

# The Effect of Tea-Cinnamon and *Melissa officinalis* L. Aqueous Extraction, on Neuropsychology Distress, Biochemical and Oxidative Stress Biomarkers in Glass Production Workers

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## Abstract

Medicinal plants are considered as natural sources of antioxidant compounds which may protect organisms against oxidative stresses. The aim of this study was to evaluate the potential of Cinnamon and *Melissa officinalis* L. (Lemon balm) on the improvement of oxidative stress in glass production workers. This clinical study was conducted on 32 glass production workers. They were asked to use 0.5 g of tea-Cinnamon and 3 g of Lemon balm as infusion for 30 days in the morning and evening each day. At the beginning and the end of the study, blood samples were taken from individuals to determine the level of fasting blood sugar (FBS), aspartate transaminase (AST), alanintransferase (ALT), triglyceride (TG), cholesterol (CL), low-density lipoprotein (LDL), high-density lipoprotein(HDL) levels, lipid peroxidation (LPO), total antioxidant capacity (TAC) and sillies were measured in workers' blood. At the end of experiment, data were subjected to the paired t-test analysis. Clinical examination was accomplished to record any abnormal signs or symptoms. After treatment, the high-density lipoprotein and TAC of serum significantly increased while the AST and LPO decreased. There were positive correlations between work history and initiative energy disorder of cognitive test. Probably tea-Cinnamon and Lemon balm possesses marked an-

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**tiioxidant activity and, therefore, it can be used to protect individuals from the oxidative stresses. Using supplementary antioxidants may be helpful in the treatment of workers.**

## Keywords

**Biochemical Toxicity Biomarkers, Glass Production Worker, Tea-Cinnamon and Lemon Balm**

## 1. Introduction

Workers who work in mines—especially coal mines as well as those working in metallurgical, chemical and construction industries are the major occupational groups who are exposed to crystalline silica dust which is one of the harmful agents to human health and crystalline is so abundant in work places. According to Maciejewska more than 2 million workers are in danger of exposure to crystalline silica in European countries [1]. IARC has ranked crystalline silica as carcinogenic to humans (group I) [2]. Workers in industries like mining, quarrying, glass making, and metal founding are more subject to crystalline silica (silicon dioxide) dust which results in disability and death among them. Inhaled silica can pile up in the body and make problem in respiratory system and other organs (like kidneys). Concomitant exposure to other minerals can change the biological effects of respirable silica. The hazard of building fibrous tissues extends when workers are exposed to high levels of silica and consequently restricts the lung disease (silicosis). Recently, the possibility of the relation between silica and silicosis and lung cancer has caused many debates [3]. Moreover, silicas and silicates are among the most frequent compounds found naturally in the earth's crust. Crystalline silica, if exposed excessively, may cause serious lung disease such as silicosis and it is believed to be associated with lung cancer [4]-[6]. Evidence suggests that chronic levels of silica dust that do not cause disabling silicosis may cause the development of chronic bronchitis, emphysema, and/or small airways disease that can result in airflow obstruction, even in the absence of radiological silicosis [7]. It is also proved that there is a certain exposure-response relationship between increasing dust exposure level and deaths from all causes, colorectal cancer, lung cancer, respiratory diseases, and pulmonary tuberculosis. The findings show that silica mixed dust in ceramic factories has harmful impact on the workers' health and their life span in ceramic factory [8]. On the other hand, increased mortality from asthma, chronic bronchitis and emphysema has already been reported among workers in the silicon carbide (SiC) industry [9] [10]. It is worth mentioning that if one is exposed to it for a long time, silica dust can cause an imbalance in the production of free radicals and in the antioxidant system, which in its turn may induce oxidative stress [11]. Exposure to SiO<sub>2</sub> nanoparticles *in vitro* state increased ROS levels and reduced glutathione levels. The increased production of malondialdehyde and lactate dehydrogenase release from the cells indicated lipid peroxidation and membrane damage. In summary, exposure to SiO<sub>2</sub> nanoparticles results in a dose-dependent cytotoxicity in cultural human bronchoalveolar carcinoma-derived cells that is closely correlated to increased oxidative stress [12]. It is well known that workers in granite, pottery factories, bioactive glass and tungsten mines suffer lung impairment, silicosis and oxidative stress [13]-[16], and they may also encounter DNA damage and carcinogens [17]-[19].

However, to keep the level of reactive oxygen species (ROS) under control, living organisms have developed antioxidant systems that contain non-enzymatic such as glutathione, ascorbic acid, tocopherol, carotene, uric acid, bilirubin as well as enzymatic scavengers like superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Antioxidants can inhibit lipid peroxidation (LPO) by decreasing localized oxygen density, scavenging free radicals, stopping initiating radical generation, decomposing peroxides, and chain breaking to prevent continued hydrogen abstraction by active radicals. Some studies in recent years have been proved that natural and synthetic antioxidants can be used to manage many diseases such as osteoporosis [20], diabetes and islet transplantation [21]-[23], inflammatory bowel diseases [24], preeclampsia [25], and pancreatitis [21] and non-alcoholic fatty liver diseases [26] and they are also applicable even in environmental toxicology [27] [28] and industrial toxicology [29]. In our previous studies, we have reported the antioxidant potentiality of Lemon balm and Cinnamon [27]-[29]. Many studies have proved the antioxidant capacity of these herbal medicines but not specifically for glass production workers; hence in the present research we aimed at exploring beneficial effects of the mixture of Lemon balm, *Cinnamomum zeylanicum* on oxidative stress in glass production workers.

## 2. Subjects

The first cohort included 32 (30 male and 2 female) workers, with age range of 22 - 54, who worked in the glass factory that is located in Esfahan. This factory has started its activity since 2007 and yearly produces about 150,000 m<sup>2</sup> of all kinds of squirt glass. The subjects were occupationally exposed to silica by inhalation. All participants were provided with specific written 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. 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 mixture infusion (1.5 and 0.25 g/100 mL) twice daily for 30 days at 7.5 a.m. and 2 p.m. every day. Doses were obtained from our previous studies [26]-[29]. 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 hours after the last dose of 30-day treatment with infusion into heparinized tubes and immediately centrifuged at 3000 g for 5 min and the plasma was separated and freeze-dried at -80°C for further analysis of oxidative stress markers and levels of Si.

## 3. Materials and Methods

Tetraethoxypropane (MDA) from Sigma-Aldrich Chemie (GmbH Munich, Germany), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol 2,4,6-tripyridyl-striazine (TPTZ), Sigma-Aldrich Chemie (GmbH-Munich, Germany) HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, HCL, NaCl, PdCl<sub>2</sub>, Cu (NO<sub>3</sub>)<sub>2</sub>·3H<sub>2</sub>O from Merck (Tehran, Iran).

### 3.1. Plant Material

The aerial parts of *Melissa officinalis* L. was collected in August 2011 from Botanical Garden of Arak University, and identified as *Melissa officinalis* L. by Dr Salehi Arjmand from Department of Medicinal Plant, Faculty of Agriculture, Arak University. Cinnamon was supplied by Arak Medicinal Plants Company and identified as *Cinnamom Zeylanicum*. The leaves of *Melissa officinalis* L. were dried in shade at room temperature for 12 days.

### 3.2. Infusion Preparation and Protocol

Lemon balm leaves were dried and cleaned and then packed with Cinnamon in 1.5 and 0.25 g tea bags. The subjects were instructed how to prepare the infusion by mixing a total of 1.5 and 0.25 g in 100 mL 98°C water for 30 minutes. A qualified expert supervised the whole procedure.

### 3.3. Assay of Oxidative Stress Markers and Silica

To measure the rate of lipid peroxidation (LPO), thiobarbituric acid TBA method was used [30]. As a result of free radicals attack, different aldehydes such as malondialdehyde (MDA) were produced from lipids which reacted with thiobarbituric acid in acidic conditions and high temperatures. The resulted complex had 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 was removed and precipitate was washed with 0.5 M sulphuric acid. Afterwards, 2.5 ml 0.5 M sulphuric acid and 3 ml 0.2% thiobarbituric acid solution were added to each tube. After preparation of 3 ml 0.2% TBA solution and 2.5 ml of each standard, all samples together with 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.

The basis of measurement of total antioxidant capacity (TAC) was to analyze the ability of plasma in reducing

Fe<sup>3+</sup> to Fe<sup>2+</sup> in the presence of TPTZ. Fe<sup>2+</sup>-TPTZ as blue complex was absorbed in 593 nm [31].

Silicon in serum samples was measured by graphite-furnace atomic absorption spectrophotometer. Solutions of silicon added to serum gave the same signal response as the aqueous standards. Thus, aqueous calibration was suitable for measurements of silicon in serum, with good recoveries from either matrix. We calibrated the instrument with two standards, prepared by diluting the primary standard solution in 10 ml/LNO<sub>3</sub> reagent to give final concentrations between 0 and 72.3 μmol/L for serum. For serum analysis we diluted 0.5 mL of standard for serum with 1.5 mL of the HNO<sub>3</sub> diluent with 1.5 mL of the HNO<sub>3</sub> diluent; for urine analysis the silicon emission used was 251.611 nm.

### 3.4. Biochemical Analysis of Serum Parameters

All biochemical serum analyses were performed in the same laboratory including AST, ALT and FBS, TG, CL, LDL and HDL.

### 3.5. Subjective Neurocognition Inventory (SNI)

The self-report questionnaire consisted of 76 items with a focus on everyday memory and attention problems. Part of the questionnaire was the “Fragebogen Erlebter Defizite der Aufmerksamkeit” (FEDA) for the measurement of self-experienced deficits of attention), and a questionnaire that taps attentional difficulties. This scale was complemented by new items constructed by [32]. Items had to be endorsed on a five-point likert scale (very frequently-never). The following domains were tested: selective attention (10 items), divided attention (4 items), long-term memory (7 items), prospective memory (7 items) and psychomotor retardation (9 items). One item tapped the forgetting of names (for a more through description see [33]-[35]).

### 3.6. Statistical Analysis

Results are presented as mean ± SD. Statistical analyses were conducted using Stats Direct 2.7.8 software. The paired t-test was applied. Relationships between parameters were determined by using Pearson correlation analysis. P value of less than 0.05 was considered statistically significant.

## 4. Results

The mean ± SD values for age and employment years and silica concentration of subjects were 30.79 ± 8.59 and 6.06 ± 2, respectively. Of subjects, 30 (93.75%) were male and 2 (6.25%) were female (Table 1).

After using the mixture infusion, LPO and AST levels significantly decreased. An increase in FBS, TAC and HDL levels was observed after the administration of the infusion (Table 2).

Table 3 showed the mean ± SD of the cognitive function and psychological distress in workers before the intervention.

There were positive correlations among work history and initiative energy disorder of cognitive test.

## 5. Discussion

The use of Lemon balm and cinnamon infusion in glass production workers resulted in significant increase in TAC and HDL and a significant reduction in LPO and AST markers. A study reported that GSH-Px and 8-hydroxydeoxyguanosine (8-OHdG) in dust-exposed group and silicosis group were significantly higher than those in control group [36]-[39]. Another study showed that serum superoxide dismutase (SOD) activity and serum levels of malondialdehyde (MDA) and glutathione (GSH) were significantly higher in silicosis patients than in controls [40]. Moreover, Quartz and vitreous silica, opposite to silica spheres, showed irregular particles with sharp edges, stable surface radicals, and sustained release of HO(\*) radicals via a Fenton-like mechanism [41].

**Table 1.** Demographic data of study subjects.

Subjects	Age (mean ± SD)	Sex	Current smokers	Employment years (mean ± SD)	Silica concentration
Exposed workers (n = 32)	30.79 ± 8.59	Male (n = 30; 93.75%); Female (n = 2; 6.25%)	N = 5 16.67%	6.06 ± 2	5 ± 3.5 μmol/dl

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

Parameters	Before Mean $\pm$ SD	After Mean $\pm$ SD	P value
TAC (mmol/mL)	2.8 $\pm$ 0.57	3.3 $\pm$ 0.39	0.0001*
LPO (nmol/mL)	4.89 $\pm$ 6.63	1.69 $\pm$ 2.14	0.018*
FBS (mg/dL)	78.7 $\pm$ 8.98	83.04 $\pm$ 10.48	0.013*
LDL (mg/dL)	84.67 $\pm$ 27.14	86.75 $\pm$ 24.92	0.53
HDL (mg/dL)	43.33 $\pm$ 7.9	46.33 $\pm$ 8.32	0.001*
ALT (U/L)	26.50 $\pm$ 16.91	24.13 $\pm$ 13.04	0.3
AST (U/L)	17.75 $\pm$ 1.24	15.54 $\pm$ 5.50	0.046*
TG (mg/dL)	151 $\pm$ 46.99	155.63 $\pm$ 44.91	0.54
CL (mg/dL)	158.21 $\pm$ 32.17	164.21 $\pm$ 29.14	0.095

\*P < 0.05. LPO: lipid peroxidation, TAC: total antioxidant capacity HDL: high density lipoprotein, LDL: low density lipoprotein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen, CL: cholesterol, TG: triglycerides, Data represent mean  $\pm$  SD.

**Table 3.** The status of cognitive function and psychological distress in workers.

Group	PS	SA	DA	VM	NVM	PM	SF	IE	TOTAL
Workers	32.5 $\pm$ 7.94	43.15 $\pm$ 10.07	17.1 $\pm$ 3.39	28.5 $\pm$ 5.04	16.45 $\pm$ 3.22	29.55 $\pm$ 4.22	21.55 $\pm$ 4.15	40.45 $\pm$ 7.28	229.25 $\pm$ 39.91

PS: psychomotor speed; SA: selective attention; DA: divided attention; VM: verbal memory; NVM: nonverbal memory; PM: prospective memory; SF: spatial functioning; and I/E: initiative/energy. Data represent mean  $\pm$  SD.

On the other hand, MDA levels of both exposed groups were significantly higher than those of the controls and were significantly higher among workers exposed to asbestos than among those exposed to silicadusts [42]. The results of previous studies as well as our findings indicated the exposure to silica in glass production workers ends in the accumulation of silicon in the workers, thus inducing free radicals and oxidative stress. An enhanced plasma level of silicon among workers confirms the existence of pollution and also absorption of this metal into body. Poor and improper protection tools seem to be the main reason for this increased plasma level of silicon in workers. In the examination of the factory and interviewing the workers, it was found that they did not use any masks and were not properly trained to use working clothes and gloves or to take shower regularly. There were not suitable bathrooms, and masks. However, in case of working clothes, gloves and shoes, more workers were inclined to use them.

Analysis did not show a significant positive correlation between silicon concentration and clinical symptoms.

Many studies show consistently increased mortality for lung and larynx cancer in the overall cohort and among makers. Stomach cancer, colon cancer, bladder cancer, brain cancer, hypertensive diseases and diseases of the genitourinary system were also increased in the overall cohort and among glass workers [43]-[45]. Also exposure to glass microfibers increases the risk of respiratory and skin symptoms and also increases the risk for cough and nasal symptoms [46]. Moreover Glass bottle workers showed an excess of upper respiratory tract symptoms, cough, and shortness of breath compared with matched hospital control workers [47]. In our present study, only those workers didn't have these clinical signs of silicon poisoning who had been employed for a short time period.

In the present study, there were no significant relationships between oxidative stress and neurocognitive disorders, either.

However, it is clear that the underlying factor in the neurological disorders is the increased oxidative stress substantiated by the findings that the protein side-chains are modified either directly by reactive oxygen species (ROS) and reactive nitrogen species (RNS) or, indirectly, by the products of lipid peroxidation [48]. Mild cognitive impairment (MCI), the phase between normal aging and early dementia, is a common problem in the elderly with many subjects developing Alzheimer's disease (AD). Oxidative damage to nuclear and mitochondrial DNA occurs in the earliest detectable phase of AD and may play a meaningful role in the pathogenesis of this disease [49]. On the other hand, Free radical-mediated oxidative damage is thought to play a role in the pathogenesis of

Alzheimer disease and the oxidative damage to lipids, proteins, DNA, and RNA in multiple brain regions in late-stage Alzheimer disease. Some studies establish oxidative damage as an early event in the pathogenesis of Alzheimer disease that can serve as a therapeutic target to slow the progression or perhaps the onset of the disease [50]. Another study shows that individuals with mild cognitive impairment (MCI) have increased brain oxidative damage before the onset of symptomatic dementia [51]. It seems that various factors influence the pathological depositions, and in general, the cause of neuronal death in neurological disorders appears to be multifactorial and most probably many other factors like workers' lifestyle may contribute to the dysfunction of cognitive disorders.

Moreover, Amin and Abd El-Twab showed that cinnamon acted as hypocholesterolemic, hepatoprotective agent and Serum ALT, AST levels were significantly lowered in *cinnamon*-treated than untreated rats group [52]. Research has also showed that cinnamon extract has potent hepatoprotective action and the elevated serum AST and ALT enzymatic activities induced by CCl<sub>4</sub> were restored towards normalization significantly by oral administration as compared to non-treated rats [53]. On the other hand, many *in vitro* and *ex vivo* studies have showed antioxidant activity of *Melissa officinalis* extracts; but the number of *in vivo* studies, especially in human, is rare. The few *in vivo* studies have just showed that *Melissa officinalis* L. extract could decrease LPO in rodents [54] and in liver tissue of hyperlipidemic rats [55] and radiology staff [28] [56]. The effects of *Melissa officinalis* L. extract on hyperlipidemic rats has previously been studied. The 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, it increased glutathione levels in the tissue [55]. Our previous studies showed that Lemon balm and cinnamon infusion had high phenolic compounds and could improve enzymatic antioxidant system and simultaneously reduce stress oxidative in healthy human, hospital workers and non alcoholic fatty liver. The main phenolic compounds that were identified in tea infusion from Lemon balm, respectively were rosmarinic acid, luteolin 7-o-glucoside, quercetin 3-rutinoside, gallic acid, quercetin 3-o-galactoside and ferulic acid. Recent studies indicated that the oral administration of Lemon balm is beneficial in protection against oxidative stress and DNA damage in subjects exposed to long-term low-dose ionizing radiation and fatty liver diseases that a significant decrease ( $P = 0.0001$ ) in LPO level and liver enzyme were observed [26]-[28] [56]. To protect glass production workers against silicon induced oxidative stress and to enhance their antioxidant defense system, the oral administration of Lemon balm and cinnamon infusion can be helpful. It seems that our infusion has phenolic compounds and due to its scavenging properties results in the reduction of free radicals and improvement of the liver by decreasing hepatic enzymes. As limitation of this study, the researchers were not able to control other antioxidants that they intake exactly because of ethical reasons.

## 6. Conclusions

This ability of Lemon balm and cinnamon infusion most probably is due to its phenolic compounds, specifically phenolic acids and flavonoids and their antioxidant activity.

These findings encourage pursuing further studies like identifying the effect of other antioxidants on silicon induced oxidative stress and searching for other natural antioxidants that can help glass production workers who are exposed to silicon chronically. Anyway, the proper use of protective tools and taking daily shower by workers can reduce the absorption of toxic elements and stop them from getting into their bodies

## Authors' Note

AA Malekirad participated in the design of the study and carried out the study and drafted the manuscript; M Rashidi participated in the design of the study and carried out oxidative stress tests; S Habibollahi participated in design of the study and; N Dolatyari carried out neuropsychological tests. Mehdi Narimani carried out assay of sillies; M Abdollahi conceived and supervised entire study and edited the manuscript.

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## Abbreviation

LPO: lipid peroxidation  
TAC: total antioxidant capacity  
HDL: high density lipoprotein  
LDL: low density lipoprotein  
AST: aspartate aminotransferase  
ALT: alanine aminotransferase  
BUN: blood urea nitrogen  
CL: cholesterol  
TG: triglycerides  
LPO: lipid peroxidation  
TAC: total antioxidant capacity  
SOD: Scavengers like superoxide dismutase  
GPx: glutathione peroxidase  
CAT: catalase

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