

**Improvement of Oxidative Stress Biomarkers in Human Plasma  
Following Consumption of *Mentha Pulegium* Decoction; a Before and  
after Clinical Trial**

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### Summary

Many plants contain natural antioxidants that act in metabolic response to the endogenous production of free radicals and other oxidant species. The present before after clinical trial was carried out to investigate the positive effects of the decoction of aerial part of *Mentha pulegium* in human. A group of 20 healthy subjects was invited to use the *M. pulegium* (60 mgkg<sup>-1</sup>) twice a day for 14 days. Blood samples before and after entering the study were measured for oxidative stress biomarkers including lipid peroxidation level (LPO), total antioxidant capacity (TAC) and total thiol molecules (TTM). A reduction of blood LPO ( $10.04 \pm 3.46$  vs.  $7.21 \pm 2.85$ ,  $P=0.03$ ) was observed after 14 days of *M. pulegium* consumption. Blood TAC ( $1.87 \pm .32$  vs.  $3.24 \pm 0.45$ ,  $P=0.0001$ ) and TTM ( $0.16 \pm 0.10$  vs.  $0.314 \pm 0.13$ ,  $P=0.004$ ) significantly improved after 14-days consumption of *M. pulegium*. The phenolic content of *M. pulegium* is thought to be responsible for positive effects of *M. pulegium*. The present study for the first time reports that *M. Pulegium* bears marked antioxidant activity in human by enhancement of TAC and TTM and reduction of LPO to protect body from free radical damage and induction of oxidative stress. Therefore use of *M. pulegium* as a dietary supplement is highly recommended.

**Key words:** antioxidant. oxidative stress. Decoction. *Mentha pulegium*.

### **Introduction**

Many plants contain natural antioxidants that act in metabolic response to the endogenous production of free radicals and other oxidant species. These responses are either due to ecological stress or promoted by toxins produced by pathogenic fungi and bacteria [1]. Recently, interest has increased in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage [2-5]. Over-production of reactive oxygen species (ROS) in human beings, by endogenous or external sources, e.g. tobacco smoke, certain pollutants, organic solvents or pesticides, leads to oxidative stress [6-9]. In healthy cells, there is an equilibrium between the production of these highly reactive species and the different defense systems, either enzymatic or non-enzymatic. When this equilibrium is disrupted, oxidative damage occurs due to free radical accumulation, defined as oxidative stress and, as a consequence, many diseases, such as cancer, arteriosclerosis and other cardiovascular problems and diabetes and even aging are promoted [10-11]. It is believed that medicinal plant can be a potential source of antioxidants and ROS scavenger molecules [12-17]. *Mentha pulegium*, due to its ketonic compounds has been shown to be a rich source of antioxidants [18]. *Mentha pulegium* L., popularly known as "*Khalvash*", is consumed mainly for its antiseptic, insect repellent, carminative, antispasmodic, diaphoretic, and anti-inflammatory properties in Iran [19]. Traditionally, total decoction of this herb has been used for the treatment of fibrosis and cervical tumors [20] and in the treatment of

flatulent dyspepsia and intestinal colic, due to its carminative and antispasmodic properties [19]. Essential oils of this plant have been shown to contain mainly pulegon and menthone [21-22]. The water extract of *M. pulegium* showed the highest radical scavenging activity in DPPH test ( $IC_{50} = 8.9 \pm 0.2 \mu gml^{-1}$ ) [23] and antioxidant activity comparable to  $\alpha$ -tocopherol [24].

Administration of essential oil of *M. pulegium* to mice caused a significantly decrease in hepatic and renal lipid peroxide levels and increased the levels of glutathione and glutathione dependent enzyme as well as enhancing the levels of antioxidant defense enzyme [25].

Regarding above-mentioned information, we were interested in performing a before after clinical trial study to explore antioxidant influences of *M. pulegium* in human by evaluating blood total antioxidant capacity (TAC), lipid peroxidation (LPO) and total thiol (SH) molecules.

## Methods

### *Plants material and total extract*

Aerial parts of *M. pulegium* were collected from Rasht (a city in north of Iran) in May 2006. A total of 1 kg air-dried aerial parts of *M. pulegium* was used to provide the decoction.

### ***Chemicals***

Dithionitrobenzoic acid (DTNB), Tris base, 1,1,3,3-tetraethoxypropane (MDA), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol, 2,4,6-tripyridyl-s-triazine (TPTZ) from Merck (Tehran), were used in this study.

### ***Subjects***

A clinical trial with a total of 20 subjects was designed. Subjects were volunteer healthy students of Arak High school, located in the south-west of Iran. Subjects were selected on a simple random basis from volunteers. The study was conducted in complete accordance with the declaration of Helsinki. All participants were provided with specific written consents obtained prior to entrance into the study. Each individual was extensively interviewed by a physician who filled in a structured questionnaire specifying gender, smoking, dietary habits, sports habits, and history of special disease before obtaining blood. Then the subjects were administered *M. pulegium* decoction (60 mg kg<sup>-1</sup>) twice daily (morning and evening) for 2 weeks. The dose was selected on the basis of a pilot study and traditional use information. A supervisor carefully checked to make sure that the volunteers were taking the decoction properly. Demographic characteristics of the subjects are presented in Table 1.

**Tab. 1.** Basic characteristics of the study subjects

| Characteristic     |                     |                  |
|--------------------|---------------------|------------------|
| Female gender      | Female<br>20 (100%) | Male<br>0        |
| Smoking            | No<br>20 (100%)     | Yes<br>0         |
| Age (year)         | <b>15-17</b>        |                  |
| Using drug         | No<br><b>0</b>      | Yes<br>20 (100%) |
| Number of subjects | 20                  | <b>0</b>         |

***Sampling and laboratory measurements***

All the blood samples were drawn by venipuncture and coded. Processing and scoring of the samples from two groups of subjects were performed blindly in the laboratory. At the end of the study, the data from the questionnaire was linked to the code number for data analysis.

***Measurement of plasma TAC***

The ability of blood in reducing  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  is the principle of the method used. In brief, the medium is exposed to  $\text{Fe}^{3+}$  and the antioxidants present in the medium start to produce  $\text{Fe}^{2+}$ . The reagent included 300 mmol/L acetate buffer, pH 3.6, and 16 mL  $\text{C}_2\text{H}_4\text{O}_2/\text{L}$  (acetic acid) of buffer solution, 10 mmol/L TPTZ in 40 mmol/L HCL, 20 mmol/L  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ .

The working reagent was prepared as required by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL FeCl<sub>3</sub> 6 H<sub>2</sub>O solution. Ten microliters of H<sub>2</sub>O diluted sample was then added to 300 µL freshly prepared reagent warmed at 37°C. The complex between Fe<sup>2+</sup> and TPTZ gives a blue color with absorbance at 593 nm.

#### ***Measurement of LPO***

For measuring the rate of LPO, the TBA-reactive substances (TBARS) were measured. In this method blood samples were mixed with TCA (20%) and the precipitate was dispersed in H<sub>2</sub>SO<sub>4</sub> (0.05 M). TBA (0.2% in sodium sulfate 2M) was added and heated for 30 min in boiling water bath. TBARS adducts were extracted by n-butanol and the absorbance was measured at 532 nm. In this method, the reaction products are reported as TBARS because in addition to MDA other aldehydes react with TBA. This reaction is formed in acidic pH and high temperature and gives a maximum absorbance with a pink color at 532 nm.

#### ***Measurement of plasma TTM***

A volume of blood (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 µl 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.

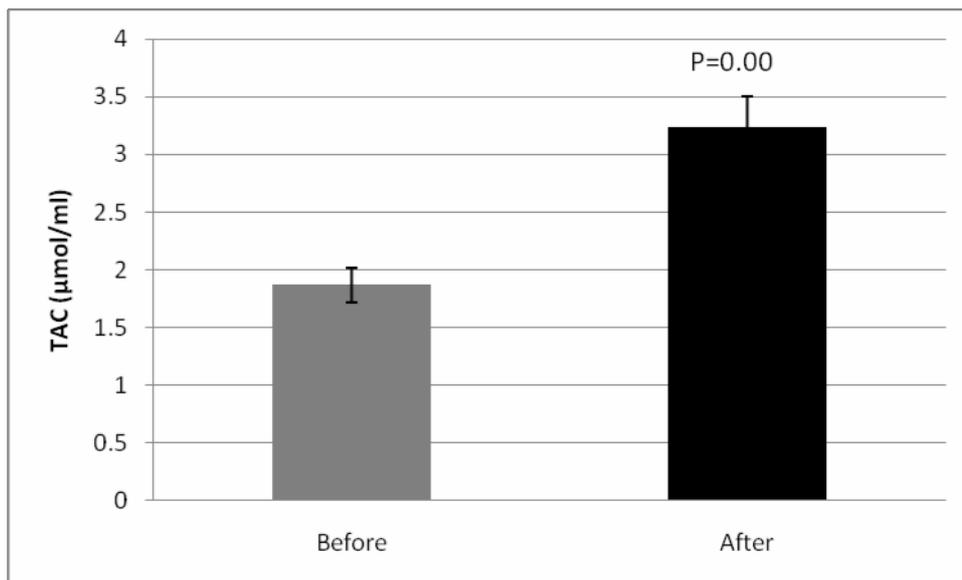
***Statistical analysis***

Nonparametric test (Wilcoxon) was used to analyze the significance of difference observed between study groups. F-test was used to determine the normal distribution of variances between groups. Type I error was chosen at a level of 5%. The power of study was 80%.

**Results and Discussion**

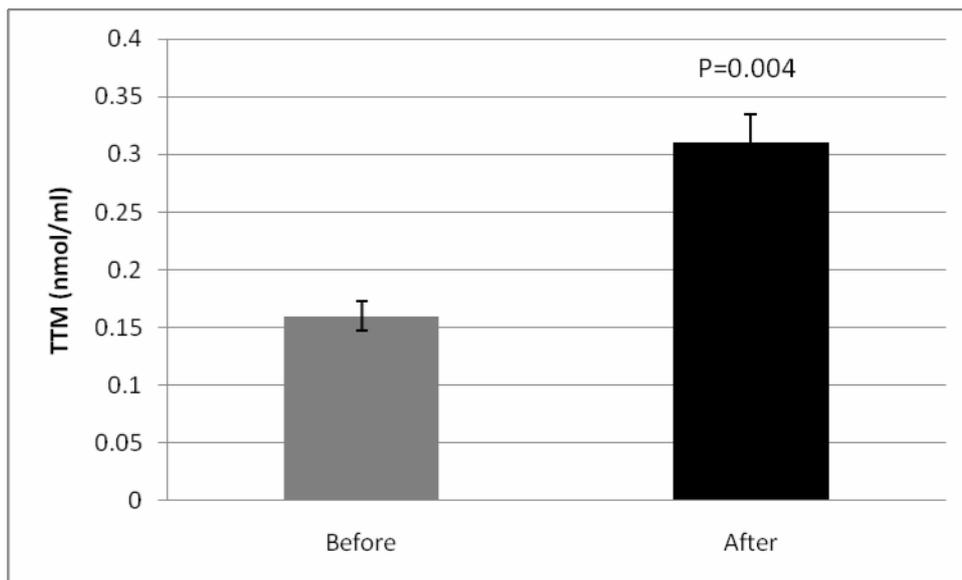
The plasma values of measured biomarkers including TAC, TBARS, and TTM are shown in Figures 1-3. A significant increase in TAC value was observed by using *M. pulegium* decoction ( $1.87 \pm 0.32$  vs.  $3.24 \pm 0.45 \mu\text{molml}^{-1}$ ,  $P=0.0001$ ). A significant increase in TTM was observed after using *M. pulegium* decoction. ( $0.16 \pm 0.10$  vs.  $0.314 \pm 0.13 \mu\text{molml}^{-1}$ ,  $P=0.004$ ). A significant reduction in plasma LPO was observed after administration of the *M. pulegium* decoction ( $10.04 \pm 3.46$  vs.  $7.21 \pm 2.85 \text{nmolml}^{-1}$ ,  $P=0.03$ ).

Overall results indicate that consumption of the decoction twice daily (morning and evening) for 2 weeks has significant improvement on the oxidative stress status of healthy subjects. There are numerous reports stating that the risk of degenerative diseases diminishes in people consuming large quantities of vegetables and fruits.



**Fig.1.** Effect of *M. pulegium* consumption on plasma total antioxidant capacity (TAC). Data are mean±SE.

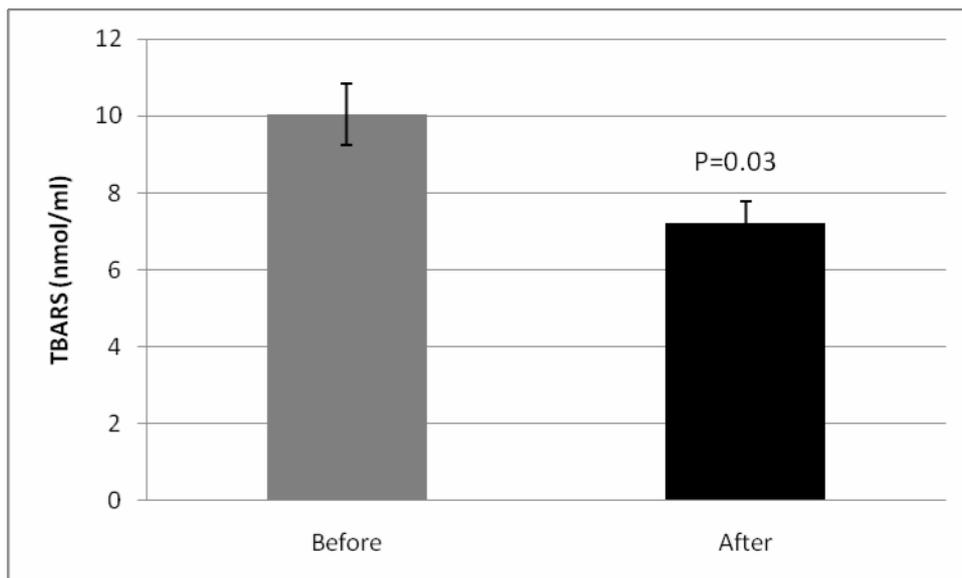
It should be taken into account that the antioxidant defense system of the human body is composed of different antioxidant compounds. The essential oil of *M. pulegium* has inhibitory effect on the contractile activity of the isolated rat myometrium [26], insecticidal effect on larvae of *spodoptera littoralis*, and exerts an and an in vitro activity against the grey mold disease [27,28]. *M. pulegium* also showed cytotoxic activity against brine shrimp, human cancer cell line [29]. Also, pennyroyal oil ingestion has been associated with severe hepatotoxicity and death [22].



**Fig. 2.** Effect of *M. pulegium* consumption on plasma total thiol molecules (TTM). Data are mean $\pm$ SE.

The phenolic content of *M. pulegium* is argued to be the possible scavenger of reactive oxygen radicals produced by the hydrogen peroxide as its anti-genotoxicity effect [18].

There are some reports supporting the present findings. Various in vitro studies indicated *M. pulegium* has antioxidative activity [24, 25]. Similarly, the study on mice showed that essential oil of aerial part of *M. pulegium* decreases malondialdehyde (MDA) levels and increases the levels of glutathione (GSH), glutathione dependent enzyme (GST) as well as enhances the levels of antioxidant defense enzyme.



**Fig. 3.** Effect of *M. pulegium* consumption on plasma lipid peroxidation (LPO) level. Data are mean $\pm$ SE.

In the body, antioxidants act as free radical scavengers. Numerous substances have been suggested to act as antioxidant in this plant. Various phenolic compounds such as pulegone have been introduced as the main constituents of *M. Pulegium* in several phytochemistry studies [21-23]. The antioxidant potential of phenolic composition has been well established [18]. Phenolic composition can highly scavenge most types of oxidizing molecules, including singlet oxygen and various free radicals, and thus act indirectly as an efficient antioxidant.

In conclusion, the present study for the first time reports that *M. Pulegium* extract bears marked antioxidant activity and via enhancement of TAC and TTM and reduction of LPO to protect body from free radical damage and induction of oxidative stress. Therefore, use of *M. pulegium* as a dietary supplement is highly recommended.

#### **Acknowledgment**

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