

A comparative study of genotoxicity and oxidative stress before and after using lemon balm and cinnamon in subjects exposed to Nickel Welding Fumes

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ABSTRACT

By producing reactive oxygen and nitrogen species, metal-induced toxicity and carcinogenicity alter DNA bases, increase lipid peroxidation, and change calcium and sulfhydryl homeostasis. The purpose of the study was to investigate putative effects of *Lemon balm* and *Cinnamomum zeylanicum* on the blood markers of welding workers. We measured nickel and chromium levels, biochemical parameters, blood oxidative stress markers, total antioxidant capacity (TAC), Myeloperoxidase (MPO), lipid peroxidation (LPO), and DNA damage. The study was conducted on 55 male workers who worked in an industrial subjects exposed to Nickel Welding Fumes. The participants were administered Lemon balm and Cinnamon extract infusion 1.5 (0.12 extract) and 0.25(0.013 extract) g/100 mL, respectively, they have drunken twice a day for 30 days at 7:00 AM and 2:00 PM every day. In order to analyze the achieved data, paired t-test and Pearson correlation coefficient have been used. After using the extract infusion, a significant increase revealed in TAC ($p= 0.007$). Also, administration of infusion decreased DNA damage but it was not statistically significant. After administration of infusion, a decrease in LPO and MPO were observed ($p= 0.014$, $p=0.000$ respectively). Also there is positive correlation between ALP and Ni with 8-oH-dG and also, between Ni and TAC. The results indicate that using infusion causes to raise in a TAC and reduce in DNA damage.

Keywords: Nickel Welding Fumes; Oxidative stress; Genotoxicity; Lemon balm; *Cinnamomum zeylanicum*.

INTRODUCTION

Reactive oxygen and nitrogen species production, metal-induced toxicity and carcinogenicity, cause a lot of different changes to DNA bases and alter calcium and sulfhydryl homeostasis and increase lipid peroxidation. While iron (Fe), copper (Cu), chromium (Cr), vanadium (V) and cobalt (Co) undergo redox-cycling reactions, metals like cadmium (Cd), mercury (Hg) and nickel (Ni) as a second group, the primary direction for their toxicity is reduction of glutathione and composing to sulfhydryl groups of proteins [1].

Many toxic substances including heavy metals, ozone, carbon monoxide, carbon dioxide, and nitrogen oxides are generated during welding [2]. A remarkable amount of chromium and nickel are detected in the fumes released during stainless

steel welding but not in the fumes from mild steel welding, with which all provocation tests were negative [3]. A study of the characteristics of aerosols presented in welding fumes reports that 30% of welding fumes are respirable dust [4].

They are all symptoms of many illnesses such as cardiovascular diseases, cancer, atherosclerosis, diabetes, neurological disorders (Parkinson 'disease, Alzheimer's disease) and chronic inflammation[5,6,7,8,9]. It would be mention that Ni, arsenic, and Cr can produce oxidative stress that is the cause of carcinogenic impacts. The expansion of oxidative stress in biological systems prepared it responsible for cellular damage or growth of illness. However, the oxidative stress-induced damage is not limited to its direct impacts on cellular mechanism like lipids, DNA and proteins. So, it can convert gene

expression, too. [10]. In pathological depositions, we can mention various effective elements and neuronal fatality in neurological disorders seems to have some factors [11]. In order to prevent frequently hydrogen abstraction through active radicals and antioxidants are able to display cellular lipid peroxidation (LPO) due to scavenging free radicals, decomposing peroxides, reduction of superficial oxygen density, chain breaking and stopping preliminary radical generation. In our last researches, the researchers mentioned the antioxidant potential of *Melissa* and *Cinnamon* [12,13,14]. The antioxidant capacity of each of these medicines have already been investigated and proved individually, however studies on their collective effects lack. The present study was conducted to perform a before-after clinical trial to investigate the probable positive influence of *Melissa officinalis* L. (Lemon balm) and *Cinnamomum zeylanicum* on the welding workers by measuring total antioxidant capacity (TAC), lipid peroxidation (LPO), Myeloperoxidase (MPO), and genotoxicity as toxicity markers.

MATERIALS AND METHODS

Plant material and preparation of infusion

The aerial parts of *Melissa officinalis* L. was gathered in August, 2013 from Botanical Garden of Arak University and identified as *Melissa officinalis* L. by Dr. SalehiArjmand, Faculty of Agriculture, Department of Medicinal Plant, Arak University. The *Cinnamon* was supplied by Arak Medicinal Plants Company and identified as *Cinnamon Zeylanicum*. The leaves of *Melissa officinalis* L. were dried in shade at room temperature for 12 days. To prepare infusion of the medicines, the leaves of *Lemon balm* were dried, cleaned and then parceled with *Cinnamon* in 1.5 (0.12 extract) and 0.25(0.013 extract) g bags. The instruction for preparation of infusion was given to the subjects; mixing a total of bags in 100 mL 98°C for 30 min. Between 7 and 8 AM on Saturday as the first day of working days, before entering the workplace, the blood samples were taken from the included subjects. The blood samples were put into test tubes and immediately centrifuged at 3000 g for 10 min, and then for further analysis of oxidative stress, genotoxicity

markers and metals level, the serum was separated and froze at -80 °C.

Subjects

The study was conducted on 55 male workers, with the age range of 27-50, who worked in a company located in an industrial part of Iran, in the Central province. All participants were provided with specific written specific information about the aims of the study before written consents were obtained, in accordance with ethical rules of Pharmaceutical Sciences Research Center (PSRC) of Tehran University of Medical Science where the study protocol was approved. Prior to blood collection, each individual was extensively interviewed by a specialized physician who filled in a structured questionnaire about disease and habit diet. 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 from questionnaires and interviews completed by each worker, with 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* and *Cinnamon* extract infusion [1.5 (0.12 extract) and 0.25(0.013 extract) g/100 mL] twice a day for 30 days at 7: 00 AM and 2: 00 PM every day. Doses were gained from our previous researchers [3,7]. A supervisor carefully checked proper taking infusion by the volunteers in order to making sure. The blood samples were collected from all the subjects into heparinized tubes before taking extract infusion and 12 hours after taking the last dose of 30-day treatment with infusion and quickly centrifuged at 3000g for 10 min and the serum was divided and freed at -800C for further analysis of oxidative stress markers and metals level.

Assay of genotoxicity and oxidative stress markers and metals

The 8-hydroxy-2-deoxy guanosine (8-OH-dG) as a measure of deoxyribonucleic acid (DNA) damage was measured by ELISA technique according to kit brochure in order to estimate genotoxicity. The kit uses an anti IgG-coated plate and a tracer consisting of an 8-OH-dG- enzyme conjugate and 8-OH-dG antibody recognizing both free 8-OH-dG and DNA-incorporated 8-OH-dG. The rate of oxidation of o-dianosidine has been measured MPO was also assayed using ELISA kit. To measure the method was used [15]. as the tool for measuring rate of LPO, thiobarbituric acid (TBA). The basis of TAC measurement was to analyze the ability of plasma in reducing Fe^{3+} to Fe^{2+} in the presence of TPTZ. Fe^{2+} -TPTZ as a blue compound was absorbed in 593 nm [16]. Levels of Ni and Cr in subject's serum were determined using an Atomic Absorption Spectrometer. All of these tests have been already conducted in our lab and was used in several experiments.

Statistical analysis

The results are accessible as Mean \pm SD. The statistical analyses were conducted by using Stats Direct 2.7.9 software.

The paired t-test has been used. By using Pearson correlation coefficient analyses, the relationships between parameters were measured. Any p value less than 0.05 was considered statistically significant.

RESULTS

The Mean \pm SD values for age and employment years of subjects were 37.31 ± 10.62 and 7.39 ± 6.42 , respectively (Table 1). All the subjects (55 cases) were male. Table 2 shows significant increase of TAC after using the extract infusion ($p=0.007$). Before and after the extract infusion, the Mean \pm SD values were 224.67 ± 132.86 and 270.84 ± 130.58 , respectively. After administration of infusion, MPO was decreased (2.90 ± 1.34 before versus 1.62 ± 0.83 after, $p=0.000$).

Table 1. Demographic data of study subjects

Subjects	Age	Sex	work history
Workers(n=55)	47.31 ± 10.62	Male (100%)	7.39 ± 6.42

Data represent mean \pm SD

Table 2. Plasma oxidative stress markers before and after treatment with Lemon balm and Cinnamon

Parameters	Before	After	p-Value
TAC ($mmol mL^{-1}$)	224.67 ± 132.86	270.84 ± 130.58	0.007
LPO ($mmol mL^{-1}$)	57.13 ± 41.16	54.40 ± 39.69	0.014
8-OH-dG (Pg/mL)	310.70 ± 113.83	300.08 ± 108.41	0.266
MPO (U/mL)	2.90 ± 1.34	1.62 ± 0.83	0.000

Data represent mean \pm SD; TAC, total antioxidant capacity; LPO, lipid peroxidation; MPO, Myeloperoxidase; 8-OH-dG, 8-hydroxy-2-deoxy guanosine.

Table 3. The association of history work, oxidative stress and heavy metal parameters with biochemical parameters

Biochemical parameters/Oxidative stress	TAC ($mmol mL^{-1}$)	LPO ($mmol mL^{-1}$)	8-OH-dG (Pg/mL)	MPO (U/mL)
AST($31.47 \pm 8.6U/L$)	-0.138	0.204	-0.133	0.053
ALT($29.8 \pm 15.33U/L$)	-0.031	0.155	-0.008	0.023
ALP($237.35 \pm 56.74U/L$)	0.171	-0.305	0.285*	-0.082
CL ($193.88 \pm 40.12mg/dL$)	0.253	-0.285	0.232	-0.055
TG ($160.07 \pm 83mg/dL$)	-0.117	0.266	-0.127	0.152
Creatinine ($1.09 \pm 0.16mg/dL$)	-0.094	0.239	-0.264	-0.121
Ni($20.24 \pm 5.21\mu g/kg$)	0.427*	-0.430	0.489**	0.196
Cr($1.33 \pm 1.65\mu g/kg$)	0.167	-0.043	0.074	-0.114
WH(7.39 ± 6.42 years)	-0.013	-0.039	-0.024	0.001

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; CL, cholesterol; TG, triglycerides; Ni, nickel; Cr, chromium; WH, work history; * $p < 0.05$, ** $p < 0.001$

After administration of infusion, 8-OH-dG was decreased (310.70 ± 113.83 before versus 300.08 ± 108.41 after, $p=0.266$). After administration of infusion, significantly decrease in LPO (57.13 ± 41.16 before versus 54.40 ± 39.69 after, $p=0.014$) was observed. There were no significant relation between work history and LPO ($R=-0.039$, $p=0.780$), TAC ($R=-0.013$, $p=0.922$), 8-OH-dG ($R=-0.024$, $p=0.865$) and MPO ($R=0.001$, $p=0.995$). The average levels of cholesterol (CL), triglycerides (TG), creatinine, aspartate aminotransferase (AST), alkaline phosphatase (ALP), Ni, Cr and alanine aminotransferase (ALT) before of intervention are shown in Table 3. Table 3 showed the associations of oxidative stress and heavy metal parameters with biochemical factors before intervention. There is positive correlation between ALP and Ni with 8-oH-dG and also, between Ni and TAC.

DISCUSSION

The findings of the present study show that using infusion can increase TAC and decrease LPO and MPO. There is positive correlation between ALP and Ni with 8-oH-dG and also, between Ni and TAC. After organization of infusion, there was decrease in 8-OH-dG but it was not significant. According to our study, the oxidative stress ratio was significantly higher in the welding exposed workers of control groups [17,18,19].

Also, in Ni-platers the level of plasma lipid peroxidation in experimental was significantly high and erythrocyte antioxidants were significantly low than controls. The level of plasma lipid peroxidation was positively and erythrocyte antioxidants were negatively significantly correlated with the urine Ni levels [20]. Other study pointed out that there were significant correlation between urine metals levels and the length of welding work and on the other side, Ni didn't significantly related to GSH [21]. Mukherjee et al. expressed that Ni, V, Cr, and Cu were significantly related with the 8-OH-dG level [22]. However, *Melissa officinalis L.* extract could reduce LPO in rodents [23] and in liver tissue of hyper lipidemic rats [24].

There are positive correlation between ALP and Ni with 8-oH-dG and also, between Ni and TAC. It seems that Ni by way of increased free radicals production results in inducing oxidative stress [1,10] and DNA damage. Moreover TAC in permanent adaption with high oxidative stress was increased that already showed in our previous study in radiology staff [25]. Some studies have shown that *Lemon balm* and *Cinnamon* infusion have high phenolic compounds and improve enzymatic antioxidant system and can reduce oxidative stress in non-alcoholic fatty liver disease, hospital, industrial workers and healthy human, concurrently [8,12,14,26]. Administering of *Lemon balm* and *Cinnamon* infusion can be effective in protecting the welding workers against oxidative stress. It can be say that the ability of *Lemon balm* and *Cinnamon* infusion is mediated via their phenolic compounds, particularly phenol acids, flavonoids and their antioxidant activity. Also, decrease in all parameters of oxidative stress especially DNA damage is not due to low work history with the factory ventilation system. So, in order to find out the effect of the other antioxidants in welding-induced oxidative stress and seeking natural antioxidants, further studies are needed.

CONCLUSION

It would be mention that the ability of *Lemon balm* and *Cinnamon* infusion is mediated through their phenol compounds, especially, flavonoids, phenol acids, and their antioxidant activity. But, in order to find out the effects of the other antioxidants in welding -induced oxidative stress and seeking natural antioxidants, further researchers are required. Also, supplying workers with special training, proper protective instruments and encouraging them to use these instruments and also, taking daily shower may decrease the risk of exposure to toxic elements and stop them from entering the workers' bodies.

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