JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Clove and eugenol in noncytotoxic concentrations exert immunomodulatory/anti-inflammatory action on cytokine production by murine macrophages

Tatiana Fernanda Bachiega[a], João Paulo Barreto de Sousa[b], Jairo Kenupp Bastos[b] and José Maurício Sforcin[a]

[a]Department of Microbiology and Immunology, Biosciences Institute, UNESP, Botucatu and [b]Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, USP, Ribeirão Preto, SP, Brazil

Keywords
clove; cytokines; eugenol; immunomodulation; *Syzygium aromaticum*

Correspondence
Jose Mauricio Sforcin, Department of Microbiology and Immunology, Biosciences Institute, UNESP, Botucatu, SP 18618-970, Brazil.
E-mail: sforcin@ibb.unesp.br

Received October 26, 2011
Accepted December 12, 2011

doi: 10.1111/j.2042-7158.2011.01440.x

Abstract

Objectives The extract and essential oil of clove (*Syzygium aromaticum*) are widely used because of their medicinal properties. Eugenol is the most important component of clove, showing several biological properties. Herein we have analysed the immunomodulatory/anti-inflammatory effect of clove and eugenol on cytokine production (interleukin (IL)-1β, IL-6 and IL-10) *in vitro*.

Methods Macrophages were incubated with clove or eugenol (5, 10, 25, 50 or 100 μg/well) for 24 h. Concentrations that inhibited the production of cytokines were used before or after incubation with lipopolysaccharide (LPS), to verify a preventive or therapeutic effect. Culture supernatants were harvested for measurement of cytokines by enzyme-linked immunosorbent assay.

Key findings Clove (100 μg/well) inhibited IL-1β, IL-6 and IL-10 production and exerted an efficient action either before or after LPS challenge for all cytokines. Eugenol did not affect IL-1β production but inhibited IL-6 and IL-10 production. The action of eugenol (50 or 100 μg/well) on IL-6 production prevented efficiently effects of LPS either before or after its addition, whereas on IL-10 production it counteracted significantly LPS action when added after LPS incubation.

Conclusions Clove exerted immunomodulatory/anti-inflammatory effects by inhibiting LPS action. A possible mechanism of action probably involved the suppression of the nuclear factor-κB pathway by eugenol, since it was the major compound found in clove extract.

Introduction

Clove (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) is an aromatic dried bud of a tree from the family *Myrtaceae*, commonly used as a spice to add flavour to food preparations.[1]

The extract and the essential oil isolated from clove are widely used due to their medicinal properties, since clove is active against a large number of bacteria.[2] Antifungal, anticarcinogenic, antiallergic and antimutagenic activities have been reported for clove also.[1,3–5] Clove essential oil increased the total white blood cell count in mice and restored cellular and humoral immune responses in cyclophosphamide-immunosuppressed mice in a dose-dependent manner.[6]

Natural products containing bioactive phytochemicals are potentially important sources of anti-inflammatory drugs.[7] Eugenol (4-allyl-2-methoxyphenol) is a phenolic compound representing the most important component of clove. In Asian countries, eugenol has been used traditionally as an antiseptic, analgesic and antibacterial agent. Its antiviral, antioxidant, anti-inflammatory and antitumoral activities have been investigated.[8–13]

Macrophages are cells of the innate immunity that respond to a variety of stimuli.[14] The innate immune response is typically triggered by pathogen-associated molecular patterns that are recognized by different receptors on the surface of macrophages, leading to intracellular pathways followed by stimulation of cytokine production, including pro-inflammatory (interleukin (IL)-1), regulatory (IL-6) and anti-inflammatory (IL-10) cytokines.[15]

The pro-inflammatory cytokines of the IL-1 family, most notably IL-1β and IL-1α, display a very important role for

antimicrobial host defense, binding the same receptor and activating the adaptive response. IL-1 is largely responsible for the acute phase response, which includes fever, acute protein synthesis, anorexia, and somnolence.[16] IL-6 is a regulatory cytokine by regulating the expression of immune/inflammatory genes and regulating cell proliferation, differentiation and survival.[17] IL-10 is a potent anti-inflammatory cytokine that is crucial for dampening the inflammatory response after pathogen invasion, protecting the host from excessive inflammation. One mechanism whereby IL-10 mediates its anti-inflammatory effect is through the down-regulation of pro-inflammatory genes such as those encoding IL-1, IL-12 and tumour necrosis factor (TNF)-α.[18]

The effects of eugenol have been investigated on arachidonic acid metabolism, inducible nitric oxide synthase (iNOS) expression, CD86 and CD54 expression, superoxide production, and cyclooxygenase-2 (COX-2) expression, providing evidence of the potential of eugenol as a possible anti-inflammatory agent. Previously, we reported that its administration to mice inhibited macrophage production of IL-1β and IL-6, suggesting the anti-inflammatory action of this spice *in vivo*.[19] Thus, this study aimed to investigate the immunomodulatory effect of clove and eugenol on cytokine production (IL-6, IL-1β and IL-10) by peritoneal macrophages *in vitro*. The concentrations of clove or eugenol that inhibited cytokine production were tested before or after macrophages were challenged with lipopolysaccharide (LPS) to verify a preventive or therapeutic effect.

Materials and Methods

Clove extract and chemical characterization by gas chromatography-mass spectrometry

Authentic flower buds of *S. aromaticum* were purchased from the local market of Botucatu, São Paulo, Brazil. Clove buds (350 g) were ground in a knife mill, and extracted in methanol 70% (v/v) at room temperature. After three days, the extract was filtered and concentrated in rotoevaporator. The dried extract was lyophilized and specific dilutions were prepared in RPMI media for each assay.

Clove chemical composition had been investigated previously by gas chromatography-mass spectrometry (GC-MS). The analyses of clove extract (10 mg/ml) were performed on a Shimadzu GC/MS – QP2010, equipped with an automatic sampler AOC – 20Si at split mode (1 : 100). Acquired data were processed by a Shimadzu system and the Wiley 7.0 spectrometric electronic library. The analyses were performed using a fused-silica capillary column (DB-5, 30 m × 0.25 mm; coating thickness 0.25 μm). Hydrogen was used as a carrier gas at 1.30 ml/min. Mass spectra were recorded in the scan mode at 70 eV (40–500 m/z). The injector temperature was 250°C and the oven temperature was programmed from 60 to 240°C at 3°C/min.

The eugenol detected in the extract sample was identified by comparing the retention indices (RI relative to C9-C20 n-alkanes), obtained by GC/MS analyses, with those reported in the literature.[19,20] A comparative study involving the extract sample in relation to the authentic standard was performed also. The mass spectra of the peak was evaluated with those either reported in the literature or available in the Wiley 7.0 data system library.[20]

Eugenol authentic standard was purchased from Sigma Chemicals (St Louis, MO, USA) and its purity was confirmed at 99% during the analytic procedures.

Animals and peritoneal macrophages

Male BALB/c mice (25–30 g; 8–12-weeks-old) were kept in rooms at 21–25°C and 50% relative humidity, with a 12 h light/dark cycle. Food and water were freely available.

Peritoneal macrophages were obtained by inoculation of 3–5 ml cold phosphate buffered saline (PBS) in the abdominal cavity. After a soft abdominal massage for 30 s, the peritoneal liquid was collected and put in sterile plastic tubes (Falcon). This procedure was repeated three or four times for each animal and the tubes were centrifuged at 200*g* for 10 min. Cells were stained with neutral red (0.02%), incubated for 10 min at 37°C and counted in a haemocytometer to obtain a final concentration of 2×10^6 cells/ml. Cells were resuspended in cell culture medium (RPMI 1640, supplemented with 10% fetal calf serum, 2 mM L-glutamine, 20 mM HEPES; Sigma, USA) and cultured in a 96-well flat-bottomed plate (Corning, New York, NY, USA) at a final concentration of 2×10^5 cells per well. Cells were incubated at 37°C and, after 2 h, nonadherent cells were removed.[21]

This work agrees with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation, and was approved in August 8, 2008 (Protocol n° 46/08-CEEA).

Cytotoxicity assay

Before in-vitro assays, cells were incubated with clove extract or eugenol in the concentrations 5, 10, 25, 50 or 100 μg/well, so as to carry out the assays with noncytotoxic concentrations only. Cytotoxicity was evaluated by the crystal violet method.[22]

The macrophage culture received the stimuli as previously described, and after 24 h, supernatants were removed and 100 μl 0.5% crystal violet solution was added to the cells. After 10-min incubation at room temperature, the plates were washed and viable crystal violet-stained cells were lysed with 1% sodium dodecyl sulphate. Optical densities (OD) were read at 492 nm in an enzyme-linked immunosorbent assay (ELISA) reader, and the percentage of cell viability was calculated using the formula: (OD test / OD control) × 100. Assays were carried out in triplicate.

In-vitro assays

Macrophages were incubated with clove or eugenol diluted in RPMI 1640 at different concentrations (5, 10, 25, 50 or 100 μg/well) for 24 h and the supernatants were harvested and stored at −70°C for cytokine (IL-1β, IL-6 and IL-10) measurement. Concentrations that were able to inhibit the production of IL-1β and IL-6 followed two other protocols, challenging the cells with LPS.

Clove and eugenol incubation before lipopolysaccharide challenge

Macrophages were pretreated with clove (100 μg/well) or eugenol (50 or 100 μg/well) at concentrations that inhibited IL-1β, IL-6 and IL-10 production for 2 h and then they were incubated with LPS (5 μg/ml) for 22 h. After this period, the culture supernatants were harvested and stored at −70°C for cytokine measurement.[23]

Clove and eugenol incubation after lipopolysaccharide challenge

Macrophages were stimulated with LPS (5 μg/ml) for 2 h and then incubated with clove (100 μg/well) or eugenol (50 or 100 μg/well) for 22 h. Afterwards, supernatants were collected and stored at −70°C for cytokine determination.[23]

Dexamethasone (10^{-4} mol/l) and LPS (5 μg/ml) were used as a negative and positive control, to inhibit and stimulate cytokine production, respectively.[24]

Determination of cytokine production

Supernatants were collected and IL-1β, IL-6 and IL-10 production were measured by enzyme-linked immunosorbent assay (ELISA), according to manufacturer's instructions (BD Biosciences, San Diego, CA, USA). Briefly, a 96-well flat bottom Nunc Maxisorp (Nunc/Apogent, Denmark) was coated with a capture antibody specific to each cytokine. The plate was washed and blocked before 100 μl of the supernatants and serially diluted specific standards were added to the respective wells. Following a series of washing, the captured cytokine was detected using the specific conjugated detection antibody. The substrate reagent was added into each well and, after colour development, the plate was read at 450 nm, using an ELISA plate reader.[25]

Statistical analysis

Data were expressed as means ± standard deviation (SD) of five to seven similar assays. Analysis of variance and Dunnet's multiple comparison method were used. A probability (P) of 0.05 was chosen as the significance level.

Results

Clove chemical composition and cytotoxicity assay

GC-MS analysis revealed that eugenol was the major component found in clove extract (Figure 1).

Clove and eugenol did not affect cell viability as determined by the crystal violet test (data not shown).

Cytokine production

Clove exerted both preventive and therapeutic action on IL-1β production

Clove (5, 10, 25 μg/well) stimulated significantly IL-1β production, whereas the concentration of 100 μg/well inhibited significantly its release ($P < 0.001$) (Figure 2a). Eugenol (5 μg/well) stimulated this production of this cytokine ($P < 0.0001$), whereas 10, 25, 50 and 100 μg/well did not affect its production ($P > 0.05$) (Figure 2b).

Thus, in the next protocol, since clove (100 μg/well) exerted an inhibitory action on IL-1β production, macrophages were challenged with LPS before or after incubation with this natural product. As seen in Figure 2c, clove prevented LPS action as well as reversing its effects, exerting

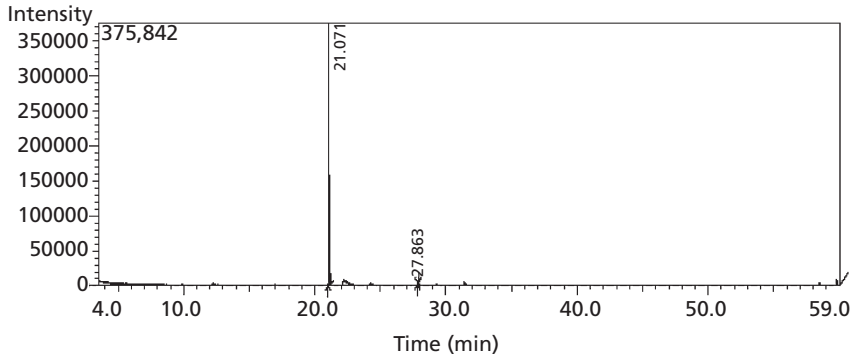

Figure 1 Chromatography profiles by gas chromatography-mass spectrometry, showing eugenol as the major component of the extract sample.

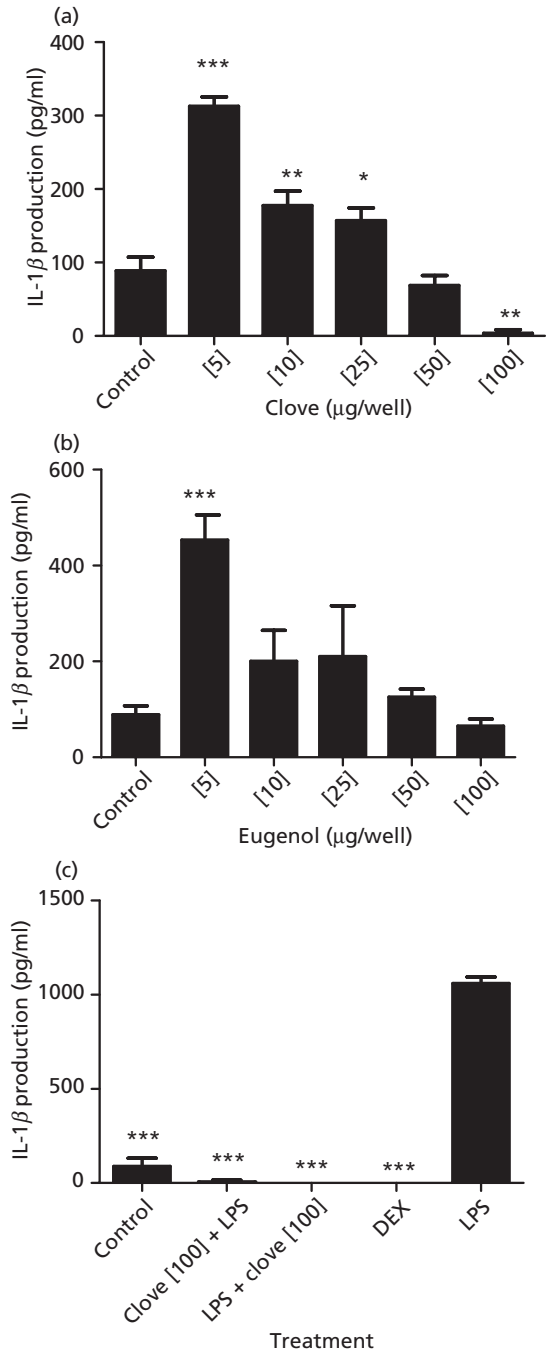

Figure 2 Interleukin-1β production by mouse peritoneal macrophages incubated with clove or eugenol. Macrophages were incubated with (a) clove or (b) eugenol at different concentrations (5, 10, 25, 50 and 100 μg/well) for 24 h at 37°C. (c) Interleukin-1β (IL-1β) production by peritoneal macrophages stimulated with lipopolysaccharide (LPS) 2 h before or after incubation with clove (100 μg/well) for 22 h. Dexamethasone (DEX; 10^{-4} mol/l) and LPS (5 μg/ml) were used as negative and positive controls, respectively. $*P < 0.01$ significantly different from control and LPS; $**P < 0.001$ significantly different from control; $***P < 0.0001$ significantly different from control (b) or LPS (c). Data are expressed as means ± SD of 5–7 similar assays.

an efficient action either before or after LPS challenge ($P < 0.0001$). Eugenol was not evaluated in LPS-challenge assays since it did not inhibit IL-1β production.

Clove and eugenol exerted both preventive and therapeutic action on IL-6 production

IL-6 production was significantly ($P < 0.0001$) increased after clove incubation for 24 h (5, 10, 25 and 50 μg/well), whereas 100 μg/well inhibited its production ($P < 0.001$) (Figure 3a). Eugenol (5, 10 and 25 μg/well) increased ($P < 0.0001$) macrophage production of IL-6, while 50 and 100 μg/well exerted an inhibitory effect on its production (Figure 3a).

The inhibitory concentrations of clove (100 μg/well) and eugenol (50 and 100 μg/well) were analysed in the next protocol. Figure 3b shows that clove prevented LPS action both before and after its challenge, and IL-6 production was lower than that induced by LPS alone ($P < 0.0001$). Eugenol prevented efficiently the effects of LPS before (100 μg/well) and after (50 and 100 μg/well) LPS addition ($P < 0.0001$) (Figure 3b).

Clove and eugenol modulated IL-10 production

Incubation with clove 5 or 10 μg/well stimulated significantly ($P < 0.01$) production of IL-10, whereas 25, 50 and 100 μg/well did not affect its production (Figure 4a). Eugenol (5 and 10 μg/well) stimulated IL-10 production ($P < 0.0001$), while 50 and 100 μg/well inhibited its release ($P < 0.01$) (Figure 4a).

Although clove did not inhibit IL-10 production, the concentration of 100 μg/well was tested in the LPS-challenge protocol because it inhibited IL-1β and IL-6 production. In fact, one may verify in Figure 4b that clove (100 μg/well) inhibited significantly LPS action ($P < 0.0001$), either before or after incubation of macrophages with LPS. Eugenol (50 and 100 μg/well) counteracted significantly LPS action when added after LPS incubation ($P < 0.0001$) (Figure 4b).

In all assays, dexamethasone and LPS exerted their inhibitory and stimulatory activities, as negative and positive controls of cytokine production, respectively.

Discussion

Several studies have pointed out the anti-inflammatory effects of clove and eugenol, reporting that these products were able to modulate several inflammatory markers such as COX-2, nitric oxide, iNOS and prostaglandin E2; leukotriene C4; mast cell degranulation and the transcription factors nuclear factor of activated T cells (NF-AT) and nuclear factor kappa B (NF-κB).[1,11,26–28]

In this study, we have investigated the immunomodulatory effects of clove and eugenol on cytokine production. Clove administration (200 mg/kg) to mice over a short term (three days) did not influence the Th1/Th2 cytokine

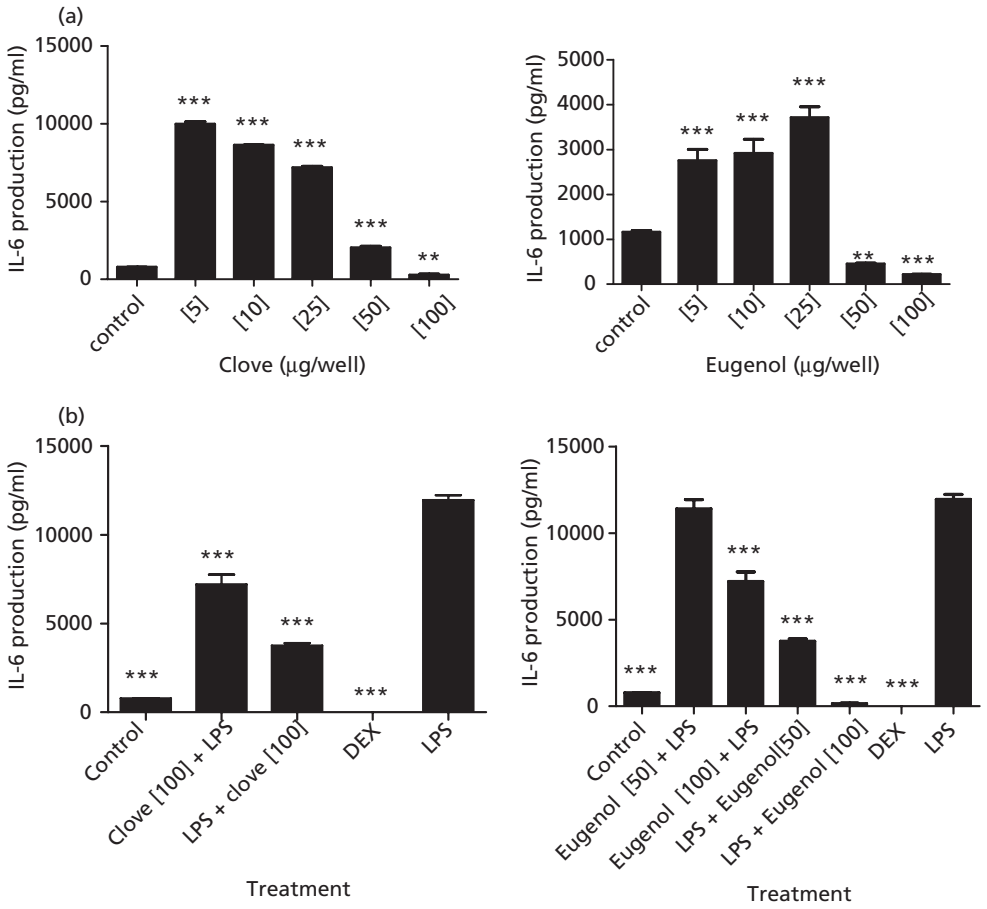

Figure 3 Interleukin-6 production by mouse peritoneal macrophages incubated with clove or eugenol. (a) Interleukin-6 (IL-6) production by peritoneal macrophages incubated with clove or eugenol at different concentrations (5, 10, 25, 50 and 100 μg/well) for 24 h at 37°C. (b) IL-6 production by peritoneal macrophages stimulated with lipopolysaccharide (LPS) 2 h before or after incubation with clove (100 μg/well) or eugenol (50 and 100 μg/well) for 22 h. Dexamethasone (DEX; 10^{-4} mol/l) and LPS (5 μg/ml) were used as negative and positive control, respectively. $*P < 0.01$ significantly different from control and LPS; $**P < 0.001$ significantly different from control; $***P < 0.0001$ significantly different from control (a) or LPS (b). Data are expressed as means ± SD of 5–7 similar assays.

balance.[29] However, eugenol and isoeugenol inhibited IL-2 expression and T cell proliferation *in vitro*, downregulating the transcription factors NF-AT and NF-κB.[30]

Work from our laboratory revealed that mice treated with water-extract of clove was found to inhibit macrophages to produce both IL-1β and IL-6. The essential oil of clove inhibited the production of such cytokines *in vitro*.[19] Those data encouraged us to investigate the effects of clove and eugenol before or after macrophages were challenged with LPS, to evaluate their efficacy as anti-inflammatory agents.

Firstly, clove and eugenol showed an immunomodulatory action: whereas lower concentrations stimulated IL-1β, IL-6 and IL-10 production, higher concentrations inhibited their generation by macrophages, which was not associated to cytotoxicity since clove and eugenol did not affect cell viability. Thus, the inhibitory concentrations of clove and eugenol were evaluated in LPS-challenge protocols, to explore their anti-inflammatory action: the incubation of macrophage with natural products before LPS addition may represent a possible preventive action, and the addition of natural products after LPS incubation could be associated to a therapeutic effect. Clove (100 μg/well) inhibited significantly IL-1β, IL-6 and IL-10 production. In LPS-challenged cells, it exerted an efficient inhibitory action on LPS stimulatory action either before or after LPS addition, suggesting a possible preventive and therapeutic action. Eugenol (50 and 100 μg/well) exerted inhibitory effects on IL-6 and IL-10 production by macrophages. The incubation of these cells with eugenol in such concentrations before and after LPS challenge revealed its potential to inhibit LPS effects as well.

A variety of intracellular and extracellular stimuli such as LPS, cytokines, reactive oxygen species, hypoxia, infections, among others, may induce phosphorylation and proteasomal degradation of inhibitory subunit IκB-alpha (IκB-α),

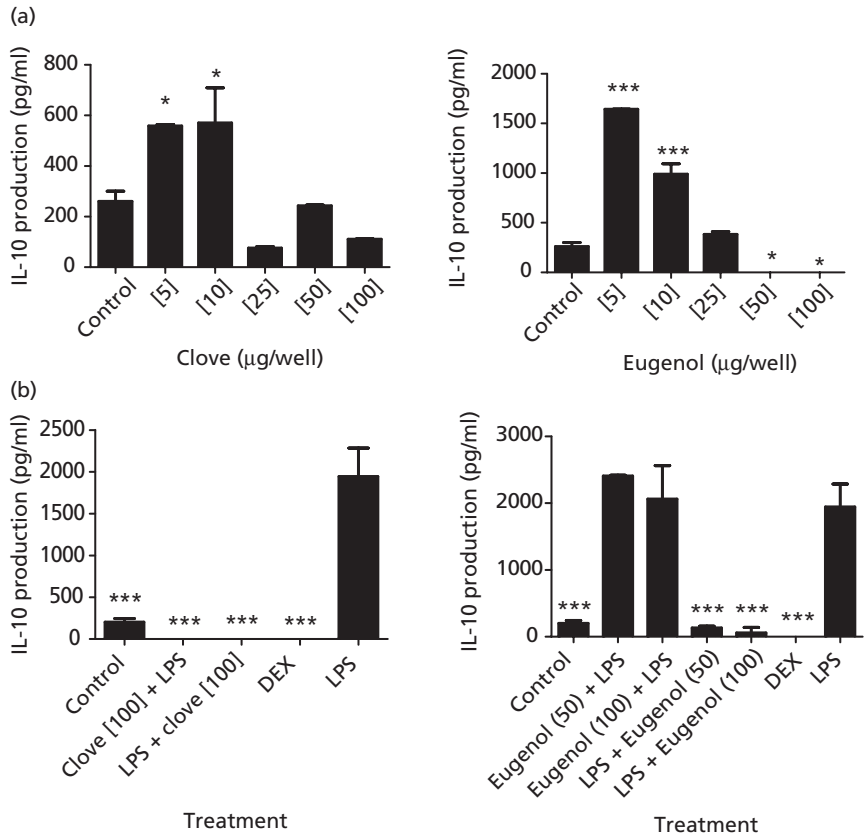

Figure 4 Interleukin-10 production by mouse peritoneal macrophages incubated with clove or eugenol. Interleukin-10 (IL-10) production by peritoneal macrophages incubated with clove or eugenol at different concentrations (5, 10, 25, 50 and 100 μg/well) for 24 h at 37°C. (b) IL-10 production by peritoneal macrophages stimulated with lipopolysaccharide (LPS) 2 h before or after incubation with clove (100 μg/well) or eugenol (50 and 100 μg/well) for 22 h. Dexamethasone (DEX; 10^{-4} mol/l) and LPS (5 μg/ml) were used as negative and positive controls, respectively. *$P < 0.01$ significantly different from control; **$P < 0.001$ significantly different from control; *** $P < 0.0001$ significantly different from control (a) or LPS (b). Data are expressed as means ± SD of 5–7 similar assays.

with release of the NF-κB heterodimer that translocates to the nucleus and binds to κB elements, regulating the expression of more than 200 genes. In the resting state, NF-κB is found sequestered in the cytoplasm as a heterodimer of p65 and p50 subunits complexed with the IκB alpha (IκBα) protein.[31] Agents that can suppress NF-κB activation would have the potential to prevent or delay the onset or treatment of NF-κB-linked diseases.[32] Eugenol and isoeugenol suppressed NF-κB activation by suppressing IκBα degradation. Based on those observations, one may speculate that the mechanism by which clove and eugenol inhibited the effects of LPS involved NF-κB suppression, since the secretion of cytokines was induced by LPS alone.

Chemoprevention by eugenol may also occur due to downregulation of IκBα phosphorylation, a critical step in NF-κB activation thereby blocking the NF-κB signalling cascade.[31] In lung inflammation, eugenol effectively improved functional and structural pulmonary changes induced by LPS, modulating lung injury by inhibition of NF-κB activation and TNF-α release.[28]

Conclusions

Clove exerted immunomodulatory/anti-inflammatory effects by inhibiting LPS action. A possible mechanism of action involved NF-κB suppression by eugenol, since it was the major compound found in clove extract. Further studies are required to investigate clove and eugenol efficacy in inflammatory diseases, to explore their potential as anti-inflammatory agents.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

The authors wish to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – 2008/06120-0) for the grant.

References

1. Kim HM *et al*. Effect of *Syzygium aromaticum* extract in immediate hypersensitivity in rats. *J Ethnopharmacol* 1998; 60: 125–131.
2. Chaieb K *et al*. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): a short review. *Phytother Res* 2007; 21: 501–506.
3. Chami F *et al*. Oregano and clove essential oils induce surface alteration of *Saccharomyces cerevisiae*. *Phytother Res* 2005; 19: 405–408.
4. Zheng GQ *et al*. Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. *J Nat Prod* 1992; 55: 999–1003.
5. Miyazawa M, Hisama M. Suppression of chemical mutagen-induced SOS response by alkylphenols from clove (*Syzygium aromaticum*) in the *Salmonella typhimurium* TA1535/pSK1002 umu test. *J Agric Food Chem* 2001; 49: 419–425.
6. Carrasco FR *et al*. Immunomodulatory activity of *Zingiber officinale* Roscoe, *Salvia officinalis* L. and *Syzygium aromaticum* L. essential oils: evidence for humor- and cell-mediated responses. *J Pharm Pharmacol* 2009; 61: 961–967.
7. Raskin I *et al*. Plants and human health in the twenty-first century. *Trends Biotechnol* 2002; 20: 522–531.
8. Morsy MA, Fouad AA. Mechanisms of gastroprotective effect of eugenol in indomethacin-induced ulcer in rats. *Phytother Res* 2008; 22: 1361–1366.
9. Benencia F, Courrèges MC. *In vitro* and *in vivo* activity of eugenol on human herpes virus. *Phytother Res* 2000; 14: 495–500.
10. Sondak VK *et al*. Allogeneic and autologous melanoma vaccines: where have we been and where are we going? *Clin Cancer Res* 2001; 12: 2337–2341.
11. Lee YY *et al*. Eugenol suppressed the expression of lipopolysaccharide-induced proinflammatory mediators in human macrophages. *J Endodontics* 2007; 33: 698–702.
12. Ghosh R *et al*. Eugenol causes melanoma growth suppression through inhibition of E2F1 transcriptional activity. *Biol Chem* 2005; 280: 5812–5819.
13. Okada N *et al*. Induction of cytotoxicity and apoptosis and inhibition of cyclooxygenase-2 gene expression by eugenol-related compounds. *Anticancer Res* 2005; 25: 3263–3269.
14. Mosser DM. The many faces of macrophage activation. *J Leukoc Biol* 2003; 73: 209–212.
15. Tzeng HP *et al*. Beta-lapachone reduces endotoxin-induced macrophage activation and lung edema and mortality. *Am J Respir Crit Care Med* 2003; 168: 85–91.
16. Netea MG *et al*. IL-1beta processing in host defense: beyond the inflammasomes. *PLoS Pathog* 2010; 6: 1–9.
17. Ahmed ST, Ivashkiv LB. Inhibition of IL-6 and IL-10 signaling and Stat activation by inflammatory and stress pathways. *J Immunol* 2000; 165: 5227–5237.
18. McCoy CE *et al*. IL-10 inhibits miR-155 induction by Toll-like receptors. *J Biol Chem* 2010; 285: 20492–20498.
19. Rodrigues TG *et al*. *In vitro* and *in vivo* effects of clove on pro-inflammatory cytokines production by macrophages. *Nat Prod Res* 2009; 23: 319–326.
20. Adams RP. *Identification of Essential Oil Components by Gas Chromatography Quadupole Mass Spectroscopy*. Carol Stream, IL: Allure Publishing Corporation, 2001.
21. Sforcin JM *et al*. Effect of a leukotriene inhibitor (MK886) on nitric oxide and hydrogen peroxide production by macrophages of acutely and chronically stressed mice. *J Pharm Pharmacol* 2007; 59: 1249–1254.
22. Ait-Mbarek L *et al*. Anti-tumor properties of blackseed (*Nigella sativa* L.) extracts. *Braz J Med Biol Res* 2007; 40: 839–847.
23. Shin HM *et al*. Suppression of interleukin-6 production in macrophages by furonaphthoquinone NFD-37. *Int Immunopharmacol* 2006; 6: 916–923.
24. Zhuo YH *et al*. Dexamethasone disrupts intercellular junction formation and cytoskeleton organization in human trabecular meshwork cells. *Mol Vis* 2010; 16: 61–71.
25. Tan EL *et al*. Quantification of Epstein-Barr virus DNA load, interleukin-6, interleukin-10, transforming growth factor-β1 and stem cell factor in plasma of patients with nasopharyngeal carcinoma. *BMC Cancer* 2006; 6: 227.
26. Kaur G *et al*. Eugenol precludes cutaneous chemical carcinogenesis in mouse by preventing oxidative stress and inflammation and by inducing apoptosis. *Mol Carcinog* 2010; 49: 290–301.
27. Raghavenra H *et al*. Eugenol the active principle from cloves inhibits 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells. *Prostaglandins Leukot Essent Fatty Acids* 2006; 74: 23–27.
28. Magalhães CB *et al*. *In vivo* anti-inflammatory action of eugenol on lipopolysaccharide-induced lung injury. *J Appl Physiol* 2010; 108: 845–851.
29. Bachiega TF *et al*. Th1/Th2 cytokine production by clove-treated mice. *Nat Prod Res* 2009; 23: 1552–1558.
30. Park KR *et al*. Suppression of interleukin-2 gene expression by isoeugenol is mediated through down-regulation of NF-AT and NF-kappaB. *Int Immunopharmacol* 2007; 7: 1251–1258.
31. Manikandan P *et al*. Eugenol induces apoptosis and inhibits invasion and angiogenesis in a rat model of gastric carcinogenesis induced by MNNG. *Life Sci* 2010; 86: 936–941.
32. Aggarwal BB, Shishodia S. Suppression of the nuclear factor-kappaB activation pathway by spice-derived phytochemicals: reasoning for seasoning. *Ann N Y Acad Sci* 2004; 1030: 434–441.