

*Full Length Research Paper*

# Immunomodulatory effects of aqueous extract of *Ocimum basilicum* (Linn.) and some of its constituents on human immune cells

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*Ocimum basilicum* Linn. (OB) is an edible plant with high concentrations of caffeic (CA) and *p*-coumaric acid (pCA). In this study, the authors evaluated the immunomodulatory activities of aqueous extract of OB, CA and pCA on human peripheral blood mononuclear cells (PBMC) by lymphoproliferation test, and defined the responding cells by flow cytometry, secretion of various cytokines by ELISA, and expression of mRNA by quantitative Real Time-PCR (qPCR) methods. At concentrations tested (0.135, 0.27, and 0.54 µg/ml of OB extract, 2.5, 5, and 10 µg/ml of CA, 5, 10, and 20 µg/ml of pCA), OB, CA, and pCA were capable of dose-dependently stimulating DNA synthesis of human PBMC. In addition, OB extract suppressed cytokines produced by T<sub>H</sub>1 (IL-2, IFN-γ, and TNF-β), T<sub>H</sub>2 (IL-5, IL-10) as well as regulatory T (TGF-β) cells, and expression of ERK2 mRNA in PBMC. OB constituents CA and pCA also suppressed some of the cytokines. These data convincingly demonstrate that OB possesses direct immunomodulatory effect on basic functional properties of human immune cells, possibly mediated by the ERK2 MAP-kinase signal pathway. Thus, aqueous extract of OB can be considered as a powerful natural immunomodulatory spice influencing various types of immune-responses and may have potential health effects.

**Key words:** Immunomodulation, *Ocimum basilicum* (Linn.), caffeic acid, immune cells, cytokines, *p*-coumaric acid.

## INTRODUCTION

Epidemiological evidence increasingly suggests that consumption of a diet rich in fruit, vegetables, and cereals has a protective effect against certain forms of chronic diseases (Arts and Hollman, 2005; Neuhouser, 2004; Rice-Evans et al., 1997). It is well-known that most spices, especially those belonging to the Lamiaceae

family, possess a wide range of biological and pharmacological activities with potential health beneficial effects. Since ancient times, they have been used to improve the flavour and the organoleptic properties of various types of food.

*Ocimum basilicum* Linn. (OB), also known as basil, belongs to the genus *Ocimum* (Lamiaceae) which contains up to 150 species of herb and shrubs in the tropical regions of Asia, Africa, and Central and South America (Simon et al., 1990). It is a popular perennial widely cultivated herb prominently featured in Italian

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cuisine, and also plays a major role in the Southeast Asian cuisines of Vietnam, Thailand, Cambodia, and Laos as a fresh herb and as a dried spice (Chang et al., 2009; Manosroi et al., 2006). In addition, it is traditionally used in alternative medicine and natural therapies such as supplementary treatment of stress, asthma, and diabetes in India. In Siddha medicine, it is used for treating pimples on the face. In China, it is used to treat a variety of infectious diseases (Lin and Kan, 1990). In local Uighur medicine, it is used as cardiogenic, anti-diarrheal drugs, and abdominal pain reliever (Upur et al., 2004).

OB is used in the pharmaceutical industries for its spasmolytic, carminative, hepatoprotective, diuretic and stimulating properties, and perfumes and cosmetics industries for its pleasant odour (Baritoux et al., 1992; Bozin et al., 2006; Brophy and Jogia, 1986; Manosroi et al., 2006). The essential oil extracted via steam distillation from the leaves and other parts of the plants of basil is used to flavour foods, snuff, dental and oral products, and a fragrance in traditional rituals and medicines (Chang et al., 2009; Kirbaslar, 2001; Simon et al., 1990).

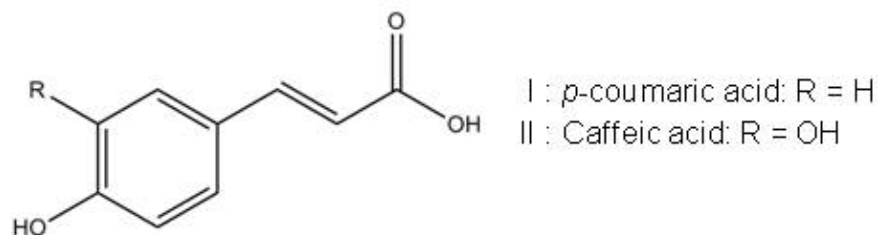
Plants produced various bioactive constituents throughout their development for a variety of reasons such as defense against microorganisms, insects, and herbivores (Crozier et al., 2006; Herms and Mattson, 1992; Rice-Evans et al., 1997). There has been much research into the health benefits conferred by OB. Olson and Bidlack (1997) found that OB has the innate ability to discourage herbivores; therefore, it can be used as an alternative pest control (Olson and Bidlack, 1997). Scientific studies also have shown that basil oil contains bioactive constituents that are antioxidant (Bozin et al., 2006), anticancer (Duke et al., 2001; Manosroi et al., 2006), antimicrobial (Bozin et al., 2006; Lis-Balchin and Deans, 1997; Ntezurubanza et al., 1984), antiviral (Chiang et al., 2005), antifungal (Bozin et al., 2006; Reuveni et al., 1984), repellent (Maganga et al., 1996; Paula et al., 2004; Popovic et al., 2006; Tawatsin et al., 2001), insecticidal (Chang et al., 2009; Chavan and Nikam, 1982; Chogo and Crank, 1981; Deshpande and Tipnis, 1977; Keita et al., 2001; Salvatore et al., 2004), or nematocidal (Chatterjee et al., 1982; de Almeida et al., 2007). In addition, basil has been shown to possess hypoglycemic effect (Agrawal et al., 1996; Aguiyi et al., 2000; Vats et al., 2002) and decrease the occurrence of platelet aggregation and experimental thrombus in mice (Tohti et al., 2006). In fact, the antimicrobial activity has formed the basis of many applications, including food preservation, pharmaceuticals, alternative medicine, and natural therapies (Lin and Kan, 1990).

Although, plants contain a variety of bioactive constituents, recent research has focused on polyphenols due to their potential therapeutic and health promoting effects (Arts and Hollman, 2005; Garcia-Lafuente et al., 2009; Neuhausser, 2004). Polyphenolic compounds are

widespread constituents of fruits, vegetables, cereals, and beverages such as tea and red wine (D'Archivio et al., 2007). Polyphenolic compounds are the most abundant natural antioxidants in their diet and their radical scavenging capabilities play an important role in preventing many chronic diseases (Garcia-Lafuente et al., 2009; Rathee et al., 2009). Polyphenols exert their protective effects by modulating various enzymes such as cyclooxygenase (Hussain et al., 2005; Lin et al., 2002; O'Leary et al., 2004), lipoxygenase (Sadik et al., 2003; Schewe et al., 2001), telomerase (Naasani et al., 2003), receptors, signal transduction pathways (Kong et al., 2000; Santangelo et al., 2007; Spencer et al., 2003; Wiseman et al., 2001), caspase-dependent pathway (Auyeung and Ko, 2010; Han et al., 2007; Monasterio et al., 2004; Way et al., 2005), and cell cycle regulation (Fischer and Lane, 2000).

Polyphenols can be divided into several classes according to the number of phenol rings and the structural elements. The main groups of polyphenols are: flavonoids, phenolic acids, phenolic alcohols, stilbenes, and lignans (D'Archivio et al., 2007). Caffeic acid (CA); (Figure 1) is the most abundant phenolic acid in dietary substances and occurs in many foods with high concentration such as *Arctium lappa* (Linn.) (CA content in the root: 2,500 parts per million, or ppm), *Helianthus annuus* (Linn.) (CA content in the seed: 1600 - 1800 ppm), *O. basilicum* Linn. (CA content in the leaf: 19,000 ppm), *Origanum vulgare* Linn. (CA content in the leaf and the plant: 1060 and 6,000 ppm, respectively), *Pyrus communis* (Linn.) (CA content in the fruit: 19,700 ppm), *Stachys officinalis* Trevisan (CA content in the plant: 5,000 ppm), and *Thymus vulgaris* Linn. (CA content in the leaf: 16,900 ppm) (Duke, 2001).

Caffeic acid has previously been shown to possess various potential health beneficial effects (Arts and Hollman, 2005), namely antioxidant, anti-inflammatory, and cancer chemopreventive activities (Facino et al., 1995; Nardini et al., 1998; Natarajan et al., 1996; Neradil et al., 2003). CA has been reported to exert an antioxidant action through reducing glutathione depletion and inhibiting lipid peroxidation *in vitro* (Nardini et al., 1998). In addition, Facino et al. demonstrated that CA protected collagen from free radical damage through a scavenging effect on reactive oxygen species (Facino et al., 1995). Moreover, CA affects leukotriene biosynthesis. Leukotrienes are involved in immunomodulation as well as in a variety of diseases, including various allergic and inflammatory conditions. Koshihara et al. (1984) demonstrated that CA is a selective inhibitor of 5-lipoxygenase, which catalyzes biosynthesis of leukotrienes from arachidonic acid (Koshihara et al., 1984). Caffeic acid phenyl ester (CAPE), a component in propolis, has been shown to possess antiviral and anticarcinogenic (Chiao et al., 1995; Demestre et al., 2009; Huang et al., 1996) properties. In immunological studies, CAPE was reported to suppress mitogen-induced T cell proliferation,



Main activity*	<i>p</i> -coumaric acid (20 µg/ml)	Caffeic acid (10 µg/ml)	<i>Ocimum basilicum</i> Linn. (0.54 µg/ml)
Lymphocyte proliferation	↑	↑	↑
Total B cell		↑	↑
Active T cell		↑	↑
CD8 <sup>+</sup> cell		↑	
NK cell	↑		
ERK-2 mRNA expression			↓

**Figure 1.** Structure and/or immunomodulatory effects of caffeic acid, *p*-coumaric acid and aqueous extract of *O. basilicum* (Linn.) on human immune cells. Note: \* Main immunomodulatory effects of caffeic acid, *p*-coumaric acid, and aqueous extract of *O. basilicum* (Linn.) on human immune cells. The drugs were evaluated on direct stimulation of resting PBMC without mitogen in triplicate of healthy volunteers. One-way ANOVA and multiple comparison of Dunnett's *t* test were used to evaluate the difference between test drug and solvent control (0.5% DMSO). Upward (↑) and downward (↓) arrows denote significant ( $p < 0.05$ ) upregulatory and downregulatory effects, respectively.

lymphokine production (Ansorge et al., 2003), and nuclear factor (NF)- $\kappa$ B activation (Natarajan et al., 1996). CAPE attenuated osteoclastogenesis and bone resorption via the suppression of RANKL-induced NF- $\kappa$ B and NFAT activities (Ang et al., 2009). CAPE also inhibited cytokine and chemokine production by human monocyte-derived dendritic cells (Wang et al., 2009).

A *p*-coumaric acid (*p*CA) (Figure 1), another constituent of OB with high concentration, can be found in a wide variety of edible plants (Duke, 2001). A *p*CA possessed radical scavenging, antioxidant (Andreasen et al., 2001; Burns et al., 2000; Yeh and Yen, 2003), antigenotoxic (Ferguson et al., 2005), and antimutagenic (Ferguson et al., 2003) properties and was believed to reduce the risk of stomach cancer by reducing the formation of carcinogenic nitrosamine (Gary, 2004).

Inappropriate immunity has been shown as a common etiology in an ever-growing array of pathological processes including infections, allergy, aging, cancer, and a variety of disorders of various organs (Peakman and Vergani, 1997). To date, there is only a modest body of knowledge about the nutraceutical active compounds in common food and their roles in promoting human health by immunomodulation. To explore the potentially health-promoting effect of OB and possible role of CA and *p*CA in this effect, the evaluated the

immunomodulatory activities of OB, CA, and *p*CA on resting mononuclear cells by lymphoproliferation test, and defined the responding cells by flow cytometry, secretion of various cytokines by ELISA, and expression of mRNA by quantitative Real Time-PCR (qPCR) methods.

## MATERIALS AND METHODS

### Crude extracts and chemicals

Fresh plants were collected in spring from Taichung County, located in Central region of Taiwan, and the authenticity of the plant was confirmed by Mau-Shing Yeh at Department of Agronomy, National Chung Shing University, Taichung, Taiwan. Fresh leaves (500 g) were extracted using Braun MP80 Juice Extractor (Kronberg, Germany). The juice was then subjected to centrifuge at 20,000 g, 20 min. The supernatant was filtered using Minisart syringe filter from Sartorius Biotech GmbH (Goettingen, Germany), concentrated in vacuum, and then lyophilized. The aqueous extract was collected and stored at  $-70^{\circ}\text{C}$  until use. Heparin, Ficoll-Hypaque, dimethylsulfoxide (DMSO), phytohemagglutinin (PHA), and caffeic acid (CA) were obtained from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). The *p*-coumaric acid (*p*CA) was obtained from MP Biomedicals (Solon, OH, U.S.A.). The purity of CA and *p*CA were more than 99 and 98%, respectively. 5-Bromo-2'-deoxyuridine (BrdU) Flow Kits were obtained from BD Biosciences (San Jose, CA, U.S.A.).

### Lymphoproliferation test

Peripheral blood from six healthy Taiwanese volunteers aged 20~35 years old was collected in a sterile syringe containing sufficient heparin to provide a final concentration of 100 units/ml. Mononuclear cells were obtained by centrifuging (25°C, 400 g, 30min) of the mixture of blood and normal saline (1/1, v/v) on Ficoll-Hypaque (2.4:1) gradients as described by the manufacturer's protocol (Sigma-Aldrich). After centrifugation, 0.05 ml ( $5.0 \times 10^5$ ) unfractionated PBMC was placed in a 5 ml test tube containing 0.15 ml fetal calf serum and 0.75 ml of RPMI 1640, with 0.05 ml of test samples, mitogen (positive control: PHA) (Severinson and Larsson, 1986), medium (negative control) or additional medium with DMSO (solvent control). After gentle mixing, 200  $\mu$ l were added into the wells of a 96-well microculture plate in triplicate. The culture plate was then allowed to incubate for 3 days at 37°C in a 5% CO<sub>2</sub> incubator. At the end of the incubation, 20  $\mu$ l per well of BrdU labeling solution were added and the culture plate was reincubated for additional 24 h at 37°C. Then, the BrdU flow cytometric analysis was completed according to the manufacturer's instruction. The study protocol was approved by the Institutional Review Boards of China Medical University, Taichung, Taiwan

### Enzyme-linked immunosorbent assay (ELISA)

The cultivation and treatment of human PBMC was completed as previously described in the lymphoproliferation test. After 3 days, particulates were removed from the supernatants by centrifugation, and the samples were stored at -70°C until used. The ELISA procedures for various cytokines using human Interleukin (IL)-2, IL-5, IL-10, interferon-gamma (IFN- $\gamma$ ), transforming growth factor- $\beta$  (TGF- $\beta$ ), and tumor necrosis factor- $\beta$  (TNF- $\beta$ ) immunoassay kits from R and D Systems Inc. (Minneapolis, MN, U.S.A.) were performed according to the manufacturer's instruction.

### Immunophenotyping

All fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, and Cy5-conjugated monoclonal antibodies (mAbs) were purchased from Becton Dickinson (San Jose, CA, USA). Optimal concentrations of mAbs were determined for each mAb by titration. The isolated PBMC were cultured in triplicate with *p*-coumaric acid (20  $\mu$ g/ml), caffeic acid (10  $\mu$ g/ml), OB (0.54  $\mu$ g/ml), PHA (5  $\mu$ g/ml), DMSO (0.5%) or medium for 3 days at 37°C in a 5% CO<sub>2</sub> incubator. Then, the numbers of NK cells (CD3<sup>-</sup>, CD16<sup>+</sup>, CD56<sup>+</sup>), CD8<sup>+</sup> T, total T cells (CD3<sup>+</sup>), total B cells (CD3<sup>-</sup>, CD19<sup>+</sup>) and activated T cells (CD3<sup>+</sup>, HLA-DR<sup>+</sup>) at 0 or 3 days were determined by standard FACSCalibur procedures with mAbs according to the manufacturer's protocol. In brief, at the indicated time period, the cultured cells were stained with 20  $\mu$ l of mAb for 30 min at 25°C. The stained cells were centrifuged (25°C, 300 g, 5 min.) and the supernatant was aspirated leaving 50  $\mu$ l of residual fluid in the tube. The cell pellet was resuspended and then 2 ml of PBS was added to each tube. Each tube was vortexed thoroughly at low speed for 3 s and then centrifuged (25°C, 200 g, 5 min). After aspiration of the supernatant until there was 50  $\mu$ l of residual fluid left, the cell pellet was resuspended and then 0.5 ml of 1% paraformaldehyde was added to each tube. Finally, the stained PBMC were analyzed on a FACSCalibur cytometer (Becton Dickinson, Cookeysville, MD, U.S.A.).

### Gene expression analysis

The ERK2 *gene* expression was investigated by relative quantitative Real Time-PCR (qPCR). Total RNA was isolated from

various test drug-treated cells using RNeasy™ Mini Kit from Qiagen GmbH (Hilden, Germany), and then treated with DNase I from Promega (Madison, WI, U.S.A.) following the manufacturer's protocols. The resulting RNA was quantified spectrophotometrically using infinite™ 200 from TECAN (Grödig, Austria), aliquoted, and stored at -70°C until further use. In each case, 1  $\mu$ g of total RNA were transcribed in a final volume of 20  $\mu$ l using ReverAid™ First Strand cDNA Synthesis Kit from Fermentas (Burlington, ON, Canada) according to the manufacturer's instruction and the reaction mixture was kept frozen at -70°C until enzymatic amplification.

All samples were analyzed in triplicate. A 20  $\mu$ l reaction mixture consisted of 10  $\mu$ l TaqMan™ *Gene* Expression Master Mix from Applied Biosystems (Carlsbad, CA, U.S.A.), 1  $\mu$ l of the RT-reaction, and 1  $\mu$ l of predesigned TaqMan™ probe for ERK2 *gene* (Assay ID: Hs00177066\_m1) from Applied Biosystems. An endogenous control of 18S rRNA *gene* (Assay ID: Hs99999901\_s1, Applied Biosystems) was used. To compare the obtained values the *ddCt* method (User Bulletin #2, Applied Biosystems) was used. Reactions were carried out in an ABI PRISM™ 7900 Sequence Detection Systems from Applied Biosystem, following manufacturer's instructions.

### Statistical analysis

Results were expressed as mean  $\pm$  standard error. The one-way ANOVA and multiple comparison of Dunnett's *t* test (> 2 groups) or 2-tails of Student's *t* test (2 groups) were used to evaluate the difference between the control and test samples by the SPSS Base 8.0 software for Windows. A *p* value of less than 0.05 was considered to be a significant difference in all experiments.

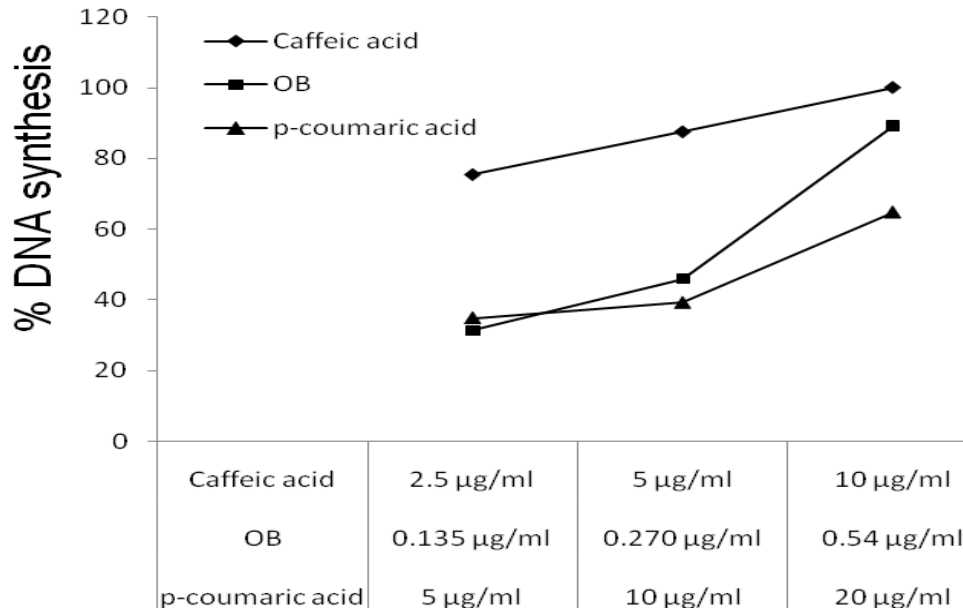
## RESULTS

### DNA synthesis of PBMC

DNA synthesis and increased cell growth of specific immune cells are preconditions of an effective adaptive immune response *in vivo*. Human PBMC were stimulated with different concentrations of CA (2.5, 5, and 10  $\mu$ g/ml), pCA (5, 10, and 20  $\mu$ g/ml), and aqueous extract of OB (0.135, 0.27, and 0.54  $\mu$ g/ml) for 72 h. DNA synthesis was measured after labeling with BrdU by flow cytometric analysis. As shown in Figure 2, DNA synthesis of PBMC was enhanced by OB extract in a concentration-dependent manner. Stimulatory effects were also obtained for some of the OB constituents like CA and pCA.

### Immunophenotyping

To determine which types of cells directing the immunostimulation, they used flow cytometric analysis. After culturing for three days, some cell fractions were changed. The immunostimulatory effects by PHA exhibited elevation of total B, activated T, and CD8<sup>+</sup> T cells. The immunostimulation by pCA, CA, and OB extract showed that pCA increased NK, CA increased CD8<sup>+</sup> T, and both CA and OB extract increased total B as



**Figure 2.** Concentration-dependent stimulation of DNA synthesis of human PBMC by caffeic acid, *p*-coumaric acid, and aqueous extract of *O. basilicum* Linn.

well as activated T cells significantly (Figure 3).

### Cytokine production/release

Immune cells synthesize and secrete soluble polypeptide factors named cytokines. Most of the secreted cytokines are then bind to specific receptors on the surface of target cells to modulate growth and/or differentiation. Special profile of cytokine production may reflect the functions of individual cell types involved in the immune system. IL-2 is produced by  $T_H1$  cells that acts in an autocrine manner to stimulate T cell proliferation. IL-5 is produced by  $T_H2$  cells and stimulates the growth and differentiation of eosinophil and activates mature eosinophils (Abbas, 2007; Ansorge et al., 2003; Romagnani, 1994). TGF- $\beta$  is produced by regulatory T cells, mononuclear phagocytes, and other cells whose main actions are to suppress the proliferation and differentiation of T cells, to suppress the activation of macrophages and to counteract the effects of proinflammatory cytokines (Abbas, 2007; Levings and Roncarolo, 2000; Sakaguchi, 2000). Given these crucial roles of cytokines in modulating various cells of the immune system, there has been some interest in the effect of OB and its constituents in regulating the production of different types of cytokines by PBMC.

PBMC were cultured for 72 h in the presence of CA (10 µg/ml), pCA (20 µg/ml) or OB extract (0.54 µg/ml). Compared to the DMSO control, in all cases a significant suppression on the production/release of cytokines produced by  $T_H1$  (IL-2, IFN- $\gamma$ , and TNF- $\beta$ ) Figure 4),  $T_H2$

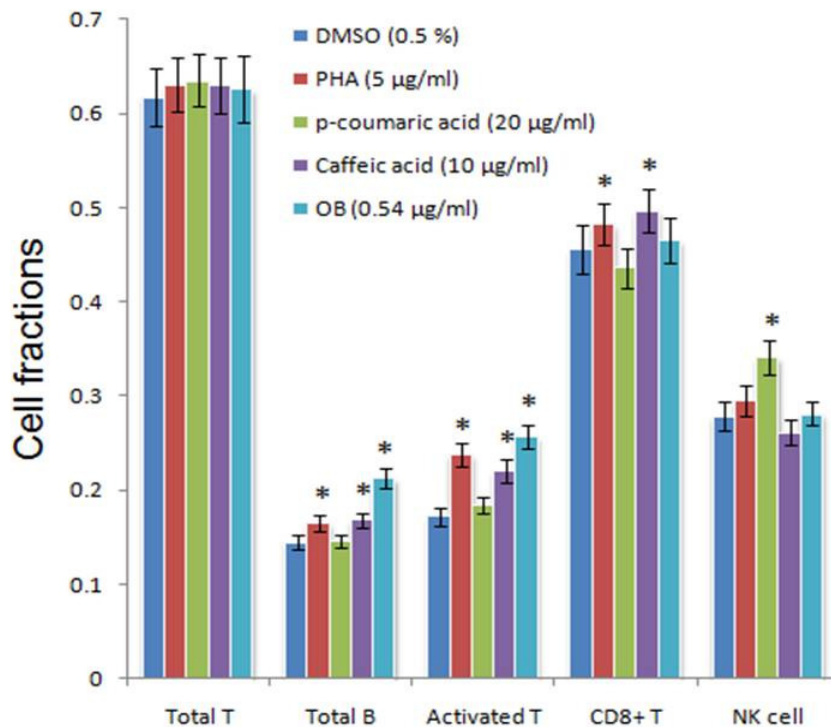
(IL-5 and IL-10) (Figure 5) as well as regulatory T (TGF- $\beta$ ) cells (Figure 6) was found with OB extracts. Suppressive effects were also found on some of the cytokines with the OB constituents CA (including IL-2, TNF- $\beta$ , IL-10, and TGF- $\beta$ ) and pCA (including IL-2, TNF- $\beta$ , IL-5, and IL-10) (Figure 4 to 6).

### Gene expression analysis

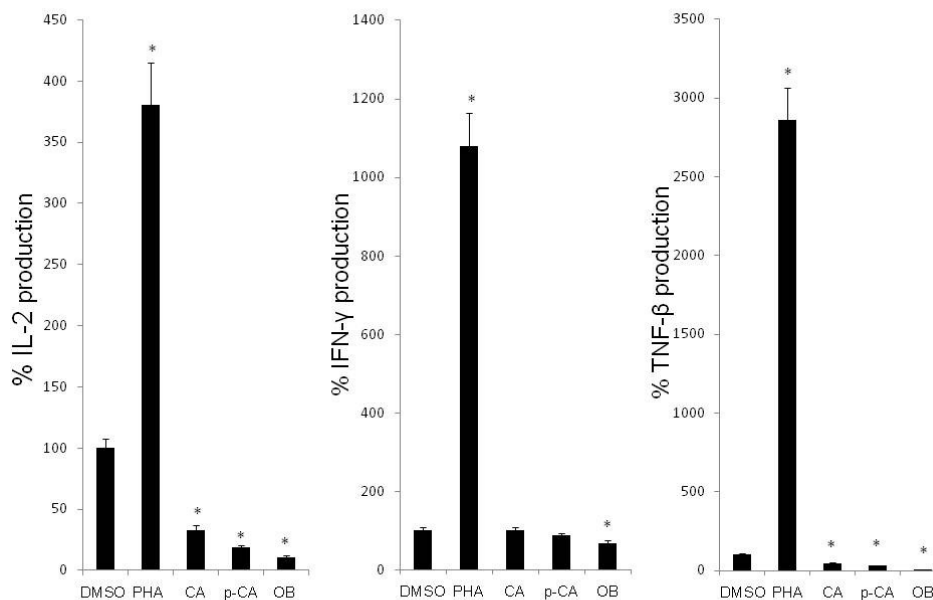
To investigate the possible molecular mechanisms responsible for the upregulation of cellular growth effect by OB extract and its components, they studied one of the MAP kinase signal pathways involved in the proliferation, differentiation, transformation, and apoptosis of cells. The authors observed the mRNA expression of the extracellular signal-regulated kinase 2 (ERK2), which is capable of regulating several transcription factors which in turn regulate critical *genes* of lymphocyte functions including that of IL-2 (Hardy and Chaudhri, 1997). In their experiments, OB extract suppressed the expression of ERK2 mRNA significantly (Figure 7).

### DISCUSSION

This study provides compelling evidence for an immunomodulatory effect of OB extract and its major constituents such as CA and pCA on human immune cells. Data shown in this study convincingly demonstrated that OB extract was capable of stimulating DNA synthesis of PBMC in a concentration-dependent manner.



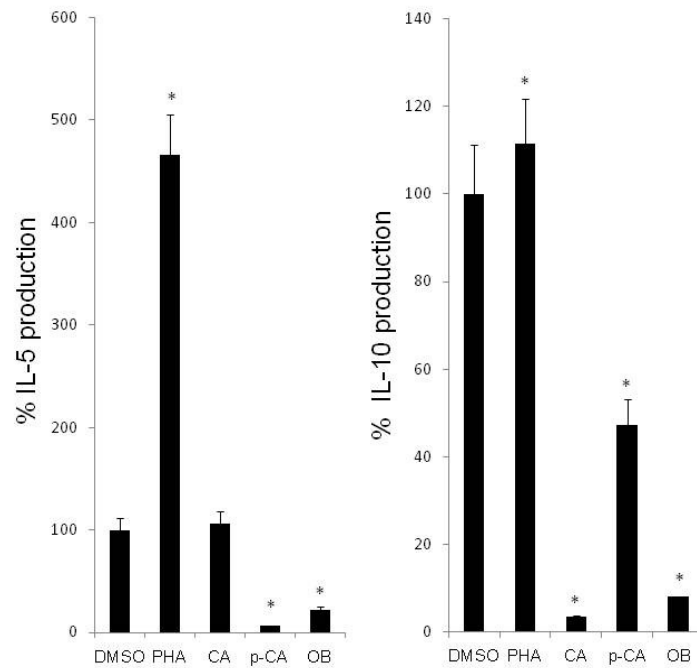
**Figure 3.** Immunomodulatory effects of aqueous extract of *Ocimum basilicum* Linn., caffeic, and *p*-coumaric acids on human PBMC.



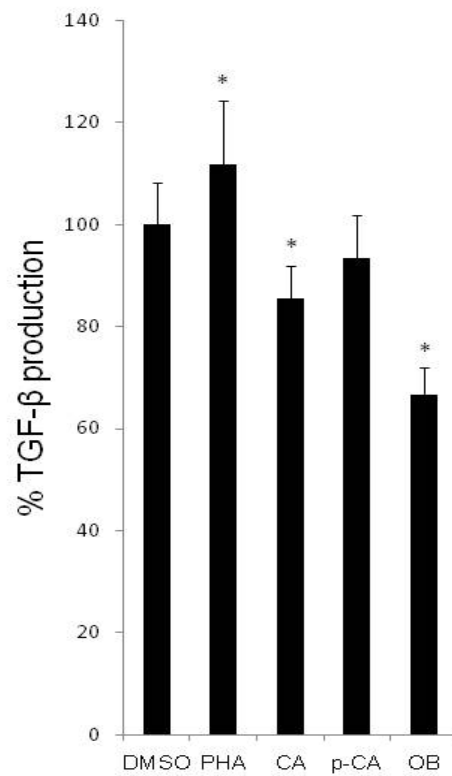
**Figure 4.** Aqueous extract of *O. basilicum* Linn., caffeic, and *p*-coumaric acids suppressed various cytokines produced by  $T_H1$  cells.

Lymphocyte proliferation was correlated to the increase of the cell fractions of total B and activated T cells. Furthermore, these effects at least in part may be mediated by some of its constituents such as CA and

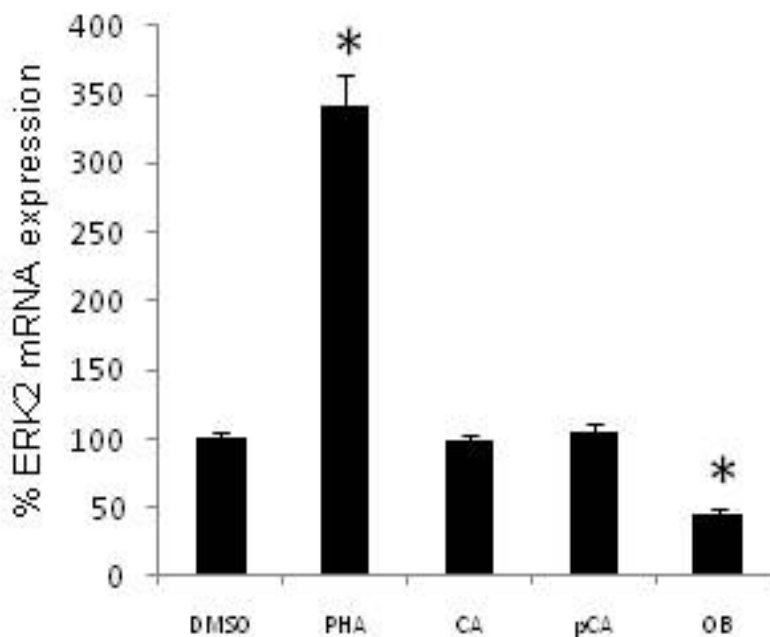
*p*CA. Considering the fact that hundreds of different substances (Duke, 2001) including various polyphenols have been identified as constituents of OB, one could speculate that the effect of OB extract on the cell cycle



**Figure 5.** Effects of aqueous extract of *O. basilicum* Linn., caffeic, and *p*-coumaric acids on the production/release of cytokines produced by  $T_H2$  cells.



**Figure 6.** Effects of aqueous extract of *O. basilicum* Linn., caffeic, and *p*-coumaric acids on the production/release of cytokines by T regulatory cells.



**Figure 7.** Aqueous extract of *O. basilicum* Linn. Suppressed the expression of ERK2 mRNA by PBMC.

has to be considered as a cumulative resultant of various negative as well as positive effectors.

*Ocimum sanctum* Linn., another species of Lamiaceae, also possesses proliferative effects in bovine subclinical mastitis with increased neutrophil and lymphocyte counts and enhanced phagocytic activity (Mukherjee et al., 2005). This clearly suggests that these spices may act via a common growth regulating mechanism which finally leads to a cell proliferation. In addition to exhibiting growth regulating activity on lymphocytes, cytokine production and/or release was also found to be influenced by OB extract and some of its constituents. Significant changes in cytokine production and/or release were found for the  $T_H1$  cytokine IL-2, IFN- $\gamma$ , and TNF- $\beta$ ; for the  $T_H2$  cytokine IL-5, IL-10 as well as for the regulatory T cell-derived cytokine TGF- $\beta$ .

Cytokines produced by  $T_H1$  (such as IL-2, IFN- $\gamma$ , and TNF- $\beta$ ) and  $T_H2$  (such as IL-5 and IL-10) cells were both targeted by OB extract. These results supported the conclusion that OB extract could be used to treat  $T_H1$ -dependent inflammatory processes and autoimmune diseases as well as  $T_H2$ -dependent allergic disorders. It has been shown that targeted disruption of the interleukin-10 *gene* (Rennick et al., 1997) or TGF- $\beta$  *gene* (Shull et al., 1992) resulted in multifocal inflammation in animal studies. This clearly implied that besides other functions both cytokines possess strong immunosuppressive effects which may partially explain the growth modulating effect of OB extract observed in this study. Changes in cell growth and cytokine production should be associated with changes in signal

transduction pathways. To investigate the possible molecular mechanism of the phenomenon observed, they studied the effect of OB extract and its constituents on the expression of ERK2 mRNA in PBMC. It has been shown that, following T cell receptor ligation, the Ras is activated, eventually leading to the activations of Raf, ERK2 and downstream transcription factors (Abbas, 2007; Hardy and Chaudhri, 1997). Raf kinase have been described as a point of convergence of other receptor-triggered signaling pathways. ERK2 plays an important role in transducing both the signal from T cell receptor and costimulatory molecules on the T cell surface, and is able to regulate several of the transcription factors controlling the expression of critical *genes*, including that for IL-2. Biological outcome of ERK2 activation is dependent on cell types, extracellular factors, and their receptors (Lahlou, 2003).

In this study, ERK-2 mRNA expression was found to be suppressed by OB extract. This clearly suggested that one way of signaling triggered by OB extract was mediated by the MAP kinase ERK2. However, this finding does not exclude that additional signal pathways and transcription factors targeted by OB extract are responsible for the results observed. Remarkably, some of the effects resulting from the application of OB extract as a complex mixture were found to be similar to those provoked by the different constituents studied here, especially CA. This is suggesting that the main effects of OB extract on the immune functions may be due, at least in part, to these particular substances.

In conclusion, their data provide a rational basis for the



beneficial usage of OB as folk medicine since ancient time. In this study we have provided evidence that OB extract and a selected number of its constituents had a direct modulatory effect on basic immune cell functions in human. OB extract was shown to be capable of downregulating T<sub>H</sub>1 and T<sub>H</sub>2 as well as regulatory T derived cytokines, possibly via the ERK2 signal pathway. The property of augmenting resting PBMC proliferation by OB extract may partly explain for the popular use of *O. basilicum* Linn. for treating infections (Chiang et al., 2005) and inflammatory diseases (Yasukawa et al., 1993).

## ACKNOWLEDGEMENTS

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**Abbreviations:** BrdU, 5-Bromo-2'-deoxyuridine; CA, Caffeic acid; DMSO, dimethylsulfoxide; ELISA, enzyme-linked immunosorbent assay; FITC, isothiocyanate; IFN- $\gamma$ , interferon-gamma; IL-2, interleukin 2; IL-5, interleukin 5; IL-10, interleukin 10; mAbs, monoclonal antibodies; OB, *Ocimum basilicum* (Linn.); PBMC, peripheral blood mononuclear cells; pCA, p-coumaric acid; PE, phycoerythrin; PHA, phytohemagglutinin; qPCR, quantitative real time-PCR; TGF- $\beta$ , transforming growth factor- $\beta$ ; TNF- $\beta$ , tumor necrosis factor- $\beta$ .

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