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# Antioxidant properties and *in vitro* immunomodulatory effects of peppermint (*Mentha x piperita l.*) Essential oils in human leukocytes

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**Abstract**

Peppermint (*Mentha x piperita L.*) is popular in folk medicine. Its immune effects however received so far limited attention. In the present study, peppermint oils from two cultivars (RAC 541 and Laimburg) grown in Northern Italy (Valtellina and Val Formazza) were investigated for their effects on human polymorphonuclear leukocytes (PMNs) and peripheral blood mononuclear cells (PBMCs), for their antioxidant activity and for chemical composition. RAC 541 oils were antioxidant at ≥1 μg/ml and Laimburg oils at ≥0,01 μg/ml. All the oils were cytotoxic for PMNs and PBMCs only at ≥0,1 μg/ml. In the 0,01-0,0001 μg/ml range, none of the oils affected spontaneous oxidative burst of PMNs, however RAC 541 oils increased stimulated burst. Oils had no effect *per se* on PBMC proliferation or production of interferon (IFN)-γ or interleukin (IL)-4. RAC 541 (but not Laimburg) oils however increased PHA-induced proliferation of PBMCs, and RAC 541 oil from Val Formazza (but not Valtellina) and Laimburg oils reduced IL-4 production. Essential oils from *Mentha x piperita L.* may thus affect the functional responses of human PMNs and PBMCs in a heterogeneous and cultivar-specific fashion.

**Key Words:** *Mentha x piperita L.*; essential oil; antioxidant activity; DPPH; TBARS; peripheral blood mononuclear cells; proliferation; interferon-γ; interleukin-4; polymorphonuclear leukocytes; reactive oxygen species.

## Introduction

Peppermint (*Mentha x piperita L.*), a perennial herb belonging to the *Lamiaceae* family, is a sterile natural hybrid derived from a cross between *Mentha aquatica* L. x *Mentha spicata* L. species. It is native in Europe and it has become both cultivated and naturalised in many European countries and in North America. Its purported benefits and uses as a folk remedy or in complementary and alternative medical therapy regard mainly digestive disorders, however a wide range of pharmacological properties is reported in the scientific literature, including antioxidant capacity, antitumor, antiallergenic, antimicrobial activities, hepatic and renal actions, chemopreventive potential, and nervous system actions (reviewed in 1 and 2). Peppermint is mentioned in Chinese traditional medicine, and dried peppermint leaves were found in Egyptian pyramids, suggesting that the use of peppermint may date back to at least 1000 BC [3].

Preparations of peppermint include leafs, leaf extracts and water, however the plant is cultivated mainly for its essential oil, which is obtained by distillation from freshly grounded leaves. Peppermint oil is composed primarily of menthol and menthone together with several other minor constituents, including pulegone, menthofuran and limonene [4], and its chemical composition may vary with plant maturity, geographical region and processing conditions [2].

Heterogeneous reports suggest that peppermint oil and some of its constituents may have immunomodulating properties [5-16], and peppermint has been included in some mixed formulations patented as effective in conditions such as arthritis and rheumatism [17]. Available evidence is however far from being clear and conclusive and the present study was therefore devised to investigate the effects of various peppermint oils on some functional properties of human peripheral blood mononuclear cells (PBMCs)

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and polymorphonuclear leukocytes (PMNs). In addition, the antioxidant activity of the oils was tested in standard chemical assays.

## Materials and methods

### *Plant material*

*M. piperita L.* var. RAC 541 was provided by the public agricultural research institute Agroscope Changins-Wädenswil ACW, Nyon, Switzerland (www.acw.admin.ch), while *M. piperita L.* var. Laimburg was provided by the Center for Agricultural and Forestal Experimentation of Laimburg (BZ), Italy (www.laimburg.it). Plants were cultivated in experimental fields in Valtellina, Sondrio province, Northern Italy, at 1.100 m above sea level and in Verbania-Cusio-Ossola province, Northern Italy, at 1300 m. above sea level. Aerial parts were harvested in June before full blooming and in September during the fruiting process and immediately processed in order to obtain the essential oils fraction.

### *Distillation of essential oils and GC-MS analysis*

Essential oil fractions were prepared in agreement with the procedures set by the European Pharmacopeia, fifth ed., vol. 5.08 (Council of Europe, Strasbourg, 2007). Briefly, 300 g of fresh crushed plant were suspended in 3 l of water in a 6 l reactor for 1 h and then submitted to hydrodistillation in a modified Clevenger apparatus for 4 h. The resulting essential oil was left to stabilize for 1 h. The distillate was collected in the graduated tube, using xylene to take up the essential oil, while the aqueous phase was automatically returned to the distillation flask. Capillary gas chromatography-mass spectrometry (GC-MS) analyses were performed using a Shimadzu GC-17Asystem coupled to a Mass Selective Detector QP5050A and equipped with an AOC-5000 autosampler (Shimadzu, Kyoto, Japan). Column: BGB-WAX (BGB-Analytik AG, Anwil, Switzerland), 60 m length, 0.25 mm i.d., 0.25 µm film; carrier gas helium; flow rate, 1.0 ml/min in constant pressure mode (122.2 kPa); the injection volume was 1 µl of essential oil diluted 1:100 in n-hexane; split mode, split ratio, 1:50; injector temperature 200°C; interface temperature 230°C; temperature program, from 60 °C (2 min) to 200 °C (8 min) at 3 °C/min. Data acquisition and analysis were performed using software of GCMSsolution (Shimadzu, Kyoto, Japan). The identification of constituents was performed by comparing their mass spectra with data from Wiley7th and NIST02 libraries. The percentage composition of individual components was computed from the GC peak areas without any correction for the relative response factor.

### *Antioxidant activity assays*

Antioxidant activity was evaluated by the DPPH methanolic free radical scavenging method and the assessment of linoleic acid peroxidation by measurement of thiobarbituric acid reactive substances (TBARS assay, as previously described [18]. For the DPPH assay 3.9 ml of DPPH radical methanolic solution 60 µM and a 100 µl aliquot test/standard reference compounds were allowed to react in the dark at room temperature for the required time. The absorbance was measured at 514 nm, and its percentage inhibition in the presence of test substances was then assessed. In the TBARS assay, the reaction mixture contained 500 µl linoleic acid 20 µM, 500 µl Tris-HCl 100 mM, 100 µl $FeSO_4 \cdot 7H_2O$ 4 mM, 100 µl ascorbic acid 2 mM and 12 µl of the test/reference substances. The reaction was terminated after 30 min by the addition of $CCl_3COOH$ 5.5%. Then 1 ml of the mixture was added with 250 µl of thiobarbituric acid 0.67%, followed by heating at 90°C for 10 min. After centrifugation, the absorbance of the supernatant at 532 nm was measured. The percentage inhibition of linoleic acid peroxidation was calculated with reference to control and to blank.

### *Isolation of human PMNs and PBMCs*

Human PMNs and PBMCs were isolated from venous blood of healthy volunteers using heparinized tubes. Whole blood was allowed to sediment on Ficoll-Paque Plus (GE Healthcare, Milan, Italy) at 37°C for 30 min.

Supernatant was recovered and PMNs and PBMCs were separated by Ficoll-Paque Plus density-gradient centrifugation. Contaminating erythrocytes were eliminated by 10 min hypotonic lysis in distilled water with added $NH_4Cl$ 8.2 g/l, $KHCO_3$ 1.0 g/l and ethylenediamine tetraacetic acid 37 mg/l. Cells were then washed three times in NaCl 0.15 M. Cell viability was always greater than 95%, as assessed by the Trypan blue exclusion test.

***Cytotoxicity assays***

Cytotoxicity was tested on PBMCs and PMNs by means of the 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) reduction method [19].

***Measurement of intracellular reactive oxygen species (ROS) levels in PMNs***

Intracellular ROS levels were assessed by use of the redox-sensitive dye C-DCFH-DA (Molecular probes, Eugene, Oregon, USA). Freshly isolated cells were suspended at the concentration of $1x10^6$ cells/ml in HBSS medium and incubated for 1 h with C-DCDHF-DA 2 μmol/l at 37°C in the dark. Cells were then washed twice with HBSS by centrifugation for 5 min (400xg, 20°C). Fluorescence measurements were performed using a spectrofluorimeter (Perkin-Elmer LS-50B, Perkin Elmer Instruments, Bridgeport, CT, USA). Excitation wavelenght was set at 488 nm and fluorescence emission was collected at 525 nm. Intracellular ROS levels were then expressed as fluorescence intensity, in arbitrary units (AU).

The effects of peppermint essential oils on ROS generation was tested on resting cells and on cells treated with 0.1 μM N-formyl-Met-Leu-Phe (fMLP; Sigma-Aldrich, Milan, Italy), a chemotactic peptide acting on membrane receptors that directly stimulates the respiratory burst [20], or with 1 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich), an activator of protein kinase C (PKC) enzymes [21]. In each experiment, infusion was added to the cells after a 60-s resting period, alone or together with fMLP or PMA, and subsequently ROS changes were calculated as the area under the ROS levels-vs-time curve (AUC), over a 30-min period.

***PBMC proliferation assay***

PBMCs were resuspended at the final concentration of $1x10^6$ cells/ml in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, and 100 U/ml penicillin/streptomycin (all from Sigma-Aldrich, Milan, Italy), at 37°C in a moist atmosphere of 5% $CO_2$. Cells were cultured alone or in the presence of 0.1 μg/ml anti-human CD3 and 0.5 μg/ml anti-human CD28 monoclonal antibodies (BD Pharmingen, Buccinasco, MI, Italy), and proliferation was measured after 2 days of culture by using a colorimetric immunoassay for the quantification of cell proliferation, based on the ELISA measurement of BrdU incorporated during DNA synthesis (Amersham, Buckinghamshire, UK). The optical density of the samples was determined by means of a spectrophotometer (Model 680, Bio-Rad Laboratories, Hercules, CA, USA) with wavelength set at 450 nm, and finally expressed as the difference between BrdU positive and negative samples, in arbitrary units (AU).

***Measurement of interferon (IFN)-γ, interleukin (IL)-4 and IL-8***

IFN-γ and IL-4 concentrations in culture supernatants were determined by using commercial ELISA assays (Amersham).

***Statystical analysis***

Data are presented as means±SEM. Statistical significance of the differences between groups was assessed by two-tailed Student's *t* test for paired data. Calculations were performed using a commercial software (GraphPad Prism version 4.00 forWindows; GraphPad Software, San Diego, CA).

## Results

***Composition of essential oils***

The hydrodistillation process was repeated ten times for a total of about 3 kg of plant material for each harvesting time and peppermint variety. The process gave 16.2ml of essential oil (yield: 0.54%) for RAC 541 (June), 13.8 ml (0.46%) for RAC 541 (September), 24.6 ml (0.82%) for Laimburg

(June) and 23.4 ml (0.78%) for Laimburg (September). The qualitative and quantitative data are summarized in Table 1.

***Antioxidant activity***

All the peppermint oils tested displayed significant antioxidant activities in both the DPPH and the TBARS test, however RAC 541 oils were antioxidant only at ≥1 μg/ml, while Laimburg oils were antioxidant already at 0,01 μg/ml at least in the TBARS assay (Figure 1).

***Cytotoxicity***

All the peppermint oils significantly affected the viability of human PMNs and PBMCs at ≥0,1 μg/ml (data not shown). In functional experiments therefore only concentrations ≤1 μg/ml were employed.

***ROS levels in PMNs***

In resting PMNs, intracellular ROS levels were 284.2±131.6 (n = 24) and increased after simulation up to 3703.5±2124.4 with fMLP (n = 42; $P<0.0001$ vs resting cells) and to 6391.8±3480.4 with PMA (n = 16; $P<0.0001$ vs resting cells).

Peppermint oils in the 0,0001-0,01 μg/ml range did not affect ROS levels in resting cells (data not shown). Oil from RAC 541 mint grown in Valtellina harvested in either June or September significantly increased fMLP-induced ROS production, while oil from RAC 541 grown in Val Formazza significantly increased ROS production induced by PMA. Oil from Laimburg mint harvested in either June or September did not exert significant effects in any of the experimental paradigms. Results are analytically shown in Figure 2.

***PBMC proliferation and cytokine production***

Treatment of PBMCs with PHA induced a strong proliferative response (resting cells: 0.069±0.016 AU, n = 32, PHA-stimulated cells: 1.071±0.185 AU, n = 32; $P<0.0001$), as well as a huge increase of the production of both IFN-γ (resting cells: 263.2±136.8 ng/ml, n = 6, PHA-stimulated cells: 10157.7±442.5, n = 6; $P<0.0001$) and IL-4 (resting cells: 0.39±0.17, n = 6, PHA-stimulated cells: 5.01±0.84, n = 6; $P<0.0005$).

None of the peppermint oils had any effect *per se* on PBMC proliferation or production of IFN-γ or IL-4 (data not shown). Oil from 541 RAC mint grown in Valtellina harvested in either June or September at 0,01 μg/ml significantly increased PHA-induced proliferation of PBMCs, without affecting cytokine production. Oil from RAC 541 grown in Val Formazza at 0,01 μg/ml significantly increased PHA-induced proliferation, and in addition reduced IL-4 production, resulting in a significant increase of the ratio IFN-γ/IL-4. Oil from Laimburg mint harvested in either June or September at 0,01 μg/ml had no effect on PHA-induced proliferation, but reduced IL-4 production and as a consenquence significantly increased the ratio IFN-γ/IL-4. Detailed results are shown in Figure 3.

**Discussion**

In the present study, all the peppermint oils tested exhibited some degree of antioxidant activity, however only at concentrations 10-100 fold higher than those which did not exert cytotoxic effects in human leukocytes. The only notable exception were essential oils from Laimburg, which in the TBARS assay were active even in the at 0,1-0,01 μg/ml range. Several lines of evidence suggest that peppermint is endowed with antioxidant properties [22,23], however the strongest antioxidant activities may be associated with the total phenolic content of the ethanolic extract [24]. On the other side, the TBARS assay is usually predictive of the ability of a given substance to inhibit lipid peroxidation, thus antioxidant properties may have physiopharmacological relevance at least in the case of essential oils from Laimburg.

PMNs play a key role in the first-line defense against invading microorganisms, however they also contribute to organ damage induced by excessive acute inflammatory responses as well as in chronic inflammatory disorders. In our experiments, peppermint oils *per se* did not affect PMN oxidative metabolism however RAC 541 oil from Valtellina increased fMLP-induced ROS production,

**Table 1.** Qualitative and quantitative analysis (relative percentage) of the essential fraction in different *Mentha* x *piperita* cultivars. (1) = RAC 541 grown in Valtellina and harvested in June; (2) = RAC 541 grown in Valtellina and harvested in September; (3) = RAC 541 grown in Val Formazza and harvested in June; (4) = Laimburg grown in Valtellina and harvested in June; (5) Laimburg grown in Valtellina and harvested in September.

| | **(1)** | **(2)** | **(3)** | **(4)** | **(5)** |
|---|---|---|---|---|---|
| menthol | 45.10 | 51.90 | 44.80 | 48.40 | 52.00 |
| menthone | 16.60 | 17.40 | 16.60 | 21.70 | 20.90 |
| eucalyptol | 5.90 | 5.06 | 5.88 | 4.66 | 3.64 |
| neo-menthol | 5.00 | 4.34 | 5.03 | 4.54 | 4.21 |
| menthofuran | 4.25 | 0.20 | 4.11 | 0.52 | 0.06 |
| menthol acetate | 3.51 | 4.03 | 3.53 | 4.78 | 5.16 |
| isomenthone | 3.12 | 3.42 | 3.27 | 3.08 | 3.04 |
| pulegone | 3.06 | 2.94 | 3.07 | 1.43 | 1.32 |
| limonene | 1.71 | 0.89 | 1.71 | 1.22 | 0.70 |
| germacrene D | 1.68 | = | 1.51 | 0.04 | = |
| terpinen-4-ol | 1.38 | 1.33 | 1.41 | 1.20 | 1.15 |
| beta-pinene | 0.94 | 0.43 | 0.94 | 0.68 | 0.33 |
| alpha-pinene | 0.64 | 0.25 | 0.64 | 0.49 | 0.19 |
| trans-sabinene hydrate | 0.62 | 0.73 | 0.64 | 0.63 | 0.63 |
| sabinene | 0.46 | 0.19 | 0.45 | 0.32 | 0.15 |
| piperitone | 0.43 | 0.46 | 0.45 | 0.45 | 0.49 |
| gamma-terpinene | 0.41 | = | 0.34 | = | = |
| linalool | 0.39 | 0.42 | 0.40 | 0.23 | 0.21 |

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| | | | | | |
|---|---|---|---|---|---|
| viridiflorol | 0.37 | 0.44 | 0.38 | 0.55 | 0.54 |
| germacrene B | 0.30 | = | 0.28 | = | = |
| p-cymene | 0.28 | 0.51 | 0.39 | 0.71 | 0.46 |
| 3-octanol | 0.25 | 0.28 | 0.25 | 0.24 | 0.24 |
| (+) alpha-terpineol | 0.25 | 0.26 | 0.26 | 0.20 | 0.18 |
| 4-carene | 0.21 | = | 0.15 | = | = |
| beta-boubornene | 0.21 | 0.23 | 0.22 | 0.31 | 0.32 |
| Z-beta-ocimene | 0.20 | = | 0.18 | = | = |
| beta-farnesene | 0.19 | 0.06 | 0.20 | 0.06 | 0.06 |
| beta-myrcene | 0.15 | 0.03 | 0.14 | 0.03 | 0.02 |
| neomenthyl-acetate | 0.15 | 0.18 | 0.15 | 0.19 | 0.20 |
| terpinolene | 0.11 | = | 0.10 | = | = |
| borneol | 0.07 | 0.04 | 0.05 | 0.05 | 0.06 |
| edulan I | 0.06 | 0.06 | 0.06 | 0.06 | 0.07 |
| cis-sabinene hydrate | 0.06 | 0.08 | 0.06 | 0.07 | 0.07 |
| 1-octen-3-ol | 0.05 | 0.06 | 0.06 | 0.08 | 0.09 |
| delta-cadinene | 0.05 | = | 0.04 | = | = |
| caryophyllene oxide | 0.05 | 0.91 | 0.07 | 0.61 | 0.94 |
| thymol | 0.05 | = | 0.06 | 0.06 | 0.03 |
| alpha-thujene | 0.04 | 0.01 | 0.05 | 0.03 | 0.01 |
| E-beta-ocimene | 0.04 | = | 0.04 | = | = |
| 3-octanol-acetate | 0.03 | 0.04 | 0.04 | 0.03 | 0.03 |
| (-) spathulenol | 0.03 | 0.20 | 0.06 | 0.17 | 0.19 |
| 2.5-diethyltetrahydrofuran | 0.02 | = | 0.02 | 0.02 | = |

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| | | | | | |
|---|---|---|---|---|---|
| camphene | 0.02 | = | 0.02 | 0.02 | 0.01 |
| psi-limonene | = | = | 0.01 | 0.01 | = |
| E-2-hexenal | = | 0.04 | = | 0.02 | 0.03 |
| n-amyl-isovalerate | = | 0.05 | = | 0.08 | 0.08 |
| pinocamphone | = | = | 0.03 | = | = |
| isopulegol | = | = | = | 0.03 | 0.04 |
| piperitone oxide | = | 0.06 | = | 0.02 | 0.06 |
| carvone | = | = | = | = | 0.02 |
| germacrene D-4-ol | = | 0.03 | = | = | = |
| other compounds | 1.63 | 2.41 | 9.26 | 2.01 | 2.10 |

**Figure 1.** Antioxidant activity of peppermint essential oils. Open symbols: plants harvested in June. Filled symbols: plants harvested in September. Concentrations are expressed in μg/ml. Each point is the mean±SEM of at least 3 separate observations. * = P<0.05 vs 0.

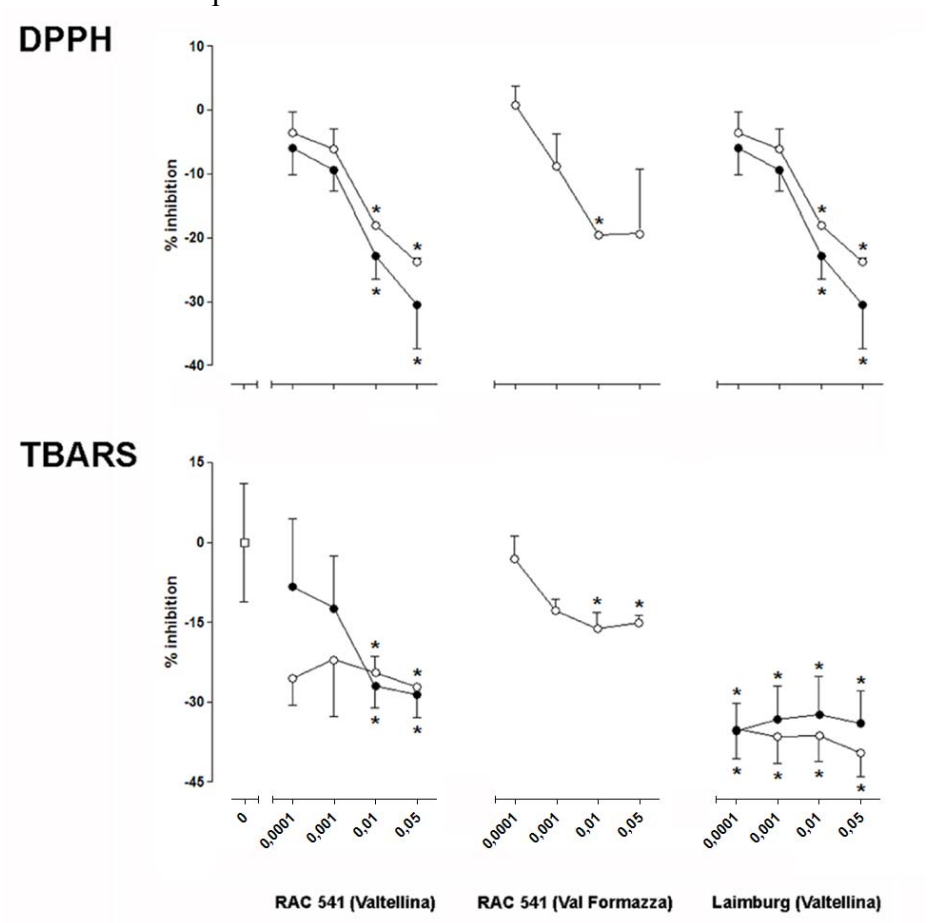


**Figure 2.** Effect of peppermint essential oils on fMLP- and PMA-induced increase of intracellular ROS levels in human PMNs. Since results obtained with oils from plants harvested in June and September were completely superimposable, in the graphs they were finally pooled. Concentrations are expressed in μg/ml. Each point is the mean±SEM of 4-9 separate observations. * = P<0.05 vs 0.

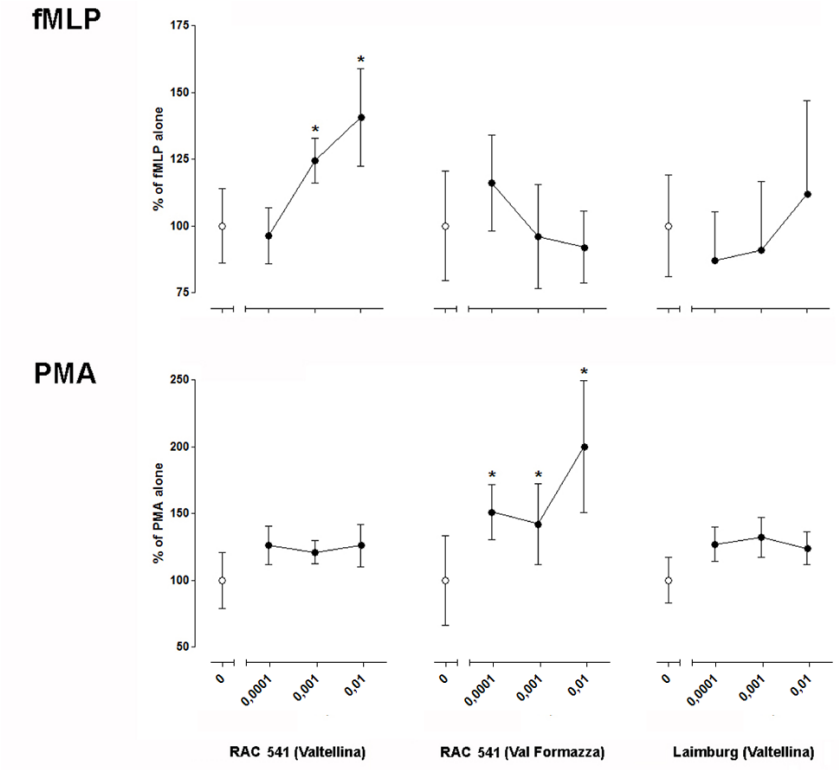
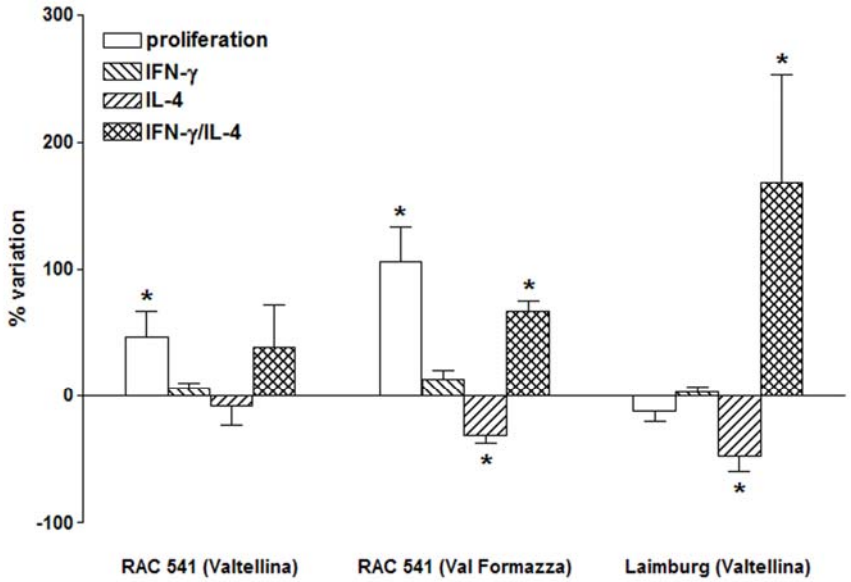



**Figure 3.** Effect of peppermint essential oils on PHA-induced functional responses of human PBMCs. Oils were added at at 0,01 μg/ml. Since results obtained with oils from plants harvested in June and September were completely superimposable, in the graphs they were finally pooled. Each point is the mean±SEM of 6-19 separate observations. * = P<0.05 vs PHA alone.



while RAC 541 oil from Val Formazza increased PMA-induced ROS production. Oil from Laimburg did not exert significant effects. We found no previous evidence regarding possible effects of Mentha preparations on human or non-human PMNs, apart from an *in vivo* study in mice showing that spearmint oil inhibits leukocyte and PMN recruitment into the peritoneal cavity [25]. Our results on the contrary indicate that at least essential oils from RAC 541 increase the respiratory burst of activated PMNs and therefore may result in enhancement of the inflammatory process.

In possible agreement with a putative proinflammatory profile, RAC 541 essential oils also increased mitogen-induced proliferation of human PBMCs. Both RAC 541 grown in Valtellina and in Val Formazza had a similar effect on cell proliferation, however while RAC 541 from Valtellina had no influence on cytokine production, RAC 541 from Val Formazza also significantly reduced the production of IL-4, with a subsequent increase of the IFN-γ/IL-4 ratio. Essential oils from Laimburg, in turn, had no effect on mitogen-induced proliferation, however they effectively inhibited IL-4 production, and therefore like RAC 541 from Val Formazza increased the IFN-γ/IL-4 ratio. The ratio IFN-γ/IL-4 is usually considered as a mirror for Th1/Th2 balance [26,27]. Th1 responses are usually associated with increased IFN-γ and tumor necrosis factor-α, and play a role in the defense against intracellular pathogens, as well as in autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, type I diabetes mellitus, and in chronic inflammation occurring in cardiovascular disease. On the other side, Th2 responses are characterized by increased IL-4 (as well as IL-5, 6, 10, 13) and are involved in atopic disorders and in autoimmune diseases such as systemic lupus erythematosus (reviewed in 28-30). The tolerability profile of peppemint oil when used on the skin or taken by mouth includes rash/hives/contact dermatitis, mouth ulcers, sores, eye irritation, heartburn, anal burning [31], and it is in agreement with the overall proinflammatory profile reported in the present study. On the other side, claims regarding beneficial effects of peppermint in arthritis and rheumatism [17] cannot be supported on the basis of our results.

The various peppermint oils tested in the present study showed different pharmacological profiles, clearly related to the botanical variety and possibly to the growing place, while no difference was observed as regards the time of harvesting. Indeed, oils prepared from the same varieties harvested in June or September had similar activities as regards both antioxidant and immunomodulating effects. On the contrary, only RAC 541 oils were somehow active on the oxidative metabolism in PMNs and on the proliferative response in PBMCs, while Laimburg oils affected only IL-4 production in PBMCs (an effect also shared by oils from RAC 541 grown in Val Formazza but not by those from the same cultivar grown in Valtellina). When dealing with complex mixtures such as essential oils, the question arises whether their pharmacological profile can be correlated with the individual chemical composition. As shown in Table 1, in the various peppermint oils the main components (relative percentage > 1) were the same, i.e.: menthol (range: 45.10-52.0), menthone (16.60-21.70), eucalyptol (3.64-5.90), neo-menthol (4.21-5.00), menthofuran (0.06-4.25), menthol acetate (3.51-5.16), isomenthone (3.04-3.12), pulegone (1.32-3.07), limonene (0.70-1.71), germacrene D (up to 1.78, undetectable in some cases), terpinen-4-ol (1.15-1.41). Comparison of different oils obtained from the same cultivar show that in the case of RAC 541 major differences (>±25%) occur only in the case of menthofuran and limonene (which are less represented in RAC 541 grown in Valtellina and harvested in September), while for Laimburg they occur only in the case of limonene and germacrene D (both less represented in the oil from plants harvested in September). In all the cases, differences regard components which represent <5% of the total composition. On the contrary, comparison between oils from RAC 541 and from Laimburg clearly shows the occurrence of major differences in several components.

In particular, oils from Laimburg contain more menthone (+26-31%) and menthol acetate (+36-47%) and less menthofuran (-78-99%), pulegone (-53-57%), and limonene (-39-59%). Together with menthol, menthone is the major component of peppermint essential oils. Chemical composition of the various oils seems thus in good agreement with the observed differences (and similarities) in their pharmacological profiles.

Current knowledge regarding the immunopharmacological effects of individual components of peppermint oil is scant and fragmentary. In one study, menthol has been reported to suppress the production of inflammatory mediators like IL-1β, LTB4 and PGE2 in human monocytes stimulated with LPS [11]. The same researchers also showed that eucalyptol reduced cytokine production in human mononuclear cells [5] and inhibited arachidonic acid metabolism in monocytes from patients with bronchial asthma [10]. Another group found that limonene and perillic acid inhibited DNA synthesis and proliferation in PHA-stimulated peripheral blood mononuclear cells [14], and perillic acid inhibited IL-2 and IL-10 production and secretion in mitogen-activated human T lymphocytes [12]. In *in vivo* murine models however limonene administration resulted in increased lymphocyte proliferation and antibody response [7,16] and increased alveolar macrophage activation [8]. On these basis it is clearly impossible to speculate about the peppermint oil constituents specifically responsible for the effects observed in our experiments, and in any case this issue was well beyond the scope of the study. Indeed, the present study suggest that even subtle differences in the composition of essential oils may result in profound changes of the pharmacological profiles.

## Conclusions

In summary, the results of the present study show that essential oils from *Mentha x piperita L.* may affect the functional responses of human PMNs and PBMCs, however in a heterogeneous and cultivar-specific fashion. As information regarding the immunomodulating properties of peppermint are still very limited, our results encourage more extensive studies to assess the potential of peppermint oils and of individual components as modulators of the immune response. In addition, since the pharmacological effects of the various oils couldn't be predicted on the basis of their chemical composition, we propose that assessment of the pharmacological properties in functional tests should be always included in their characterization.

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