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Anti-inflammatory Activity of a Honey Flavonoid Extract on Lipopolysaccharide-Activated N13 Microglial Cells

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ABSTRACT: Neuroinflammation is an important contributor to pathogenesis of age-related neurodegenerative disorders such as Alzheimer's or Parkinson's disease. Accumulating evidence indicates that inhibition of microglia-mediated neuroinflammation may become a reliable protective strategy for neurodegenerative processes. Flavonoids, widely distributed in the vegetable kingdom and in foods such as honey, have been suggested as novel therapeutic agents for the reduction of the deleterious effects of neuroinflammation. The present study investigated the potential protective effect of a honey flavonoid extract (HFE) on the production of pro-inflammatory mediators by lipopolysaccharide-stimulated N13 microglia. The results show that HFE significantly inhibited the release of pro-inflammatory cytokines such as TNF-α and IL-1β. The expressions of iNOS and the production of reactive oxygen intermediates (ROS) were also significantly inhibited. Accordingly, the present study demonstrates that HFE is a potent inhibitor of microglial activation and thus a potential preventive−therapeutic agent for neurodegenerative diseases involving neuroinflammation.

KEYWORDS: *honey, flavonoids, anti-inflammatory, microglia, LPS, neurodegeneration*

INTRODUCTION

Neuroinflammation is an important contributor to pathogenesis of age-related neurodegenerative disorders such as Alzheimer's or Parkinson's disease,[1,2] which represent a major health problem in moderm societies. One of the hallmarks of neurodegeneration is the presence of activated microglia, the resident macrophages in the central nervous system, which represents the first line of defense after tissue damage and/or infection. However, activated microglia may also contribute to neurodegeneration through the release of pro-inflammatory and/or cytotoxic factors such as IL-1β, TNF-α, NO, and reactive oxygen intermediates (ROS) among others.[3,4] High levels of ROS production in the respiratory burst system of activated microglia may lead to oxidative stress-mediated inflammation that contributes to neuronal death in the neurodegenerative processes.[1] Therefore, after initial damage to neurons microglia become activated and in turn release cytotoxic soluble factors. Thus, a "self-propelling" cycle causing prolonged inflammation is created.[1,4] In this context, it has been proposed that attenuation of excessive microglial activation may be therapeutic in neurodegenerative diseases.[5]

Recently, flavonoids have been suggested as novel therapeutic agents for the reduction of the deleterious effects of neuroinflammation in the brain and thus also as potential preventive drugs for neurodegenerative disease development.[6] This protective effect may reside in a number of physiological functions, including their antioxidant properties.[7] Interestingly, it is widely reported that polyphenolic flavonoids attenuate the inflammatory response after stimulation of macrophage−microglia cells.[8−15]

In the present study we have analyzed a flavonoid extract (HFE) obtained from raw multifloral honey that contains flavonoids such as luteolin, quercetin, apigenin, kaempferol, isorhamnetin, acacetin, tamarixetin, chrysin, and galangin.[16] We have already demonstrated HFE beneficial properties as antioxidant[17] and antifungal agents.[18,19] The aim of the present study was to determine whether HFE treatment might reduce the induction of pro-inflammatory mediators in lipopolysaccharide (LPS)-activated N13 microglia. The N13 cell line was used in the current study because this cell line produces a repertoire of cytokines similar to primary microglia after stimulation with LPS,[20] a component of the Gram-negative bacterial cell wall that has been shown to be a potent activator of microglia and inducer of brain inflammation-associated proteins and pro-inflammatory cytokines in many in vivo and in vitro experimental models.[21] To elucidate whether treatment with HFE could attenuate the production of inflammatory mediators at early (IL-1β, TNF-α, TLR-4) and late (iNOS) stages of LPS-induced N13 microglial activation, we analyzed the mRNA of these factors by real-time PCR and used a fluorescent method to assay the production of the ROS.

MATERIALS AND METHODS

Chemicals. Dimethyl sulfoxide (DMSO), LPS, and 2′,7′-dichlorofluorescin diacetate (DFCH-DA) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). RPMI 1640 and PBS

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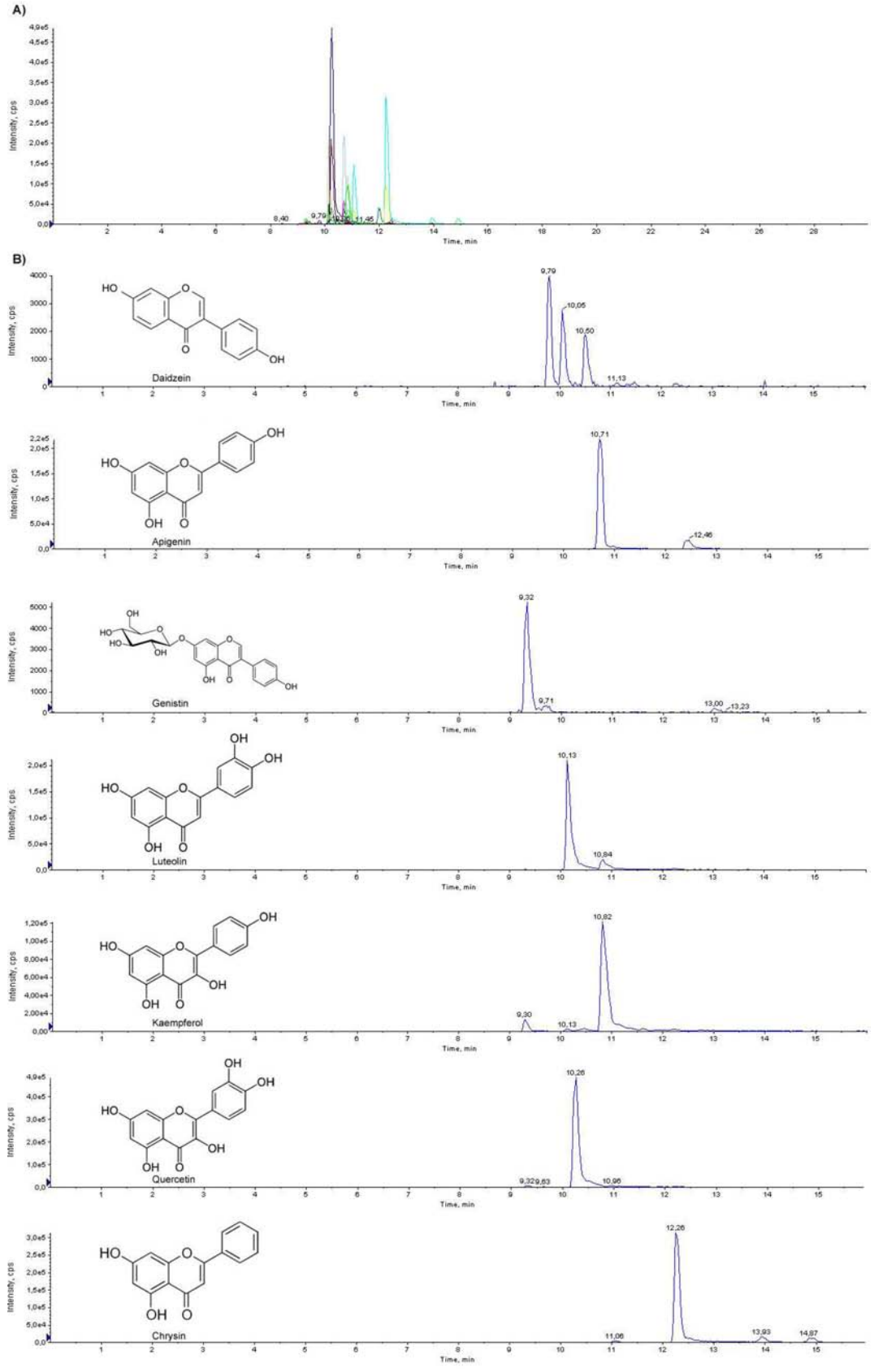

Figure 1. (A) Revese-phase chromatogram of HFE. The flavonoid fragmental ions from HFE were observed in total ion chromatography (TIC). (B) Identification of the major flavonoids was performed by mutiple reaction monitoring (MRM). The main flavonoid mass ionic fragments include daidzein (**253.1→133.1**; 253.1→ 222.9), apigenin (**269.1→117.0**; 269.1→151.0), genistin (**431.2→268.0**; 431.2→239.1), luteolin (**285.1→133.0**; 285.1→151.0), kaempferol (285.0→121.0; 285.0→135.0), quercetin (**301.1→151.0;** 301.1→179.0);, and chrysin (**253.0→63.0**; 253.0→145.0).

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was purchased from Oxoid (Basingstoke, UK). All other reagents were of analytical grade.

Unprocessed multifloral honey was locally obtained from Associazione Marchigiana Apicoltori (A.M.A., Marche, Italy) with guarantee of genuiness and known history. The sample was harvested in 2010 and stored in darkness at room temperature to minimize any alterations. As previously reported by us,[22] we have analyzed different multifloral and acacia honeys from the Marche regione (Italy), and we have chosen for the study the honey samples with higher antioxidant activities and higher concentration in flavonoids.[16−18]

Preparation and HPLC-MS Analysis of HFE. Phenolic compounds were extracted from the whole honey by use of a nonionic polystyrene resin (Amberlite XAD-2; Sigma), and more hydrophobic flavonoids were separated by diethyl ether and analyzed as previously reported.[16,17,22] Briefly, a qualitative and quantitative study of the polyphenol content was performed in a Perkin-Elmer 200 Micro HPLC system (Perkin-Elmer, USA) and a QTRAP 2000 mass detector (Sciex Applied Biosystems, Toronto, Canada), which consists of an electrospray interface and an ion trap mass analyzer. The software for the control of the equipment, acquisition, and treatment of data was Analyst, version 1.4.2.

ESI-MS/MS detection was performed in negative ionization mode. Interface conditions were optimized with tuning parameters for each standard (temperature of the capillary, 250 °C; spray voltage, 4.5 kV; capillary voltage, 25 V; focus gas flow, 80 (arbitrary units); and auxiliary gas flow, 10 (arbitrary units)).

Chromatography was performed in a Zorbax Eclipse XDB-18 column (150 × 2.1 mm i.d., 3.5 μm particle size) (Agilent Technologies, Santa Clara, CA, USA). The chromatographic conditions were as follows: flow rate of 0.25 μL/min, sample injection volume of 20 μL, and mobile phases A (10% acetonitrile, 0.1% formic acid, 89.9% water) and B (95% acetonitrile, 0.1% formic acid, 4.9% water). The identification of individual polyphenolic compounds was performed on the basis of their retention times and spectra matching with respect to standards.

The HFE was stored at −80 °C until further analysis. Just before using, aliquots were diluted with DMSO. The final DMSO concentration in cultures never exceeded 0.1% (v/v), and it did not interfere with N13 cell growth.

Determination of the Total Content of Flavonoids of HFE. The concentration of total flavonoids was determined as previously.[16,17,22] Total flavonoids were expressed as milligram catechin equivalents (CE). Samples were analyzed in three replications.

Determination of Cell Viability. After 24 h of HFE treatment (0.5 and 1 μg/mL of flavonoids), cells were washed with 1 mL of PBS and stained for 60 min with 1 mL of a 1% crystal violet solution. After careful aspiration of the crystal violet solution, the plates were washed with deionized water and dried prior to the solubilization of the bound dye with 1 mL of a 1% aqueous SDS solution. The optical density of the plates was measured at 590 nm in a microplate spectrophotometer.

N13 Cell Culture and Immunostimulation Assays. The N13 microglial line was a kind gift of Dr. David Pozo-Pérez (Dpto. Bioquímica Médica y Biología Molecular, Facultad de Medicina, Universidad Sevilla). After stimulation with LPS, N13 microglia produce a repertoire of cytokines similar to primary microglia.[20] Cells were grown in RPMI 1640 (PAA, Linz, Austria) supplemented with 2 mM glutamine (PAA), 5% (v/v) fetal bovine serum (PAA), 100 U/mL penicillin, and 100 μg/mL streptomycin (PAA) at 37 °C and 5% CO_2. For subculture, cells were removed from the culture flask with a scraper, resuspended in the culture medium, and subcultured in 6-well plates for experiments (Nunc, Thermo Fisher Scientific, USA) in culture medium at a density of 5.0 × 10^5 cells/well/2 mL. After adhering, cells were treated with HFE (0.5 and 1 μg/mL flavonoids) and/or LPS (0.01 μg/mL) and finally collected at different times after stimulation (30 min and 1, 4, and 6 h) to extract RNA and proteins. Cells treated only with vehicle but no HFE or LPS were used as control.

RNA Extraction and Reverse Transcription. For PCR analysis, total RNA was extracted using the Tripure Isolation Reagent (Roche, Germany), according to the instructions of the manufacturer. Whole cells were collected by adding 0.5 mL/well of Tripure. This procedure allows the isolation of total RNA, DNA, and protein fractions from a single sample. After isolation, the integrity of the RNA samples was assessed by agarose gel electrophoresis. The yield of total RNA was determined by measuring the absorbance (260/280 nm) of ethanol-precipitated aliquots of the samples.

Reverse transcription (RT) was performed using random hexamers primers, 3 μg of total RNA as template, and the High-Capacity cDNA Archive Kit (Applied Biosystems) following the manufacturer's recommendations as previously described.[23]

Real-Time PCR. After RT, the cDNA was diluted in sterile water and used as template for the amplification by the polymerase chain reaction. For real-time RT-PCR, each specific gene product was amplified using commercial TaqMan probes using the ABI Prism 7000 sequence detector (Applied Biosystems, Madrid, Spain) as previously described.[23] The cDNA levels were determined using GAPDH as housekeeper. The amplification of the housekeeper was done in parallel with the gene to be analyzed. Thus, the results were normalized to GAPDH expression. Threshold cycle (Ct) values were calculated using the software supplied by Applied Biosystems.

Immunoblot. Protein pellets obtained with Tripure isolation reagent according to the manufacturer's instructions were resuspended in 4% SDS and 8 M urea in 40 mM Tris-HCl. The total recovery of these fractions was determined according the method of Lowry et al.[24] and SDS−polyacrylamide gel electrophoresis (SDS-PAGE). Immunoblots were done as described elsewhere.[25] Briefly, proteins from cell culture samples were loaded on a 12% polyacrylamide gel for electrophoresis (SDS-PAGE, Bio-Rad, USA). Then, proteins were transferred to a nitrocellulose membrane (Hybond-C Extra, Amersham, Sweden). After blocking, membranes were incubated overnight at 4 °C, with the rabbit polyclonal antibody anti-iNOS (BD Bioscience, USA, 1/1000) or the mouse monoclonal antibody against β-actin (Sigma-Aldrich) at a dilution of 1/10000. The blots were developed using the ECL-plus detection method (Amersham).

Determination of ROS Levels. The intracellular ROS content was determined as described.[19] N13 microglial cells (200 μL; 0.5 × 10^5 cells/well) were inoculated in their growth medium and pretreated with 0.5 and 1 μg/mL of HFE for 30 min prior to treatment with LPS (2.5 ng/mL) for 1 h. After the incubation, 20 μM DFCH-DA (final concentration) was added. In each experiment, one well with no DFCH-DA was used as blank. The fluorescence intensities (FIs) were measured using a Spectrafluor instrument (Perkin-Elmer, Norwalk, CT; LS-5) with λ_{ex} 485 nm and λ_{em} 520 nm. The kinetic measurement of ROS was continued for 2 h after administration of DFCH-DA. ROS production was calculated by subtracting the FI value of cells not treated with DCFH-DA (blank) from the FI value of cells treated with DCFHDA. The values are expressed as percentage of production of ROS with respect to the control without LPS and HFE.

Statistics. Data were expressed individually, as the mean ± SD, or as a percentage with respect to control. For data comparison, LPS-stimulated N13 cells were compared with control nonstimulated cells. Similarly, LPS-stimulated cells treated with HFE were compared with LPS-stimulated cells. At least three independent experiments were conducted and analyzed statistically using Student's t test. Different levels of significance (*, $p < 0.05$; **, $p < 0.001$) are considered to be statistically significant.

RESULTS

Characterization an Quantification of Flavonoid Compounds in HFE. Figure 1 shows the individual chromatograms of the main phenolic compounds identified in HFE. The identification of individual polyphenolic compounds was performed on the basis of their retention times and the parent mass of the compound for MS/MS fragmentation and then specifically monitoring for two single fragment ions. Libraries comprising retention times and MS/MS data for major polyphenolic compounds expected in honey extract were made by subjecting solutions of each polyphenolic standard

to reverse phase chromatography−ESI-MS/MS analysis. Thus, the main flavonoid compounds present in HFE are daidzein, apigenin, genistin, luteolin, kaempferol, quercetin, and chrysin. We have also detected other minor flavonoids at low levels such as fisetin, isorhamnetin, acacetin, 8-methoxykaempferol, rutin, and galangin.

The main flavonoids found in HFE were quantified by reverse phase chromatography−ESI-MS/MS using a standard curve of each polyphenol that we have previously identified; the quantification is shown in Table 1.

Table 1. Quantification of Main Flavonoids in HFE[a]

	μg/mg of HFE
kaempherol	50384.72 ± 143
quercetin	11502.62 ± 378
chrysin	11356.814 ± 380
luteolin	785.74 ± 41
apigenin	544.34 ± 17
genistein	63.66 ± 12
daizdein	34.23 ± 2

[a]Total flavonoid contents are expressed as micrograms in 1 mg of HFE. All data are the mean ± SD of three independent determinations.

HFE Does Not Induce Cytotoxicity to N13. Concentrations of HFE tested on LPS-treated N13 cells showed no significant decrease in cell viability compared to untreated controls (Figure 2a). Also, HFE did not induce TNF-α or iNOS production (Figure 2b,c), demonstrating that the extract alone has no effect on N13 microglia cells.

HFE Attenuates Activation of N13 Microglia after LPS Stimulation. The model of LPS-activated microglia has been widely used as an in vitro system for the study of mechanisms underlying neuron damage by various mediators released from activated microglia. LPS signals through its Toll-like receptor (TLR)-4, leading to a cascade of intracellular events such as transcription of inflammatory genes.[26] Thus, to investigate whether HFE might attenuate the activation of microglia, we evaluated the transcriptional expression of the pro-inflammatory factors TNF-α, IL-1β, TLR-4, and iNOS in murine N13 microglia cells stimulated with LPS. The time points after LPS stimulation were chosen according to previous assays (data not shown) that determined the maximun induction for each factor. As would be expected, LPS stimulation up-regulated the mRNA expression of the pro-inflammatory factors studied. In detail, it reached maximum values at 30 min for TLR-4, at 1 h for TNF-α, at 4 h for IL-1β, and at 6 h for iNOS.

Treatment with HFE (0.5 and 1 μg/mL on flavonoids) decreased the LPS-induced mRNA expression of pro-inflammatory factors in a dose-dependent manner (Figure 3). In detail, HFE (0.5 μg/mL) decreased the expression of TNF-α up to 15%, and up to 50% with 1 μg/mL, with respect to control LPS-stimulated cells ($p < 0.05$; Figure 3a). An even greater inhibition was found in the expression of IL-1β in the presence of HFE, decreasing the mRNA expression up to 85% of control with 1 μg/mL extract ($p < 0.001$; Figure 3b).

LPS stimulation also up-regulated the mRNA expression of iNOS in N13 microglia (Figure 1c), but the induction was observed later than for TNF-α. Also, HFE strongly inhibited the LPS-induced up-regulation of iNOS mRNA in a dose-dependent manner, producing significant decreases of 66% ($p < 0.05$) and 82% ($p < 0.001$), with respect to LPS treatment, with 0.5 and 1 μg/mL of HFE, respectively (Figure 3c). Importantly, analysis of proteins isolated from N13 cells also showed a decrease in iNOS protein expressions after HFE treatment. Immunoblots revealed that HFE significantly decreased the expression of iNOS protein in a similar manner as that observed for mRNA, with both 0.5 and 1 μg/mL HFE ($p < 0.05$; Figure 4).

However, the effect on TLR-4 mRNA was less obvious. Although the mRNA of TLR-4 decreased to 73% with 1 μg/mL HFE, it was not statistically significant ($p > 0.05$; Figure 3d).

HFE Attenuates the Induction of ROS after LPS Stimulation of N13. Besides pro-inflammatory cytokines and enzymes such as iNOS, high levels of ROS are also produced in the respiratory burst system of activated microglia.[1] Thus, we also evaluated the intracellular ROS content as previously described.[19] The linearity of ROS production was good for times between 30 min and 2 h after treatment (data not shown), and the time point 1 h was selected for subsequent experiments. As shown in Figure 5, LPS stimulation increased the production of ROS up to 50% after 1 h of treatment. This effect was significantly attenuated by pretreatment with HFE. In fact, both concentrations of

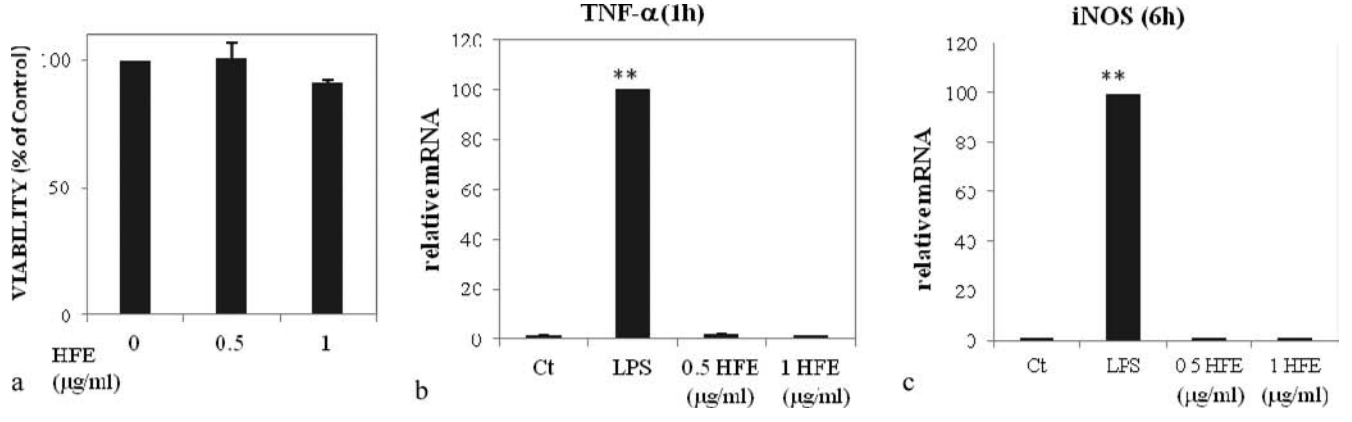

Figure 2. Cell viability was examined by crystal violet solution (a). Data are expressed as percentage of viability with respect to control cells (0) nontreated with HFE. Panels b and c show the effect of LPS (0.01 μm/mL) or HFE (0.5 and 1 μm/mL of flavonoids) on TNF-α (b) and iNOS (c) production by N13 microglia cells, 1 h (b) or 6 h (c) after treatment. Data are expressed with respect to control (Ct) nontreated cells. Results are the mean ± SD of three different experiments. (*) $p < 0.05$ and (**) $p < 0.001$, significant differences compared to control (Ct or 0).

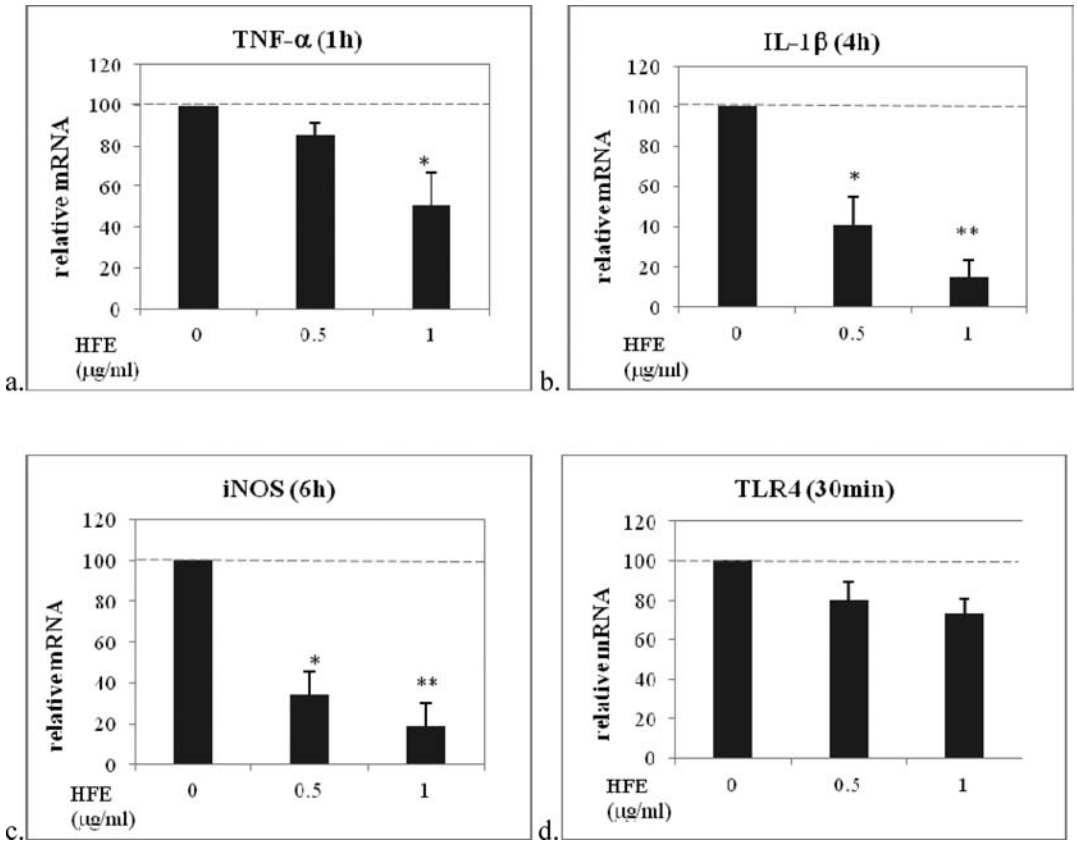

Figure 3. Protective effect of HFE on the expression of TNF-α (a), IL-1β (b), iNOS (c), and TLR4 (d) in N13 microglia cells stimulated with LPS (1 μg/mL) (cell density = 0.5 × 10^6 cells/well/2 mL). The effect of HFE on the expression of TNF-α, IL-1β, iNOS, and TLR4 in N13 cells was evaluated by detecting mRNA levels by real-time PCR after 30 min and 1, 4, and 6 h of LPS treatment. All data are presented as the mean ± SD of three independent experiments. (*) $p < 0.05$ and (**)$p < 0.001$, significant differences compared to treatment with LPS alone.

flavonoids inhibited ROS production induced by LPS ($p < 0.001$).

DISCUSSION

Nowadays it is widely accepted that inflammation plays a pivotal role in neurodegenerative process such as Parkinson's or Alzheimer's disease.[1,2] The hallmark of neuroinflammation is the activation of microglia, but although microglial activation is the brain's major defense against immune challenge, activated microglia may also contribute to neurodegeneration through the release of pro-inflammatory and/or cytotoxic factors such as IL-1β, TNF-α, NO, and ROS among others. Thus, it can be assumed that a degree of brain inflammation is required for repair of damaged tissue, but excessive inflammation causes neuronal cell death, and in this sense, it will be interesting to search for molecules that could help to control inflammation in the central nervous system.[1−4] In this context, it is known that naturally occurring food chemicals such as flavonoids are able to exert neuroprotective actions (at low physiological concentrations) via their interactions with critical neuronal/glial intracellular signaling pathways pivotal in controlling neuronal resistance to neurotoxins, including oxidants[27] and inflammatory mediators.[28] There is also evidence showing a close link between antioxidant and anti-inflammatory activities.[29] In this sense, both antioxidant and anti-inflammatory activities have been shown in bioactive natural compounds as extracts of Chinese medicinal plants rich in phenolics and flavonoids.[30] Interestingly, antioxidant, antimicrobial, and anti-inflammatory activities are associated with phenolic compounds in honey.[31−35] Because HFE is rich in flavonoids and possesses beneficial properties such as antioxidant[17] and anticandidal activities,[18,19] in this study we have evaluated the potential anti-inflammatory property of HFE. For that we used an in vitro model of inflammation by stimulating N13 microglia cells with LPS.

LPS has been shown to be a potent activator of microglia and an inducer of brain inflammation-associated proteins and pro-inflammatory cytokines in many in vivo and in vitro experimental models.[21] In this sense, intracerebral injection of LPS has been widely used to induce degeneration of dopaminergic cells in vivo,[25,36−38] and nowadays challenge with LPS in *Substantia nigra* is accepted as an animal model for studying Parkinson's disease (see ref 39 for an extensive review). We have previously reported two stages in the inflammatory response induced by LPS, both in vivo[25] and in vitro.[40] Thus, in the present work the time points after LPS stimulation of N13 were chosen according to the reported works, as well as to preliminary assays not shown here. The peak for TNF-α mRNA was studied at 1 h poststimulation with LPS, followed by up-regulation of IL-1β at 4 h and of iNOS at 6 h. Cytokines are very potent inductors of iNOS in glial cells;[41] thus, it is conceivable that after stimulation with LPS, pro-inflammatory cytokines trigger iNOS induction. Our results show that the HFE, rich in polyphenols, deeply attenuates the induction of iNOS mRNA and protein in N13 microglia after LPS stimulation. Our findings are in agreement with previous reporting that polyphenolic flavonoids attenuate the inflammatory response after stimulation of macrophage−microglia cells.

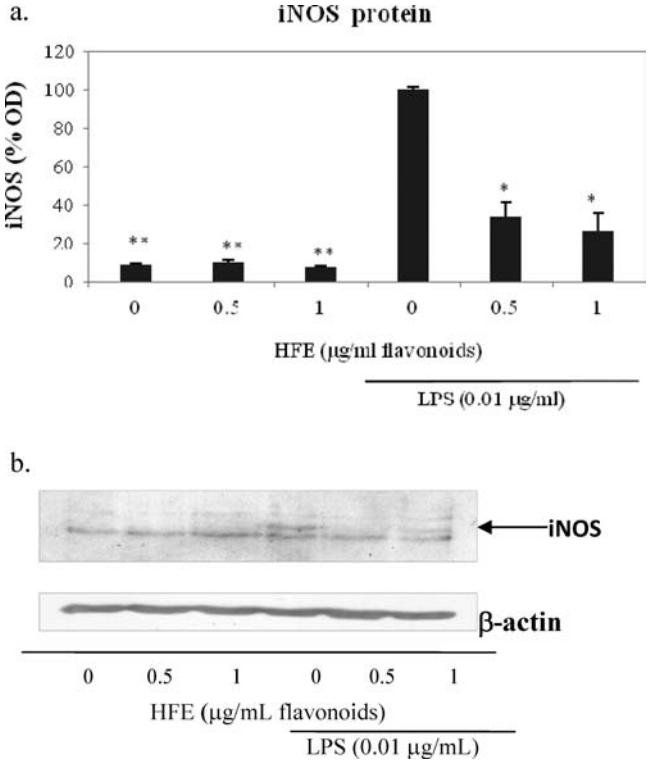

Figure 4. Expression of iNOS protein. Both concentrations of HFE (0.5 and 1 μg/mL in concentration of flavonoids) clearly decrease the expression of iNOS protein at 6 h after LPS treatment (0.01 μg/mL). Bands were scanned, and the relative density of iNOS (iNOS/β-Actin) is shown (a). Results are the mean ± SD of three different experiments. (∗) $p < 0.05$ versus LPS alone stimulated cells (0). The lower panel shows a representative immunoblot in which iNOS is detected (b).

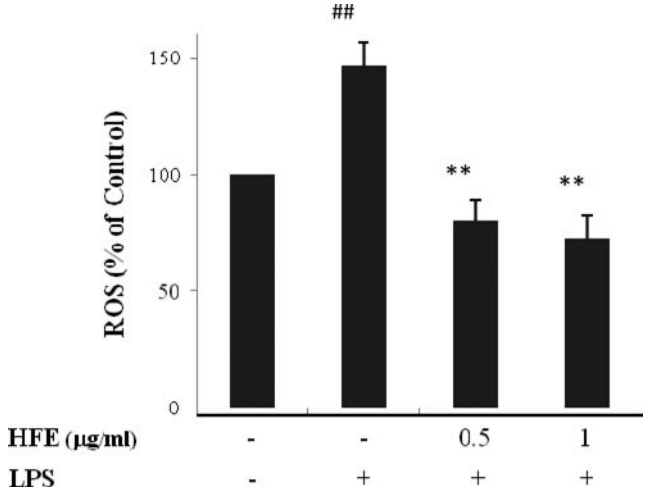

Figure 5. Effect of HFE on the LPS-induced intracellular accumulation of reactive oxygen species (ROS) in N13 microglial cells. Cells were pretreated with 0.5 and 1 μg/mL of HFE for 30 min prior to the treatment with 2.5 ng/mL of LPS for 1 h. Values are the mean ± SD from three separate experiments that represent relative fluorescence intensity. (#) $p < 0.05$ and (##)$p < 0.001$, significant differences compared with untreated controls; (∗) $p < 0.05$ and (∗∗) $p < 0.001$, significant differences compared with LPS-treated group.

Therefore, a polyphenol-enriched extract of blueberry inhibits the production of pro-inflammatory mediators after LPS activation of BV2 microglia.[8] Engeletin and astilbin exhibited remarkable inhibitory effects on LPS-stimulated mouse J774A.1 macrophage cells.[9] Clovamide, found in cocoa beans, strongly inhibited the inflammatory response after phorbol 12-myristate 13-acetate (PMA) activation in human monocytes.[10] Chrysin, a natural flavonoid found in many plant extracts, honey, and propolis, possesses an anti-inflammatory effect in murine-activated macrophages[11] and microglia.[12,13] Apigenin, another polyphenolic flavonoid present in honey, also attenuated LPS-induced nitric oxide and tumor necrosis factor production in murine microglia and macrophages.[14] Similarly, it has been demonstrated that luteolin possesses an inhibitory effect on LPS−interferon (IFN-γ)-induced NO and pro-inflammatory cytokine production in rat primary microglia and BV-2 microglial cells.[15] Although the precise components of the inflammatory response that induces neurodegeneration are not clearly elucidated and depend on the experimental model, it is worth noting that iNOS/NO from activated microglia play a central role in neuronal death in the LPS-induced neurodegeneration of dopaminergic cells.[25,42−44] Importantly, using an in vitro model of neurodegeneration after immunostimulation of BV2 microglia cells, it has been shown that NO is, at least partially, responsible for proximity-dependent microglial-mediated neural toxicity.[45] Therefore, suppression of iNOS expression may be an important strategy for prevention of neurodegenerative diseases.

However, in addition to iNOS/NO, activated microglia also produce cytokines such as TNF-α and IL-1β that may contribute to neuronal damage. In fact, cytokines not only enhance the expression of iNOS and increase NO production but also may contribute to neuronal death via their binding to specific cell surface receptors expressed in neurons that activate pro-apoptotic pathways.[46,47] Importantly, our data also show that treatment of N13 cells with HFE significantly attenuated the production of both TNF-α and IL-1β in a dose-dependent-manner. Even more, we also found that HFE strongly inhibited the ROS production induced by LPS in N13 cells. Besides pro-inflammatory cytokines and enzymes such as iNOS, high levels of ROS are also produced in the respiratory burst system of activated microglia.[1] ROS are considered to act as neurotoxin, playing a central role in the oxidative stress in the nervous system. As we mentioned above, there is a close link between antioxidant and anti-inflammatory activities, and it has been stated that the mechanistic basis of the neuroprotective activity of antioxidants relies not only on the general free radical trapping or antioxidant activity per se in neurons but also on the suppression of genes induced by pro-inflammatory cytokines and other mediators released by glial cells.[29] Thus, it has been proposed that combinations of agents which act at sequential steps in the neurodegenerative process can produce additive neuroprotective effects.[29] In this sense, both the high antioxidant capacity of HFE[17] and the anti-inflammatory effect described here make the HFE a good candidate to prevent neurodegenerative processes linked to inflammation.

On the whole, our results show that HFE exhibits pharmacological activities via an inhibitory effect on the production of LPS-induced inflammatory mediators by activated microglia. Therefore, on the basis of the high content of flavonoids of HFE, our results suggest that flavonoids could be considered as potential preventive−therapeutic agents that ameliorate the deleterious effects associated with microglial activation in the brain.

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Notes

The authors declare no competing financial interest.

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