JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 8873–8883 8873
DOI:10.1021/jf902255h

Saponins in Yerba Mate Tea (*Ilex paraguariensis* A. St.-Hil) and Quercetin Synergistically Inhibit iNOS and COX-2 in Lipopolysaccharide-Induced Macrophages through NFκB Pathways

Sirima Puangpraphant and Elvira Gonzalez de Mejia*

Department of Food Science and Human Nutrition, 228 ERML, MC-051, University of Illinois at Urbana−Champaign, 1201 West Gregory Drive, Urbana, Illinois 61801

Yerba mate tea (*Ilex paraguariensis*) is growing in popularity around the world. The objective of this study was to investigate the potential anti-inflammatory effect of yerba mate tea (MT) extracts as well as some of its phytochemicals and their interactions. MT and decaffeinated MT extracts [1−300 μM chlorogenic acid (CHA) equiv]; CHA, caffeine from MT (matein), and mate saponins (1−300 μM); quercetin (1−200 μM); and ursolic and oleanolic acids (1−100 μM) were tested by measuring their ability to inhibit COX-2/PGE_2 and iNOS/NO pathways in LPS-induced RAW 264.7 macrophages. Mate saponins (IC_{50} = 20 μM) and oleanolic acid (IC_{50} = 80 μM) significantly inhibited iNOS/NO pathways, whereas ursolic acid showed low or no inhibition at 100 μM. Quercetin was the most potent inhibitor of pro-inflammatory responses at a concentration 10 times lower than the concentrations used of other compounds (IC_{50} = 11.6 μM for NO, 7.9 μM for iNOS, and 6.5 μM for PGE_2). Combination of quercetin/mate saponins (0.001:0.004, molar ratio) resulted in synergistic interaction inhibiting both NO and PGE_2 production. It also suppressed IL-6 and IL-1β production and resulted in reduction of LPS-induced nuclear translocation of nuclear factor-κB subunits. MT extract did not have a potent anti-inflammatory effect perhaps due to the antagonistic effect of some of its compounds. However, whole MT consumption still has a promising anti-inflammatory outcome mainly through the PGE_2/COX-2 pathway. To the authors' knowledge, this is the first study demonstrating the efficacy, interactions, and mechanisms of some MT phytochemicals in inhibiting pro-inflammatory responses.

KEYWORDS: Cyclooxygenase-2 (COX-2); prostaglandin E_2 (PGE_2); NFκB; quercetin; RAW264.7 macrophages; saponins; yerba mate tea phytochemicals

INTRODUCTION

Yerba mate tea (*Ilex paraguariensis*) (MT) is rich in many phytochemical compounds such as theobromine, caffeine (matein), caffeoylquinic acid derivatives, dicaffeoylquinic acid derivatives, rutin, luteolin, quercetin, and saponins (*1*). **Figure 1** presents the chemical structures of some of the important phytochemicals in MT.

Several studies have shown that flavonoids in MT have anti-inflammatory activity; this has important health implications because chronic inflammation can develop into several diseases such as arteriosclerosis, cancer, diabetes, and neurodegenerative diseases (*2*−*5*). Published reports suggest that MT has chemopreventive activity against some types of cancer (*6*−*8*). However, at the cellular level, it is unclear which compounds in MT are responsible for this effect. Administering agents that cause colitis in healthy rodents or genetically engineered cancer-prone mice accelerated the development of colorectal cancer (*9*). These observations support the role of inflammation in carcinogenesis. Anti-inflammatory properties of phytochemicals such as quercetin (*10*−*13*) and saponins (*14*) are documented. However, the anti-inflammatory mechanism of MT and its components has not been studied.

Pro-inflammatory cytokines are involved in the formation of toxic peroxynitrite by increasing the activity of inducible nitric oxide synthase (iNOS). Prostaglandin E_2 (PGE_2) is also an important mediator of inflammatory reaction produced by the enzyme cyclooxygenase-2 (COX-2). COX-2 and iNOS are induced during inflammatory conditions and are overexpressed in colonic tumors in humans, as well as in animals (*9*). iNOS has been shown to be involved in regulating COX-2 production, which plays an important role in colon tumorigenesis. Hence, suppression of enzyme induction and the activities of iNOS/COX-2 is an important approach to preventing carcinogenesis in several organs including the stomach and colon (*15*).

Interleukin (IL)-1β and IL-6 are pro-inflammatory cytokines that can promote acute to chronic inflammation through activation of nuclear factor-κB (NFκB). NFκB is a transcription factor that has five protein subunits: RelA (p65), Rel B, c-Rel, NFκB1 (p50), and NFκB2 (p52). These subunits may form homo- or heterodimers. The activated NFκB is a heterodimer and consists of p65 and p50. In human cells, NFκB is bound to the inhibitor

*Author to whom correspondence should be addressed [telephone (217) 244-3196; fax (217) 265-0925; e-mail edemejia@illinois.edu].

Published on Web 09/09/2009

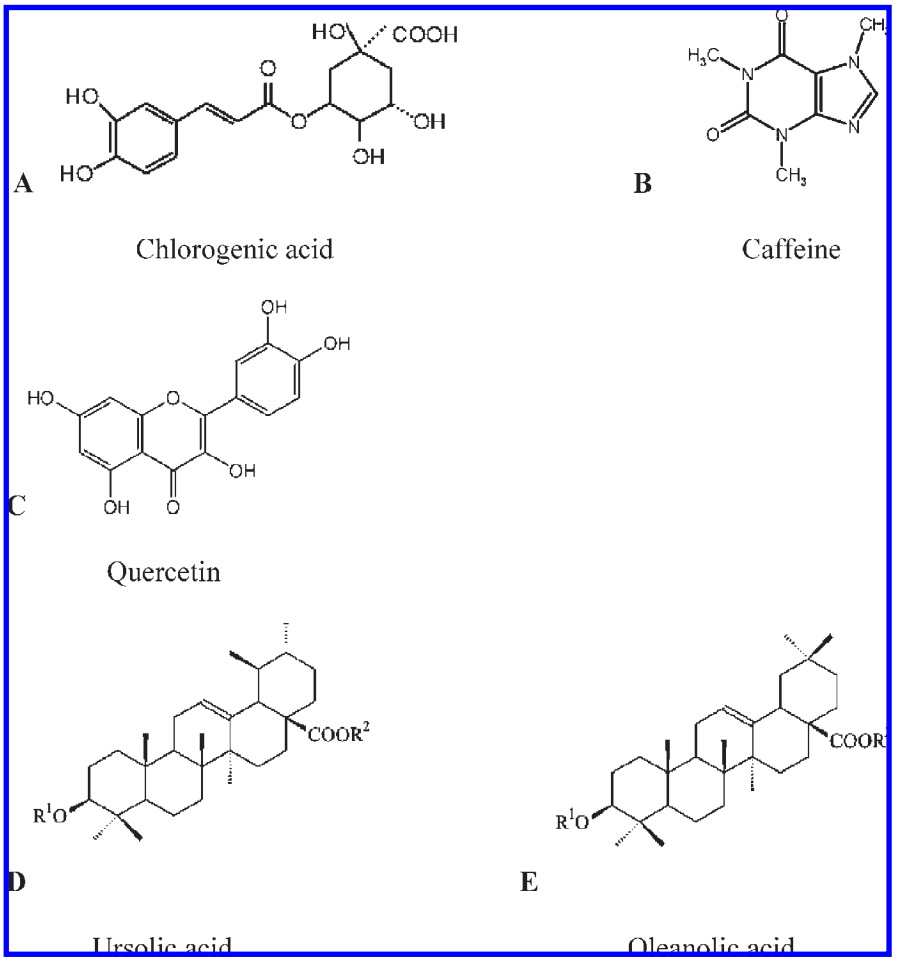

Figure 1. Chemical structures of (**A**) chlorogenic acid, (**B**) caffeine (matein), (**C**) quercetin, (**D**) ursolic acid, and (**E**) oleanolic acid.

κBα (IκBα) in the cytoplasm (*15*). IκB kinase complex (IKK) can be activated in response to many different signals which result in phosphorylation of IκBα followed by its ubiquitination and proteosomal degradation, causing the IκBα to dissociate from NFκB. Degradation of IκBα releases NFκB (p65 and p50), resulting in translocation of the activated NFκB into the nucleus, which then regulates the transcription of target genes (*16*). NFκB regulates several genes involved in immunity, inflammation, and apoptosis and promotes the expression of cytokines such as IL-1β, IL-6, and TNFα, and up-regulates inflammatory enzymes such as COX-2 and iNOS. Thus, NFκB is an emerging target for the prevention of cancer and inflammation (*15*). For instance, ursolic acid reduces pro-inflammation cytokines through down-regulation of NFκB (*17*).

We hypothesized that phytochemicals present in MT will be able to inhibit inflammation through NFκB pathways. The objective of this research was therefore to determine the effect of selected MT phytochemicals for their ability to suppress inflammation. The anti-inflammatory properties of MT extract, decaffeinated MT extract, chlorogenic acid (CHA), quercetin, matein, mate saponins, ursolic acid, and oleanolic acid were evaluated in vitro using lipopolysaccharide-induced RAW 264.7 macrophages. COX-2/PGE_2 and iNOS/NO pathways as well as the interaction between MT phytochemicals in inhibiting NO and PGE_2 production were investigated. On the basis of the results, a mechanism of action was suggested.

MATERIALS AND METHODS

Chemicals. Chlorogenic acid (≥95% CHA), ursolic acid (≥90%), quercetin (≥98%), oleanolic acid (≥97%), sodium nitrite, sulfanilamide, *N*-1-(naphthyl)ethylenediamine-diHCl, and lipopolysaccharide (LPS) from *Escherichia coli* O55:B5) were purchased from Sigma (St. Louis, MO). Macrophage RAW 264.7 cell line and Dulbecco's Modified Eagle Medium with L-glutamine (DMEM) were purchased from American Type Culture Collection (Manassas, VA). Fetal bovine serum was purchased from Invitrogen (Grand Island, NY). COX-2, iNOS, NFκB p50, and NFκB p65 mouse monoclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-mouse IgG conjugated horseradish peroxidase secondary antibody was purchased from GE Healthcare (Buckinghamshire, U.K.). Luciferase assay kit and TransFast transfection reagent were purchased from Promega (Madison WI) and pNF-κB-luciferase vector (pNF-κB luc vector) was purchased from Clontech (Mountain View CA). All other chemicals were purchased from Sigma, unless otherwise specified.

Mate Tea Preparation. Organically grown mate (*Ilex paraguariensis*) leaves from Paraguay were kept in plastic bags and refrigerated at 4 °C until used. MT was prepared by using the traditional North American procedure of preparing tea. Briefly, 2.5 g of dry tea leaves (DL) was soaked in 250 mL of boiling water for 10 min. The infusion was cooled to room temperature before filtration using Whatman paper no. 2 to eliminate dry tea leaves and then freeze-dried in a Labconco, Shell Freeze System (Fisher Scientific, Pittsburgh, PA). The freeze-dried material called MT extract, equivalent to instant tea, was kept at −20 °C protected from light. The phytochemical composition of MT under investigation was previously published by Heck et al. (*35*).

Preparation of Decaffeinated Mate Tea. Matein and decaffeinated MT were obtained by supercritical CO_2 extraction of MT leaves. The supercritical extraction apparatus was provided by the U.S. Department of Agriculture (Peoria, IL). Matein was extracted from organic MT using a water-saturated CO_2 supercritical extraction. The temperature for the extraction vessel was 70 °C with a pressure of 398 bar. The temperature of the receiver vessel was 50 °C with a pressure of 75 bar. The flow rate was

466 mL/min of liquid CO_2. A total of 1.5 kg of MT leaves was placed in the CO_2 supercritical extractor, and it took approximately 8 h for the total procedure (*18*). The final product was freeze-dried.

Extraction and Purification of Mate Saponins. Mate saponins were isolated from the MT infusions as performed by Gnoatto et al. (*19*). Briefly, MT infusion was prepared by boiling 15 g of dry leaves for 10 min in 100 mL of water. The infusion was then filtered, brought to 100 mL with water, treated with hydrochloric acid, and then refluxed for 3 h. Mate saponins were extracted four times with 50 mL of chloroform. The chloroform fraction was evaporated to dryness (77.6 mg). A 1050 Hewlett-Packard (Palo Alto, CA) gradient liquid chromatograph equipped with a 1050 HP autosampler, a gradient pump, and a photodiode array detector was used to estimate saponin concentration. HPLC analysis was performed using a C_{18} RP guard column and a C_{18} RP Phenomenex Prodigy ODS column (250 mm × 4.6 mm × 5 μm). The variable-wavelength detector was set at 203 nm. The mobile phase consisted of acetonitrile/water (70:30 v/v). Flow rate = 1.0 mL min^{-1} and sensitivity = 0.001 AUFS. Mate saponins were calculated to have 73% purity. A molecular mass of 1000 g/mol was used in the calculation of the molar concentration of mate saponin.

Macrophage Culture and Cell Viability Assay. Macrophage cell line RAW 264.7 was cultured in growth medium containing DMEM, 1% penicillin/streptomycin, 1% sodium pyruvate, and 10% fetal bovine serum at 37 °C in 5% CO_2/95% air. The cell viability assay was conducted using the CellTiter 96 Aqueous One Solution proliferation assay kit using the novel tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS), and an electron coupling reagent, phenazine ethosulfate (PES) (Promega Corp., Madison, WI). Briefly, 5×10^4 cells were seeded in a 96-well plate, and the total volume was adjusted to 200 μL with growth medium. Cells were allowed to grow for 24 h at 37 °C in 5% CO_2/95% air. Cells were then treated with different concentrations of MT phytochemicals for 24 h, the growth medium was replaced by 100 μL of fresh growth medium, and 20 μL MTS/PES was added to each well. The plate was incubated for 2 h at 37 °C, and the absorbance was read at 515 nm. The percentage of viable cells was calculated with respect to cells treated with 0.3% DMSO (for MT extract, decaffeinated MT extract, CHA, matein, quercetin, ursolic acid, oleanolic acid) and 0.3% 1:1 DMSO/methanol (for mate saponins). Solvents used at these concentrations showed no cytotoxicity (viability > 80%). DMSO at < 0.3% presented 95.8% cell viability.

Treatments. Cell treatment was conducted by seeding 2×10^5 cells in a 6-well plate, and cells were allowed to grow for 48 h at 37 °C in 5% CO_2/95% air. After 48 h of incubation, the cells were treated with different concentrations of MT phytochemicals (1, 10, 100, 200, 300 μM) and 1 μg/mL of LPS for 24 h. All MT phytochemicals were sterilized using 0.22 μm nylon filters before the cells were treated. After 24 h of treatment, the spent medium was collected and analyzed for NO, PGE_2, IL-6, and IL-1β. Cell lysates were used to study the effect of MT phytochemicals on the expressions of COX-2 and iNOS.

Nitrite and PGE_2 Measurement. After 24 h of treatment and LPS induction, culture supernatant was collected. For nitrite measurement, 100 μL of the spent medium was plated in 96-well plate and an equal amount of Griess reagent (1% sulfanilamide and 0.1% *N*-1-(naphthyl)ethylenediamine-diHCl in 2.5% H_3PO_4) was added. The plate was incubated for 5 min, and the absorbance was measured at 550 nm. The amount of NO was calculated using sodium nitrite standard curve. For PGE_2 measurement, ELISA kit monoclonal was used following the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI).

Interaction Study and Isobolographic Analysis. Possible interactions between MT phytochemicals in inhibiting NO and PGE_2 production by LPS-induced RAW 264.7 were tested. The molar ratio of phytochemicals tested was approximately equal to its concentration in one cup of tea (1.5 g of DL/150 mL of water); 2.3:0.86 (CHA/matein), 2.3:0.004 (CHA/mate saponins), 2.3:0.001 (CHA/quercetin), 0.004:0.001 (mate saponins/quercetin), 0.004:0.86 (mate saponins/matein), 0.001:0.86 (quercetin/matein). Calculated and experimental percent inhibition values from combination studies were compared to determined expected interactions. Experimental values that were significantly different from the calculated values at 95% confidence interval were defined as synergistic or "more than additive" interactions as previously reported (*20*). To further understand the interactions, an isobolographic analysis was performed (*20*). IC_{35} values for MT phytochemicals were used in constructing isobolograms. A straight line connecting the IC_{35} values of these MT phytochemicals predicts the IC_{35} value of an additive effect. If results of the isobolographic analysis deviated significantly to the left of the additivity line with 95% confidence interval, the interaction was confirmed as a synergy; when it deviated to the right, the interaction was antagonistic. If it fell within the confidence limit for the additivity line, the interaction was said to be additive (*20*). The IC_{30} and IC_{50} values for MT phytochemicals were calculated by linear regression using Prism 4.0 (GraphPad Software, San Diego, CA).

Measurement of iNOS and COX-2 Protein Expressions. COX-2 and iNOS expressions were determined in cell lysates. Briefly, treated cells were washed with ice-cold DMEM and ice-cold phosphate-buffered saline before treatment with 200 μL of Laemmli buffer (Bio-Rad Laboratories, Hercules, CA) with 5% β-mercaptoethanol as lysing buffer. The cell lysates were boiled for 5 min, and approximately 25 μg of proteins was loaded in 4−20% Tris-HCl ready gels (Bio-Rad Laboratories) for protein separation. Separated proteins were transferred in PVDF membrane and blocked with 5% nonfat dry milk in 0.1% Tris-buffered saline Tween 20 (TBST) for 1 h at 4 °C. After blocking, the membrane was washed with 0.1% TBST (five times, 5 min each) and incubated overnight with either COX-2 or iNOS mouse monoclonal antibody (1:1000) at 4 °C. The membrane was washed again and incubated with anti-mouse IgG antibody conjugated to horseradish peroxidase secondary antibody for 3−4 h at room temperature. After incubation and repeated washings, the expression of COX-2 and iNOS was visualized using a chemiluminescent reagent (GE Healthcare) following the manufacturer's instructions. The membrane picture was taken with a Kodak Image station 440 CF (Eastman Kodak Co., New Haven, CT).

IL-6 and IL-1β Measurement. For IL-6 and IL-1β measurements, a Bio-Plex singleplex cytokine assay was used (Bio-Rad Laboratories). Briefly, a 96-well filter plate was prewetted with 100 μL of assay buffer and the buffer was removed by vacuum filtration. Then, 50 μL of the bead working solution (IL-6 and IL-1β) was added, and the buffer was removed by vacuum filtration. The plate was washed with 100 μL of wash buffer, and 50 μL of diluted standard or sample was added to each well. The plate was then covered with sealing tape and aluminum foil and incubated at room temperature for 30 min with shaking. After incubation, the buffer was removed and washed with 100 μL of wash buffer three times. Twenty-five microliters of detection antibody was added, and the plate was incubated at room temperature for 30 min with shaking. The plate was washed again, and 50 μL of streptavidin-PE was added and incubated for 10 min at room temperature with shaking. After incubation with streptavidin PE, the plate was washed three times with wash buffer, and the beads were resuspended with 125 μL of assay buffer. The plate was then read using a Bioplex suspension array system using high photomultiplier tube setting standards measuring 100 beads per region. The concentrations of IL-6 and IL-1β were calculated using IL-6 and IL-1β standard curves, respectively.

Transfection of RAW 264.7 Macrophage Cell Line with pNF-κB-Luciferase Vector. RAW 264.7 macrophage cell line was transiently transfected with a pNF-κB-luciferase vector or pTAL-Luc vector (Clontech) using TransFast transfection reagent (Promega) following the manufacturer's protocol. The pNF-κB-luciferase vector contained the firefly luciferase gene from *Photinus pyralis*, whereas the pTAL-Luc vector served as a negative control. Briefly, 5×10^4 cells were plated in a 24-well plate and allowed to grow to 80−90% confluency for 24 h at 37 °C in 5% CO_2/95% air. The day before transfection, the transfection reagent was resuspended in 400 μL of nuclease-free water and stored at −20 °C overnight. The cells were then treated with 200 μL of DNA-Transfast reagent mixture and incubated for 1 h at 37 °C in 5% CO_2/95% air. After 1 h of incubation, each well was then overlaid with 1 mL of complete growth medium, and transfection was carried out for 48 h at 37 °C in 5% CO_2/95% air.

Measurement of Luciferase Activity. After transfection, cells were treated with quercetin (1 μM), mate saponins (4 μM), quercetin/mate saponins combination (1:4 μM), and 1 μg/mL LPS for 24 h. Luciferase activity from the cells was measured using a luciferase assay system (Promega) following the manufacturer's protocol and a Femtomaster FB 12 luminometer (Zylux Corp.). Briefly, growth medium was removed

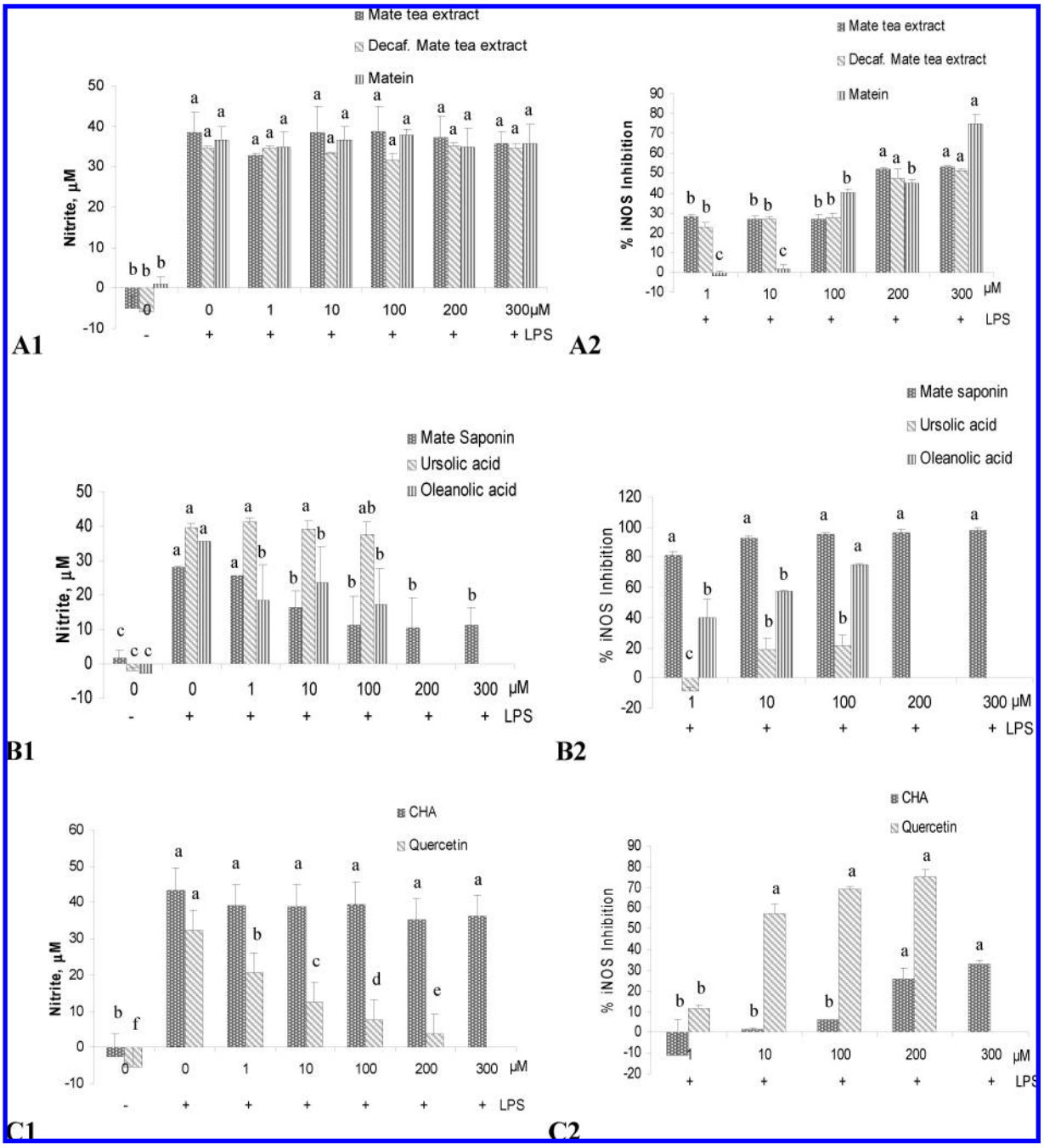

Figure 2. Effect of different concentrations of MT phytochemicals on NO (**1**) production and iNOS (**2**) expression by LPS-induced RAW 264.7 cells: (**A**) MT extract, decaffeinated MT extract, matein; (**B**) mate saponins, ursolic acid, oleanolic acid; (**C**) CHA, quercetin. The data represent the mean ± SD from two independent experiments and at least a triplicate analysis. Different letters indicate significant differences, $P < 0.05$.

and the cells washed with 1 mL of ice-cold PBS. After complete removal of ice-cold PBS, 100 μL of passive lysis buffer was added and the plate was incubated at room temperature for 15 min with shaking. After incubation, luciferase activity was measured by adding 20 μL of cell lysate in 100 μL of luciferase assay reagent and immediately reading the flash using the Femtomaster luminometer. Luciferase activity was normalized on the basis of protein content as determined by Bio-Rad protein assay.

Western Blotting of NFκB p50 and p65 Protein Expression. NFκB p50 and p65 expressions were determined in cell cytoplasm and nucleus. Nuclear and cytoplasmic proteins were isolated with a buffer extraction system and centrifugation NE-PER nuclear and cytoplasmic extraction reagents (Pierce Biotechnology, Rockford, IL) according to the manufacturer's recommendations. Briefly, RAW 264.7 macrophages were plated at a density of 2×10^5 cells per well and allowed to confluence for 48 h at 37 °C in 5% CO_2/95% air. Confluent cells were then treated with quercetin (1 μM), mate saponins (4 μM), and quercetin/mate saponins combination (1:4 μM) and 1 μg/mL LPS for 24 h. After 24 h of treatment, cells were harvested in 200 μL of 0.25% trypsin solution. The lysates were then transferred in a 1.5 mL microcentrifuge tube and pelleted by centrifugation at 500*g* for 2−3 min. After centrifugation, the cell pellet was treated with 200 μL of ice-cold cytoplasmic extraction reagent I, vortexed for 15 s, and incubated on ice for 10 min and then with treated 11 μL of ice-cold cytoplasmic extraction reagent II, vortexed for 5 s, and incubated on ice for 1 min. After incubation, the tube was vortexed for 5 s and centrifuged at 16000*g* for 5 min at 4 °C. The supernatant (cytoplasmic extract) was immediately transferred to a clean prechilled 1.5 mL microcentrifuge tube. The pellet was then resuspended in 100 μL of nuclear extraction reagent, vortexed for 15 min, and incubated on ice for 40 min with vortexing for 15 s every 10 min. The mixture was centrifuged at 16000*g* for 10 min at 4 °C, and the supernatant (nuclear extract) was

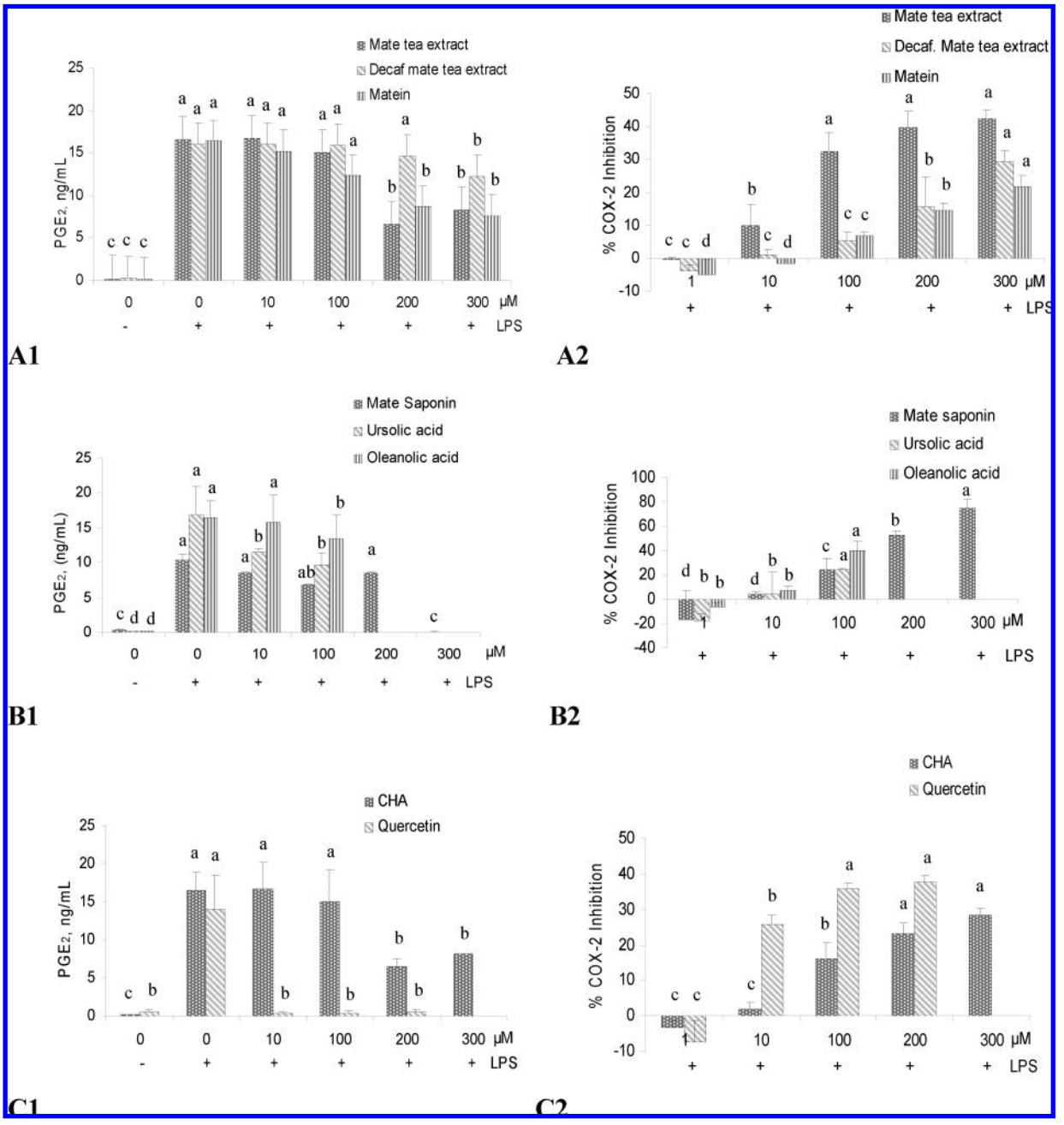

Figure 3. Effect of different concentrations of MT phytochemicals on PGE_2 production (**1**) and COX-2 expression (**2**) by LPS-induced RAW 264.7 cells: (**A**) MT extract, decaffeinated MT extract, matein, (**B**) mate saponins, ursolic acid, oleanolic acid, (**C**) CHA, quercetin. The data represent the mean ± SD from two independent experiments and at least a triplicate analysis. Different letters indicate significant differences, $P < 0.05$.

collected. Both cytoplasmic and nuclear extracts were stored at −80 °C until use for Western blot analysis. For the determination of p50 and p65 nuclear translocation, approximately 10 μg of protein was loaded in 4−20% Tris-HCl ready gels (Bio-Rad Laboratories) for protein separation. The separated proteins were transferred in PVDF membrane and blocked with 5% nonfat dry milk in 0.1% Tris-buffered saline Tween 20 (TBST) for 1 h at 4 °C. After blocking, the membrane was washed with 0.1% TBST (five times, 5 min each) and incubated overnight with either p50 or p65 mouse monoclonal antibody (1:200) at 4 °C. The membrane was washed again and incubated with anti-mouse IgG horseradish peroxidase conjugate secondary antibody for 3−4 h at room temperature. After incubation and repeated washing, the expression of p50 ad p65 was visualized using chemiluminescent reagent (GE Healthcare) following the manufacturer's instructions. The membrane picture was taken with a Kodak Image station 440 CF (Eastman Kodak Co.).

Statistical Analysis. Data are presented as means ± SD for the indicated number of independently performed experiments. Data were analyzed using one-way ANOVA, and means were considered to be significantly different at $P < 0.05$ as determined by least significant differences (LSD).

RESULTS

Effect of Different Mate Tea Phytochemicals on Viability of RAW 264.7 Cells. Cells showed a survival rate of >80% when incubated with MT extract (92.8%), decaffeinated MT extract (93.5%), CHA (91.2%), matein (91.2%), and mate saponins (82.7%), at a concentration ≤300 μM. Therefore, in this study, a concentration of 1−300 μM was used to treat the cells and thus prevent the compounds from having a cytotoxic effect. Concentrations ranging from 1 to 200 μM, from 1 to 100 μM, and from 1 to 100 μM were used for quercetin, ursolic acid, and oleanolic acid, respectively, because their survival rates were 96.4, 80.8, and 87.6%, respectively.

Table 1. IC_{10}, IC_{35}, and IC_{50} of Mate Tea Compounds against NO and iNOS Production and Expression[a]

	NO[b] (μM)			iNOS[b] (μM)		
compound	IC_{10}	IC_{35}	IC_{50}	IC_{10}	IC_{35}	IC_{50}
mate tea extract	>300	>300	>300	16.5 ± 10.2	198.0 ± 10.2	>300
decaffeinated mate tea extract	>300	>300	>300	24.8 ± 3.5	208.0 ± 3.5	>300
chlorogenic acid	123.4 ± 1.6	>300	>300	198.8 ± 0.8	250.8 ± 0.8	>300
matein	>300	>300	>300	147.5 ± 0.3	203.0 ± 0.3	223.7 ± 0.3
quercetin	0.5 ± 0.3	1.5 ± 0.3	11.6 ± 0.3	1.1 ± 0.1	4.5 ± 0.1	7.9 ± 0.1
mate saponins	4.8 ± 0.5	10.1 ± 0.5	20.8 ± 0.5	2.3 ± 0.5	6.2 ± 0.5	8.4 ± 0.5
ursolic acid	101.5 ± 1.6	>100	>100	81.2 ± 0.5	>100	>100
oleanolic acid	16.7 ± 5.4	37.8 ± 5.4	79.7 ± 5.4	8.3 ± 0.5	22.6 ± 0.5	30.9 ± 0.5

[a] IC_{10}, IC_{35}, and IC_{50} are the concentrations (μM) that resulted in 10, 35, and 50% reduction, respectively, of production/expression of pro-inflammatory responses (mean ± SD, $n = 2$). [b] Abbreviations: NO, nitric oxide; iNOS, inducible nitric oxide synthase.

Table 2. IC_{10}, IC_{35}, and IC_{50} of Mate Tea Compounds against PGE_2 and COX-2 Production and Expression[a]

	PGE_2[b] (μM)			COX-2[b] (μM)		
compound	IC_{10}	IC_{35}	IC_{50}	IC_{10}	IC_{35}	IC_{50}
mate tea extract	173.6 ± 2.0	220.0 ± 2.0	280.6 ± 2.0	111.8 ± 0.2	206.3 ± 0.2	>300
decaffeinated mate tea extract	225.0 ± 3.2	>300	>300	205.5 ± 2.5	>300	>300
chlorogenic acid	100.1 ± 2.4	130.6 ± 2.4	231.6 ± 2.4	186.4 ± 0.2	>300	>300
matein	137.5 ± 0.5	215.6 ± 0.5	252.6 ± 0.5	213.7 ± 0.5	>300	>300
quercetin	0.5 ± 2.2	1.5 ± 2.2	6.5 ± 2.2	52.2 ± 0.2	179.9 ± 0.2	>200
mate saponins	122.7 ± 16.3	208.7 ± 16.3	227.1 ± 16.3	153.3 ± 0.4	209.6 ± 0.4	227.7 ± 0.4
ursolic acid	74.2 ± 12.5	>100	>100	>100	>100	>100
oleanolic acid	>100	>100	>100	>100	>100	>100

[a] IC_{10}, IC_{35}, and IC_{50} are the concentrations (μM) that resulted in 10, 35, and 50% reduction, respectively, of production/expression of pro-inflammatory responses (mean ± SD, $n = 2$). [b] Abbreviations: PGE_2, prostaglandin E_2; COX-2, cyclooxygenase-2.

Effect of Different Mate Tea Phytochemicals on NO Production and iNOS Expression by LPS-Induced RAW 264.7 Cells. Figure 2 shows the effect of different concentrations of MT phytochemicals on NO production (**A1**, **B1**, **C1**) and iNOS expression (**A2**, **B2**, **C2**) by LPS-stimulated RAW 264.7 cells.

MT extract, decaffeinated MT extract, and matein did not show any effect on NO production (**Figure 2A1**) but inhibited iNOS expression (**Figure 2A2**) at the conditions tested. Mate saponins and oleanolic acid significantly decreased NO production at 10 and 1 μM, respectively (**Figure 2B1**). Mate saponins and oleanolic acid significantly inhibited iNOS expression (80%) at 1 and 100 μM, respectively (**Figure 2B2**). Ursolic acid did not present a significant inhibition of NO and did present an extremely low inhibition for iNOS. Quercetin significantly inhibited NO production, in a dose-dependent manner, starting at a very low concentration (1 μM) (**Figure 2C1**). CHA had no effect in reducing NO production. Quercetin significantly inhibited iNOS expression (60%) at 10 μM, whereas CHA inhibited only 30% at a higher concentration (200 μM) (**Figure 2C2**).

Effect of Different Mate Tea Phytochemicals on PGE_2 Production and COX-2 Expression by LPS-Induced RAW 264.7 Cells. Figure 3 shows the effect of different concentrations of MT phytochemicals on PGE_2 production (**A1**, **B1**, **C1**) and COX-2 expression (**A2**, **B2**, **C2**) by RAW 264.7 macrophages induced by LPS.

MT extract (> 50%), decaffeinated MT extract (20%), and matein (50%) inhibited PGE_2 production at 200, 300, and 200 μM, respectively (**Figure 3A1**). MT extract significantly inhibited COX-2 expression (30%) at 100 μM, but decaffeinated MT extract (30%) and matein (25%) inhibited at concentration of 300 μM (**Figure 3A2**). Ursolic acid significantly decreased PGE_2 production (20%) at 10 μM, whereas mate saponins (90%) and oleanolic acid (10%) showed inhibition at higher concentrations (300 and 100 μM, respectively) (**Figure 3B1**). Mate saponins (50%), ursolic acid (20%), and oleanolic acid (40%) inhibited COX-2 expression at concentrations of 200, 100, and 100 μM, respectively (**Figure 3B2**). Quercetin significantly inhibited PGE_2 production (80% at 10 μM) (**Figure 3C1**) and COX-2 expression (25% at 10 μM), whereas CHA showed PGE_2 inhibition (50%) at higher concentration (200 μM) (**Figure 3C1**).

Tables 1 and **2** present the concentrations (μM) of each MT phytochemical that resulted in 10, 35, and 50% reductions against pro-inflammatory parameters. Quercetin was the most potent inhibitor of pro-inflammatory responses at a concentration 10 times lower than the concentrations used for the other compounds ($IC_{50} = 11.6$ μM for NO, 7.9 μM for iNOS, and 6.5 μM for PGE_2). Mate saponins had a high capacity in preventing inflammation through the NO/iNOS pathway ($IC_{50} = 20.8$ μM for NO and 8.4 μM for iNOS). Among the eight MT phytochemicals studied, quercetin and mate saponins exhibited the lowest IC_{50} values for inhibition of iNOS, NO, and PGE_2 production, indicating that they are the most potent phytochemicals in MT in preventing inflammation.

Interaction Study and Isobolographic Analysis. Figures 4 and **5** show the effect of combined MT phytochemicals on the production of NO (**A1**, **B1**, **C1**) and PGE_2 (**A2**, **B2**, **C2**) by LPS-induced RAW 264.7. More than additive effect was observed in the inhibition of NO production when CHA and quercetin were combined (**Figure 4A1**). Additive effects in inhibiting PGE_2 production were observed in the combination of CHA/quercetin (**Figure 4A2**). Combinations of CHA/mate saponins (**Figure 4B1**, **B2**) and CHA/matein (**Figure 4C1**, **C2**) resulted in an antagonistic effect in inhibiting NO and PGE_2 production. Combinations of quercetin/matein (**Figure 5A1**) resulted in an antagonistic effect in inhibiting NO production but resulted in an additive effect in PGE_2 production (**Figure 5A2**). Combinations of quercetin/mate saponins (**Figure 5 B1**,**B2**) resulted in synergistic effects in inhibiting both NO and PGE_2 production. More than an additive effect was observed in the inhibition of NO production when mate saponins/matein were combined (**Figure 5C1**). A slight decrease

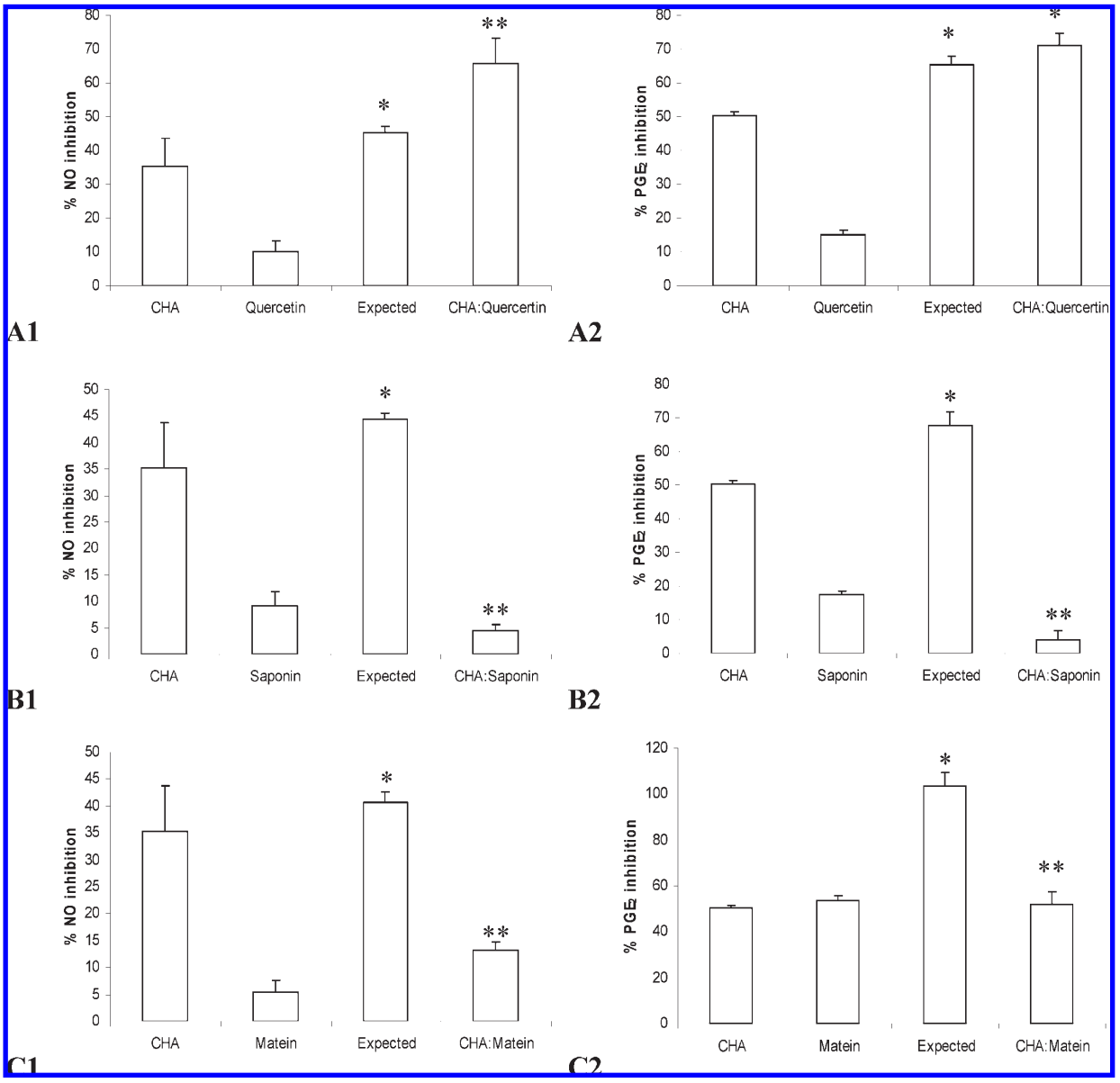

Figure 4. Inhibition (%) of NO (**1**) and PGE_2 (**2**) production by LPS-induced RAW 264.7 macrophages after treatment with (**A1**, **A2**) CHA (200 μM), quercetin (1 μM), and combination of CHA/quercetin (2.3:0.001); (**B1**, **B2**) CHA (200 μM), saponins (4 μM), and combination of CHA/saponins (2.3:0.004); and (**C1**, **C2**) CHA (200 μM), matein (200 μM), and combination of CHA/matein (2.3:0.86). The data represent the mean ± SD from two independent experiments and at least a triplicate analysis. Significant differences between observed values and additive interaction are reported at $P < 0.05$.

between the combinations of mate saponins/matein in inhibiting PGE_2 production was also shown but was not statistically significant (**Figure 5C2**). Interactions were confirmed by isobolographic analysis as shown in **Figure 6**. Quercetin and mate saponins synergistically interacted to inhibit NO (**Figure 6A1**) and PGE_2 (**Figure 6A2**), whereas CHA/mate saponins (**Figure 6B1**) and CHA/matein (**Figure 6B2**) presented an antagonistic interaction.

Effect of Mate Saponins and the Combination of Quercetin and Mate Saponins on IL-1β and IL-6 Protein Levels and on the Transcriptional Activity of NFκB and NFκB Nuclear Translocation by LPS-Induced Macrophages. Mate saponins and the combination of quercetin and mate saponins significantly inhibited IL-1β (**Figure 7A**) and IL-6 (**Figure 7B**). Transient transfection experiments using the NFκB vector indicated that quercetin and mate saponins inhibited the transcriptional activity of NFκB but that the combination of quercetin and mate saponins showed no statistical differences by measuring firefly luciferase activity (**Figure 8A**). Quercetin, mate saponins, and their combination significantly inhibited nucleus translocation of the NFκB p65 subunit (**Figure 8B**). Although quercetin and mate saponins did not statistically inhibit the nucleus translocation of NFκB p50 subunit (**Figure 8C**), their combination significantly inhibited the nucleus translocation of this subunit.

DISCUSSION

MT has been reported to have several biological functions, mostly related to antioxidant activity (*6*, *7*, *21*−*26*), interfering with glucose absorption, which might help lower the risk for type 2 diabetes (*27*) and obesity (*28*). Although a recent paper has shown that MT could be useful as an anti-inflammatory agent in lungs damaged by cigarette smoke exposure (*29*), the anti-inflammation mechanism of MT has not been studied yet. To our knowledge, this is the first study that evaluated and compared the inflammatory properties of different MT phytochemicals and investigated the potential interactions between phytochemicals using iNOS/NO and COX-2/PGE_2 mechanistic pathways.

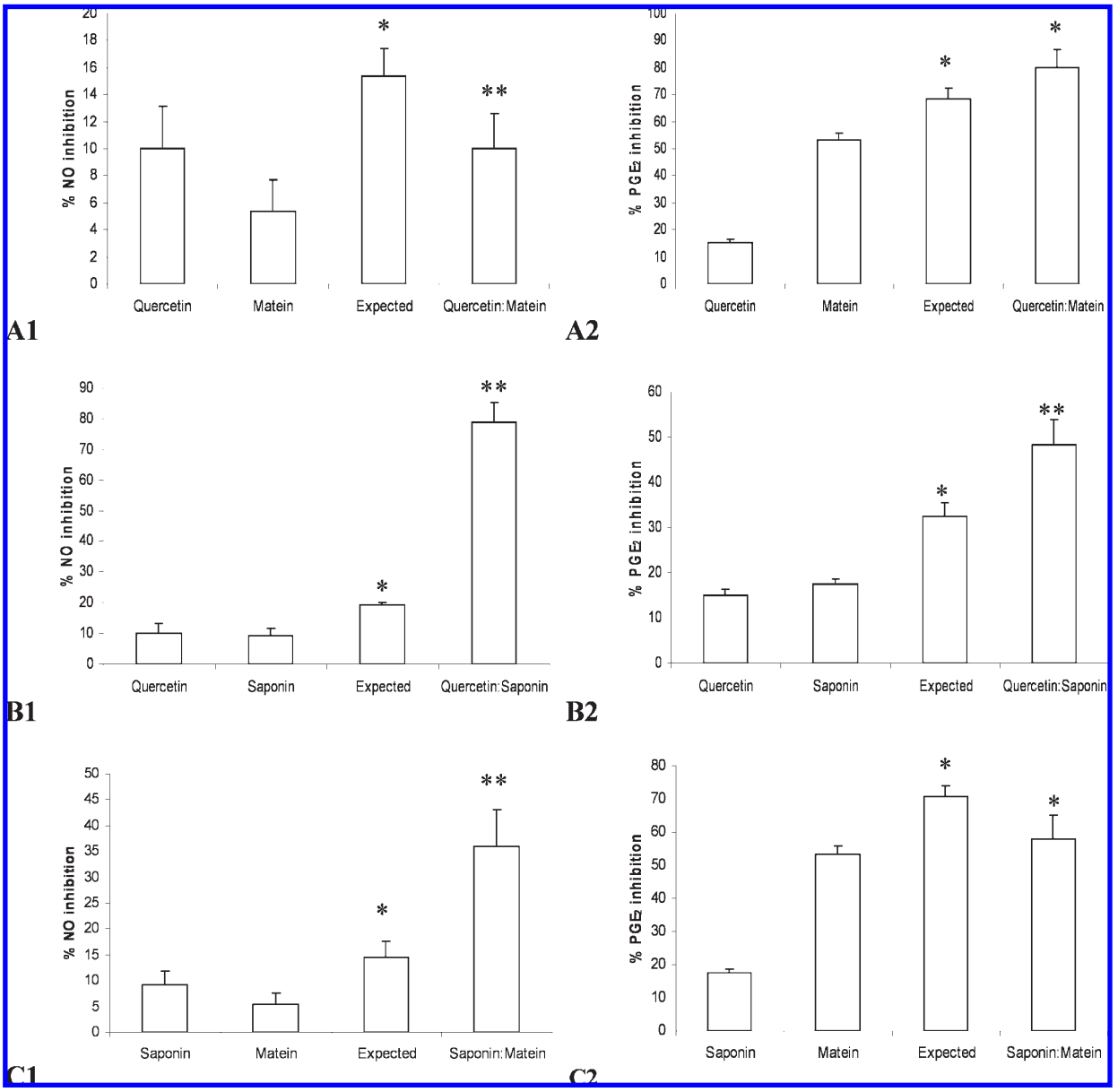

Figure 5. Inhibition (%) of NO (**1**) and PGE_2 (**2**) production by LPS-induced RAW 264.7 macrophages after treatment with (**A1**, **A2**) quercetin (1 μM), matein (200 μM), and combination of quercetin/matein (0.001:0.86); (**B1**, **B2**) quercetin (1 μM), saponins (4 μM), and combination of quercetin/saponins (0.001:0.004); and (**C1**, **C2**) saponins (4 μM), matein (200 μM), and combination of saponins/matein (0.004:0.86). The data represent the mean $\pm$ SD from two independent experiments and at least a triplicate analysis. Significant differences between observed values and additive interaction are reported at $P < 0.05$.

The results suggest that different MT phytochemicals have different capacities in inhibiting inflammation through iNOS/NO and COX-2/PGE_2 pathways. MT extract, decaffeinated MT extract, and matein showed no reduction of NO production and a slight inhibition of iNOS expression in a concentration-dependent manner. The observed slight inhibition in iNOS expression showed that these compounds are capable of inhibiting the translation of the enzyme. However, the amount of NO produced was not significantly inhibited by these compounds, which might be attributed to their incapability of inhibiting the enzyme activity. Even though the expression of iNOS was slightly inhibited, the enzyme was still present and as such NO was still produced. In addition, matein did not show a potent anti-inflammatory effect. Thus, the inhibitory effect on pro-inflammatory responses by MT may not be attributed to matein. Recently, caffeine (5−50 mg/kg) did not show an inhibitory effect on PGE_2 synthesis in inflamed tissue of male Sprague−Dawley rats (*30*).

MT contains a significant amount of triterpenoid saponins, which exist as ursolic and oleanolic acid (*19*). Our results showed that mate saponins and ursolic acid inhibited inflammation through the iNOS/NO pathway more than through the COX-2/PGE_2 pathway. Sur et al. (*14*) found that two saponins (10 mg/kg) isolated from aqueous alcohol extract of tea root had an anti-inflammatory effect by inhibiting carrageenan-induced paw edema in rats. Crude saponin extracts and purified saponin extracts (100 mg/kg) of *Hedera colchica* were shown to be potent inhibitors of acute and chronic inflammation in carrageenan- and cotton pellet-induced inflammation models in rats (*31*). It has been reported that ursolic acid (100 μM) suppressed the expression of LPS-induced pro-inflammatory mediators in RAW 264.7 macrophages that resulted from inhibition of NFκB activation (*17*, *32*). Although ursolic acid is known to have numerous pharmacological activities, the potency of ursolic acid is relatively weak (*32*), an observation in accordance with our results.

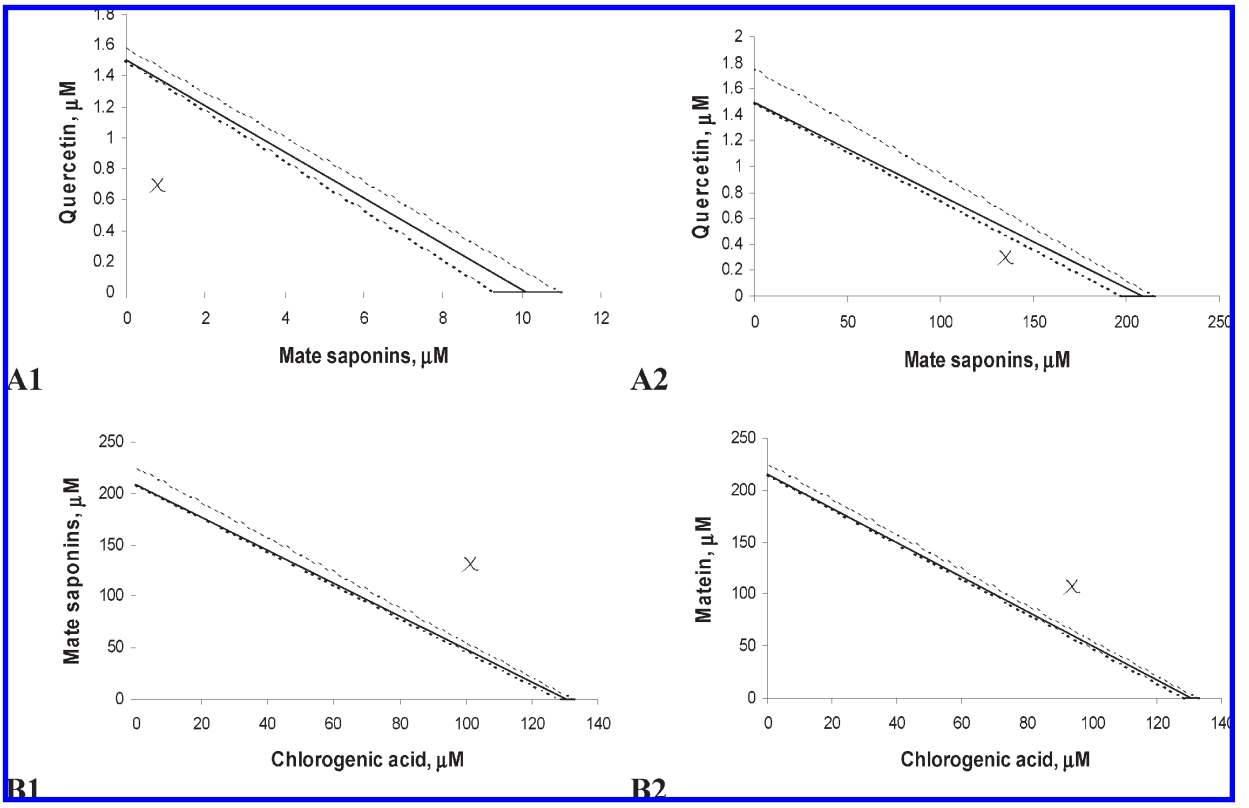

Figure 6. IC_{35} isobolograms for the synergistic interaction of (**A**) quercetin and mate saponins in inhibiting NO (**1**) and PGE_2 (**2**) production and for the antagonistic interaction of (**B1**) CHA and mate saponins and (**B2**) CHA and matein in inhibiting PGE_2 production by LPS-induced RAW 264.7 cells. The line of additivity (solid line) is shown with its 95% confidence interval (dotted lines).

CHA had a weak inhibition on pro-inflammatory markers in RAW 264.7 cells, even though it showed a very strong antioxidant capacity in vitro. These results are in agreement with those of Wang and Mazza (*33*) using the same model system. We found that CHA inhibited only the COX-2/PGE_2 pathway; this is consistent with the findings of Shan et al. (*34*). COX-2 can be activated by both AP-1 and NFκB, but iNOS is activated only by NFκB and negatively regulated by AP-1. This may explain why CHA did not alter LPS-induced iNOS expression.

Quercetin revealed a very potent anti-inflammatory effect via inhibition of NO/iNOS pathway. This finding is in accordance with Mu et al. (*10*), who showed that quercetin (10 μM) significantly reduced LPS-induced RAW 264.7 macrophage NO production in a concentration-dependent manner through down-regulating the expression of iNOS. Furthermore, Liang et al. (*11*) found that quercetin (12.5 μM) slightly enhanced the COX-2 protein level, but significantly inhibited iNOS protein level expression in LPS-induced macrophages. They suggested that the two OH groups on the B ring (**Figure 1**) decrease the inhibitory potency of quercetin on COX-2 activity (*11*). Our results showed that quercetin had less capacity to inhibit COX-2 expression. However, quercetin has been shown to suppress COX-2 expression in J774A.1 macrophages (50 μM) (*12*) and in rat aberrant crypt foci formation (4.5 g/kg) (*13*).

The combined concentrations of MT phytochemicals used in this study were equivalent to the concentrations in one cup of tea (1.5 g of DL/150 mL of water). We found that the mixture of quercetin/mate saponins exhibited the highest potency in inhibiting pro-inflammatory responses, among other combinations tested. The presence of quercetin in the mixture resulted in higher potency as compared to individual MT phytochemicals, suggesting a possible synergistic and/or additive effect. On the other

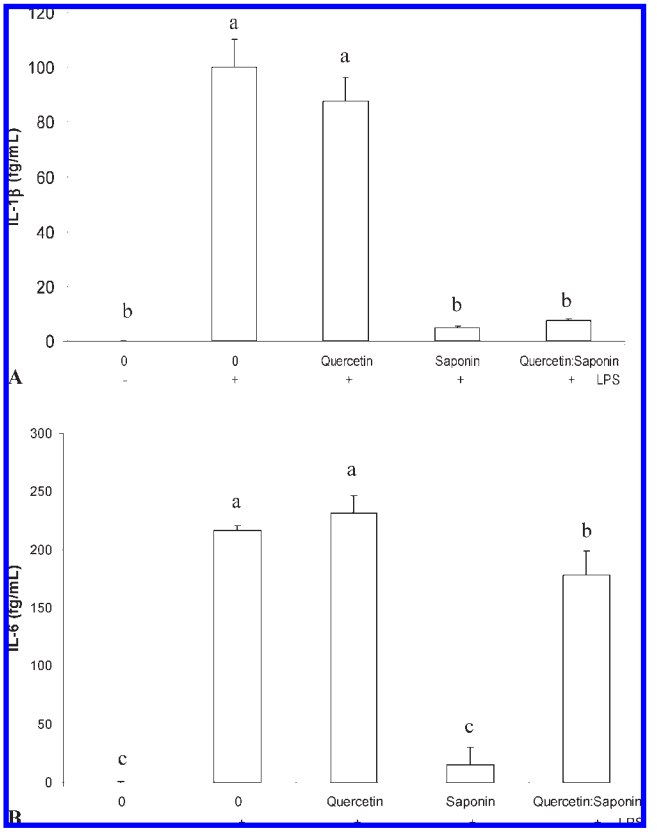

Figure 7. Effect of quercetin, mate saponins, and their combination on (**A**) IL-1β and (**B**) IL-6 production by LPS-induced RAW 264.7 macrophages. The data represent the mean ± SD from two independent experiments. Different letters indicate significant differences, $P < 0.05$.

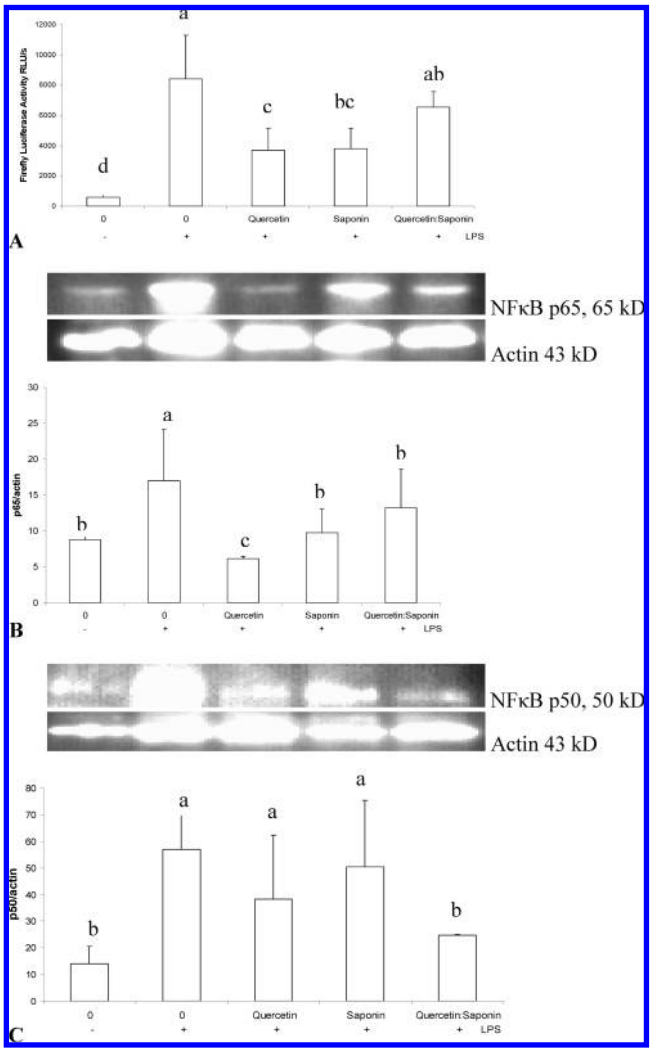

Figure 8. Effect of quercetin, mate saponins, and their combination on NFκB transactivation by LPS-induced RAW 264.7 macrophages as measured by luciferase assay (**A**) and effect of quercetin, mate saponins, and their combination on (**B**) p65 nuclear translocation and (**C**) p50 nuclear translocation by LPS-induced RAW 264.7 macrophages. The data represent the mean ± SD from two independent experiments. Different letters indicate significant differences, $P < 0.05$.

hand, combination of CHA/mate saponins and CHA/matein resulted in an antagonistic inhibition of NO and PGE_2 production. The MT extract not having a potent anti-inflammatory effect may be due to the antagonistic effect of some of its compounds. However, whole MT consumption still has a promising anti-inflammatory effect mainly through the PGE_2/COX-2 pathway.

The data suggest that the anti-inflammatory effect of MT might be due to the presence of quercetin and mate saponins even though their concentration in MT is low (*35*). The synergistic or antagonistic effects of the mixtures may depend on the chemical structures of the molecules and the possible formation of stable intermolecular complexes (*36*). These interactions could be due to attachment between the aromatic ring of phenolic acid and the B ring of flavonol, and hydrogen-bonding effects would also help to form the complex. Further studies in vivo are important to determine the bioavailability and the potential adverse effects (*37*).

Mate saponins and the combination of quercetin and mate saponins had a strong anti-inflammatory activity due to their ability to inhibit the overexpression of proinflammatory cytokine IL-1β, suggesting inhibition in the activation IKK, the enzyme responsible for the phosphorylation of IκB protein kinase. In addition, quercetin, mate saponins, and their combination inhibited the nuclear translocation of NFκB p65 in accordance with the decrease in NO and PGE_2 and reduced production of IL-6 by saponins and the mixture.

Quercetin and mate saponins reduced significantly ($P < 0.05$) the activation of NFκB in transiently transfected RAW 264.7 macrophages, suggesting a reduction in the binding of NFκB to its target DNA, so inhibiting transcription of genes with pro-inflammatory properties. Furthermore, the combination significantly inhibited nuclear translocation of NFκB p50.

In summary, our results suggest that quercetin and mate saponins are the most potent inflammation inhibitors in MT and, when combined, they have a synergistic effect. The quercetin and saponin combination, at a concentration found in one cup of MT, inhibited inflammation through suppression of NFκB pathways by inhibiting production of IL-1β, thereby suggesting prevention in the activation of IκB kinase, by blocking the translocation of p50 and p65 NFκB subunits resulting in the decreased production of IL-6, PGE_2, and NO. To our knowledge this is the first study demonstrating the efficacy and interactions of phytochemicals in MT inhibiting pro-inflammatory markers.

ABBREVIATIONS USED

CHA, chlorogenic acid; COX-2, cyclooxygenase-2; DL, dry leaves; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; NO, nitric oxide; NFκB, nuclear factor-κB; PGE_2, prostaglandin E_2; MT, mate tea.

LITERATURE CITED

(1) Heck, C. I.; Gonzalez de Mejia, E. Yerba mate tea (*Ilex paraguariensis*): a comprehensive review on chemistry, health implications, and technological considerations. *J. Food Sci.* **2007**, *72*, 138–151.

(2) Lafuente, A. G.; Guillamon, E.; Villares, A.; Rostagno, A.; Martinez, J. A. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflammation Res.* **2009**, *58*, 537–552.

(3) Agarwal, S.; Reddy, G. V.; Reddanna, P. Eicosanoids in inflammation and cancer: the role of COX-2. Expert review. *Clin. Immunol.* **2009**, *5*, 145–165.

(4) Kundu, J. K.; Surh, Y. Inflammation: gearing the journey to cancer. *Mutat. Res. Rev. Mutat. Res.* **2008**, *659*, 15–30.

(5) Granic, I.; Dolga, A. M.; Nijholt, I. M.; van Dijk, G.; Eisel, U. L. M. Inflammation and NF-κB in Alzheimer's disease and diabetes. *J. Alzheimer's Dis.* **2009**, *16*, 809–821.

(6) Ramirez-Mares, M. V.; Chandra, S.; de Mejia, E. G. In vitro chemopreventive activity of *Camellia sinesis*, *Ilex paraguariensis* and *Ardisia compressa* tea extracts and selected polyphenols. *Mutat. Res.* **2004**, *554*, 53–65.

(7) de Mejia, E. G.; Song, Y.; Ramirez-Mares, M. V.; Kobayashi, H. Effect of yerba mate (*Ilex paraguariensis*) tea on topoisomerase inhibition and oral carcinoma cell proliferation. *J. Agric. Food Chem.* **2005**, *53*, 1966–1973.

(8) Gnoatto, S. C. B.; Dassonville-Klimpt, A.; Da Nascimento, S.; Galéra, P.; et al. Evaluation of ursolic acid isolated from Ilex paraguariensis and derivatives on aromatase inhibition. *Eur. J. Med. Chem.* **2008**, *43*, 1865–1877.

(9) Itzkowitz, S. H.; Yio, X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2004**, *287*, G7–17.

(10) Mu, M. M.; Chakravortty, D.; Sugiyama, T.; Koide, N.; et al. The inhibitory action of quercetin on lipopolysaccharide-induced nitric oxide production in RAW 264.7 macrophage cells. *J. Endotoxin Res.* **2001**, *7*, 431–438.

(11) Liang, Y. C.; Huang, Y. T.; Tsai, S. H.; Lin-Shiau, S. Y.; et al. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis* **1999**, *20*, 1945–1952.
(12) Raso, G. M.; Meli, R.; Di Carlo, G.; Pacilio, M.; Di Carlo, R. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A.1. *Life Sci*. **2001**, *68*, 921–931.
(13) Warren, C. A.; Paulhill, K. J.; Davidson, L. A.; Lupton, J. R.; et al. Quercetin may suppress rat aberrant crypt foci formation by suppressing inflammatory mediators that influence proliferation and apoptosis. *J. Nutr*. **2009**, *139*, 101–105.
(14) Sur, P.; Chaudhuri, T.; Vedasiromoni, J. R.; Gomes, A.; Ganguly, D. K. Antiinflammatory and antioxidant property of saponins of tea Camellia sinensis root extract. *Phytother. Res*. **2001**, *15*, 174–176.
(15) Aggarwal, B. B.; Shishodia, S. Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem. Pharmacol*. **2006**, *71*, 1397–1421.
(16) Dixit, V.; Mak, T. W. NF-κB Signaling: many roads lead to Madrid. *Cell* **2002**, *111*, 615–619.
(17) Shishodia, S.; Majumdar, S.; Banerjee, S.; Aggarwal, B. B. Ursolic acid inhibits nuclear factor-κB activation induced by carcinogenic agents through suppression of IκBα kinase and p65 phosphorylation: correlation with down-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1. *Cancer Res*. **2003**, *63*, 4375–4383.
(18) Assis Jacques, R.; dos Santos Freitas, L.; Flores Peres, V.; Dariva, C.; et al. Chemical composition of mate tea leaves (*Ilex paraguariensis*): a study of extraction methods. *J. Sep. Sci*. **2006**, *18*, 2780–2784.
(19) Gnoatto, S.; Schenkel, E. P.; Bassani, V. L. HPLC method to assay total saponins in *Ilex paraguariensis* aqueous extract. *J. Braz. Chem. Soc*. **2005**, *4*, 723–726.
(20) Gessner, P. K. Isobolographic analysis of interactions: an update on applications and utility. *Toxicology* **1995**, *105*, 161–179.
(21) Bracesco, N.; Dell, M.; Rocha, A.; Behtas, S.; et al. Antioxidant activity of a botanical extract preparation of Ilex paraguariensis: prevention of DNA double-strand breaks in *Saccharomyces cerevisiae* and human low-density lipoprotein oxidation. *J. Altern. Complement. Med*. **2003**, *9*, 379–387.
(22) Lunceford, N.; Gugliucci, A. *Ilex paraguariensis* inhibit AGE formation more efficiently than green tea. *Fitoterapia* **2005**, *76*, 419–427.
(23) Chandra, S.; de Mejia, E. G. Polyphenolic compounds, antioxidant capacity, and reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*llex paraguariensis*) and green (*Camellia sinensis*) teas. *J. Agric. Food Chem*. **2004**, *52*, 3583–3589.
(24) Bastos, D. H. M.; Ishimoto, E. Y.; Marques, M. O. M.; Ferri, A. F.; Torres, E. A. F. S. Essential oil and antioxidant activity of green mate and mate tea (*Ilex paraguariensis*) infusions. *J. Food Compos. Anal*. **2006**, *19*, 538–543.
(25) Menini, T.; Heck, C.; Shulze, J.; de Mejia, E.; Gugliucci, A. Protective action of Ilex paraguariensis extract against free radical inactivation of paraoxonase-1 in high density lipoprotein. *Planta Med*. **2007**, *73*, 1141–1147.
(26) Miranda, D. D. C.; Arcari, D. P.; Pedrazzoli, J., Jr.; Carvalho, P. d. O.; et al. Protective effects of mate tea (*Ilex paraguariensis*) on H_2O_2-induced DNA damage and DNA repair in mice. *Mutagenesis* **2008**, *23*, 261–265.
(27) Oliveira, D. M.; Freitas, H. S.; Souza, M. F.; Arçari, D. P.; et al. Yerba maté (*Ilex paraguariensis*) aqueous extract decreases intestinal SGLT1 gene expression but does not affect other biochemical parameters in alloxan-diabetic Wistar rats. *J. Agric. Food Chem*. **2008**, *56*, 10527–10532.
(28) Pang, J.; Choi, Y.; Park, T. *Ilex paraguariensis* extract ameliorates obesity induced by high-fat diet: potential role of AMPK in the visceral adipose tissue. *Arch. Biochem. Biophys*. **2008**, *476*, 178–185.
(29) Lanzetti, M.; Bezerra, F. S.; Romana-Souza, B.; Brando-Lima, A. C.; et al. Mate tea reduced acute lung inflammation in mice exposed to cigarette smoke. *Nutrition* **2008**, *24* (4), 375–381.
(30) Fernández-Dueñas, V.; Sánchez, S.; Planas, E.; Poveda, R. Adjuvant effect of caffeine on acetylsalicylic acid anti-nociception: prostaglandin E2 synthesis determination in carrageenan-induced peripheral inflammation in rat. *Eur. J. Pain* **2008**, *12*, 157–163.
(31) Gepdiremen, A.; Mshvildadze, V.; Suleyman, H.; Elias, R. Acute and chronic anti-inflammatory effects of *Hedera colchica* in rats. *J. Ethnopharmacol*. **2004**, *94*, 191–195.
(32) Suh, N.; Honda, T.; Finlay, H. J.; Barchowsky, A.; et al. Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. *Cancer Res*. **1998**, *58*, 717–723.
(33) Wang, J.; Mazza, G. Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor in LPS/IFN-γ-activated RAW 264.7 macrophages. *J. Agric. Food Chem*. **2002**, *50*, 4183–4189.
(34) Shan, J.; Fu, J.; Zhao, Z.; Kong, X.; Huang, H.; Luo, L.; Yin, Z. Chlorogenic acid inhibits lipopolysaccharide-induced cycloxygenase-2 expression in RAW 264.7 cells through suppressing NF-κB and JNK/AP-1 activation. *Int. Immunopharmacol*. **2009**, *9*, 1042–1048.
(35) Heck, C. I.; Schmalko, M.; de Mejia, E. G. Effect of growing and drying conditions on the phenolic composition of mate teas (*Ilex paraguariensis*). *J. Agric. Food Chem*. **2008**, *56*, 8394–8403.
(36) Peyrat-Maillard, M.; Cuvelier, M.; Berset, C. Antioxidant activity of phenolic compounds in 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidation: synergistic and antagonistic effects. *J. Am. Oil Chem. Soc* **2003**, *80*, 1007–1012.
(37) Lambert, J. D.; Sang, S.; Yang, C. S. Possible controversy over dietary polyphenols: benefits vs risks. *Chem. Res. Toxicol*. **2007**, *20*, 583–585.

Received June 30, 2009. Revised manuscript received August 21, 2009. Accepted August 24, 2009. This research was supported by the University of Illinois Research Board and by a Royal Thai Government Scholarship (to S.P.).