani-Ranjbar *et al*., 2009). A recent study indicated that Lemon balm is beneficial in protection against oxidative stress and DNA damage in subjects exposed to long-term low-dose ionizing radiation (Zeraatpishe *et al*., 2011).

Regarding above information, we aimed to evaluate possible benefit of Lemon balm on oxidative stress status of workers of an Al production factory who are chronically exposed to Al.

MATERIALS AND METHODS

Materials: The main chemicals used in this study were dithiobisnitrobenzoic acid (DTNB), Tris base,1,1,3,30-tetraethoxypropane (MDA), 2-thiobarbituric acid (TBA),

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trichloroacetic acid (TCA), n-butanol and 2,4,6-tripyridyl-s-triazine (TPTZ) that were obtained Merck Chemical Co. (Tehran).

Plant material: The aerial parts of *Melissa officinalis* L. were collected in August 2009 from Botanical Garden of Shaheed Beheshti University and identified as *Melissa officinalis* L. by Dr. M.A. Vakili from Department of Biology, Faculty of Science, Islamic Azad University, Jiroft Branch. The leaves of *Melissa officinalis* L. was dried in shade at room temperature for 12 days.

Study population: The study was conducted on 30 male workers, with age range of 22–65, who worked in the Al production factory located in an industrial part of the Iran in Arak province. The factory has started its activity since 1972 and the product of this factory is 175000 tons per year of all kinds of Al bar. Now about 1700 workers are working in the factory. Al is produced by electrolysis of alumina (Al_2O_3) in electrolytic cells (pot). Al is reduced by carbon from the Na_3AlF_6. The subjects were occupationally exposed to Al by inhalation. All participants of the study before entering the study were provided with specific written information about the aims of the study and then were asked to give written consents in accordance with ethical rules of Pharmaceutical Sciences Research Center (PSRC) of Tehran University of Medical Science (TUMS) where the study protocol was approved. Information on occupational history, socioeconomic status (salary, education) and lifestyle information (smoking, alcohol consumption, drug uses, consumption of vitamin or antioxidant supplements and dietary habits) were obtained by a questionnaires and a direct interview with each worker by a trained interviewer. All subjects were submitted to complete clinical examination to detect any signs or symptoms of chronic diseases such as arterial hypertension, heart failure, cancer, thyroid disturbance, asthma, diabetes and anemia. Individuals with chronic disease, alcohol consumption, antioxidant consumption and/or under drug treatment, or exposure to other toxic materials, radiation therapy, or substance abuse were excluded from the study. The included subjects were administered Lemon balm infusion (1.5 g/100 mL) twice per day for 30 days at 7.5 a.m. and 2 p.m. every day. Doses were obtained from our previous studies (Malekirad *et al*., 2012; Zeraatpishe *et al*., 2011). A supervisor carefully checked to make sure that the volunteers were taking infusion properly. Blood samples were collected from all subjects before using infusion and 12 h after the last dose of 30-day treatment with infusion.

Plasma preparation: Blood samples were collected from all subjects before using Lemon balm infusion and 12 h after the last dose of 30-day treatment with infusion. Five milliliter of 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.

Infusion preparation and protocol: Lemon balm infusion was prepared according to a standard protocol. To 3 g of plant leaves, 200 mL of distilled water was added. The initial temperature of added water was 98°C. Infusion was left to stay at room temperature without additional heating. Infusion time was 30 min (Zeraatpishe *et al*., 2011).

Measurement of plasma TAC: Plasma TAC was determined by measuring the ability of plasma to reduce Fe^{3+} to Fe^{2+}. The complex between Fe^{2+} and TPTZ gives a blue color with absorbance at 593 nm that is measured by spectrophotometer.

Measurement of plasma TTM: A volume of plasma (0.20 mL) was mixed with 0.6 mL of Tris-EDTA buffer (Tris base 0.25 M, EDTA 20 mM, pH 8.2) and 40 μL of 10 mM of DTNB in methanol. The final volume of the reaction mixture was made up to 4 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.

Measurement of plasma LPO: LPO of plasma was determined by the reaction of TBA with MDA and other lipid peroxides. Briefly, plasma samples were mixed with TCA (20%) and the precipitate was dispersed in H_2SO_4 (0.05 M). TBA (0.2% in sodium sulfate) was added and heated for 30 min in a boiling water bath. LPO adducts were extracted by n-butanol and absorbance was measured at 532 nm.

Statistical analysis: All data were analyzed with stats direct 2.7.8. A paired t-test was used for statistical comparisons of biochemical parameters. Pearson correlation coefficient was used to study the association between variables. P values lower than 0.05 were considered statistically significant.

RESULTS

Table 1 shows demographic information of subjects recruited in the study. The average levels of subjects ages and years of employment were 35.8±5.6 and 15±5.7, respectively.

Table 1: Demographic data of study subjects

Subjects	Age (Mean±SD)	Sex	Current smokers	Employment years (Mean±SD)
Exposed workers (n= 30)	35.8±5.6	Male (n=30) (100%)	N=5 16.67%	15±5.7

Table 2: Plasma oxidative stress and hematological markers before and after treatment with Lemon balm

Parameters	Before	After	p-value
TTM (mM)	0.150±0.086	0.198±0.068	0.015
TAC (mmol mL^{-1})	0.27±0.77	3.13±0.53	0.0001
LPO (nmol mL^{-1})	18.95±19.76	18.87±16	0.89
ALT (U L^{-1})	16.3±6.8	16.83±8	0.61
AST (U L^{-1})	31.5±10.44	21.7±8.4	0.0001
Triglyceride (g L^{-1})	180.4±122.1	146.6±81.1	0.036
Cholesterol (g L^{-1})	179.77±47.37	144.4±30.46	0.0001

Values are as Mean±SD

Table 2 shows the average levels of TAC, LPO, TTM, AST, ALT, triglyceride and cholesterol. The mean levels of TAC and TTM were significantly raised while the reduction of LPO was not statistically significant. The mean levels of AST, triglyceride and cholesterol significantly decreased after using infusion.

No linear correlation was found between age or years of employment and plasma biochemical parameters.

DISCUSSION

Use of Lemon balm infusion in Al workers caused a significant increase in blood TAC, TTM and a significant reduction in hepatic and lipid markers.

The first think that comes to mind is that accumulation of Al in the workers induces free radicals and oxidative stress. Poor and improper protection tools seem to be the main reason for increased oxidative stress. In our examination of the mine and interview with workers, it was clear that they did not use proper masks and were not properly trained to use working cloth and gloves or to take shower regularly. The only safety tools that they used was paper masks. Though there were suitable bathrooms, the workers did not use them so often. However, in case of working cloth, gloves and shoes, more workers were inclined to use them. Our team in another study have proved that workers of this factory have higher level of blood Al and oxidative stress (Ranjbar *et al*., 2008) that explains existence of exposure to Al.

In support of the present findings, an increase in the activities of AST and ALT and a decline in the activity of acid phosphatase (ACP) were previously reported (Yousef *et al*., 2007). The effect of *Melissa officinalis* L. extract on hyperlipidemic rats was also studied. Administration of *Melissa officinalis* L. extract reduced total cholesterol, total lipid, ALT, AST and ALP levels in serum and LPO levels in liver tissue, moreover increased glutathione levels in the tissue (Bolkent *et al*., 2005).

Many *in vitro* and *ex vivo* studies have shown antioxidant activity of *Melissa officinalis* extracts but *in vivo* studies especially in human are rare. *In vivo* studies just showed that *Melissa officinalis* L. extract could decrease LPO in rodents (Birdane *et al*., 2007) and in liver tissue of hyperlipidemic rats (Bolkent *et al*., 2005) and in radiology staff (Zeraatpishe *et al*., 2011). *Melissa officinalis* L. extract has been useful as rich source of antioxidants (Dastmalchi *et al*., 2008). The main phenolic compounds which were identified in tea infusion from Lemon balm were rosmarinic acid, luteolin 7-O-glucoside, quercetin 3-rutinoside, gallic acid, quercetin 3-O galactoside and ferulic acid.

Hence, it seems that Lemon balm, due to its antioxidant components and scavenging properties could increase the activity of antioxidant defense and decrease oxidative stress and triglyceride, cholesterol and AST in Al workers.

CONCLUSION

The oral administration of Lemon balm infusion may be useful for the protection of the Al worker from toxic effects of Al that is mediated through oxidative stress.

These findings encourage pursuing further studies such as determination of the effect of other antioxidants in Al induced oxidative stress and searching for natural antioxidants for Al workers in long-term Al exposures. Anyway, if workers use suitable protective tools and take daily shower, the absorption and entrance of Al into body would be reduced.

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